Brain Research, 270 (1983) 231–235
Elsevier

Characterization and Regional Distribution of Serotonin S_2-Receptors in Human Brain

A. SCHOTTE, J. M. MALOTEAUX and P. M. LADURON*

Institute of Cellular Pathology (ICP), Louvain-en-Woluwe, Brussels and Department of Biochemical Pharmacology, Janssen Pharmaceutica, B-2340 Beerse (Belgium)

(Accepted February 15th, 1983)

Key words: serotonin S_2-receptors — regional distribution — human brain — [^{3}H]ketanserin binding

[^{3}H]Ketanserin binding was characterized in vitro in human brain homogenates and the regional distribution of the sites was determined.

In human brain, [^{3}H]ketanserin was found to bind on serotonin (5-HT) S_2-receptors; only 5-HT antagonists competed with the labelled ligand at nanomolar concentrations; other drugs were much less active or inactive. Special attention was paid to the choice of a displacer, here methysergide, to determine the blank value (non-displaceable binding). [^{3}H]Ketanserin binding in human brain displayed similar binding characteristics to the S_2-receptor in the rat frontal cortex, high affinity (K_d 0.69 nM) and relatively slow dissociation rate.

The regional distribution of serotonin S_2-receptors labelled with [^{3}H]ketanserin was studied in 30 different regions of human brain. The highest number of receptors was measured in the cortex. However, within the cortex the distribution was also inhomogeneous, a much lower number of sites being found in the pre- and post-central gyri. In the dopaminergic areas and the cerebellum the number of sites was quite low, and only few binding sites were detected in the corpus callosum, the medulla and the hypophysis.

The large number of serotonin S_2-receptors in the human cortex suggests that serotonin has an important role in this brain region.

INTRODUCTION

In the central nervous system, histochemical studies have shown that serotonin (5-HT)-containing neurones originate in the raphe nuclei of the brainstem and form a very diffuse system projecting to different brain areas, but most especially to the cortex and the spinal cord[5]. The existence of postsynaptic 5-HT receptors was postulated and it was suggested that they play an important role in numerous behavioural systems.

Recent biochemical binding studies revealed the existence of two distinct classes of 5-HT binding sites[18]; first, [^{3}H]5-HT has been found to label with high affinity sites[1,2,6,15] which have been called serotonin S_1-sites[18]. Since their physiological role has not yet been elucidated, they must be called binding sites or acceptor sites or recognition sites for indole derivatives rather than receptor sites[11]. In contrast, the serotonin S_2-sites labelled with [^{3}H]spiperone in the frontal cortex[12,13,16,19] and with [^{3}H]ketanserin in various brain regions[14] may be called receptors; indeed, they are involved in various behavioural and pharmacological effects such as the antagonism of tryptamine-induced clonic seizures in the rat[13,14] and the antagonism of 5-hydroxytryptophan and mescaline-induced head twitches[14,18] and the antagonism of 5-HT-induced vasoconstriction in rat caudal arteries[14]. In these tests, the ED_{50} values of numerous drugs correlate with their affinities for [^{3}H]spiperone and [^{3}H]ketanserin binding in the rat frontal cortex.

More recently, the serotonin S_2-receptor has been obtained in a dispersed form after solubilization with lysolecithin[9–11].

Previous attempts to characterize 5-HT binding sites in human brain were carried out using either [^{3}H]5-HT[3,7,8], thus a ligand for serotonin S_1-sites, or [^{3}H]LSD[4,7,8], which is known to bind to both S_1- and S_2-sites[17] and to dopamine receptors in the striatum[7].

Since [^{3}H]spiperone also binds to dopaminergic receptors in different brain regions[12,13,16,19], [^{3}H]ketanserin was likely to be a more appropriate ligand for a

* To whom correspondence should be addressed at: Department of Biochemical Pharmacology, Janssen Pharmaceutica, B-2340 Beerse, Belgium.

study on the distribution of serotonin S_2-receptors in brain.

We now report on the characterization and the regional distribution of serotonin S_2-receptors in human brain using [^{3}H]ketanserin as ligand.

MATERIALS AND METHODS

Human brains were removed 3–10 hours after death (Cliniques Universitaires Saint-Luc, U.C.L., Brussels). Exclusion criteria were treatment with serotonin antagonists or death following prolonged coma or neurological disease. Age of the patients varied between 30 and 72 years.

Dissection of brain regions was carried out on ice and tissue samples were stored at —80 °C until the time of assay. After thawing, the samples from different brain regions were homogenized in 10 vol. of 50 mM Tris-HCl buffer, pH 7.6, by means of a Potter-Elvejhem homogenizer with a conical motor-driven teflon pestle. The total homogenate was centrifuged at 16,000 rpm for 10 min in a Sorvall SS34 rotor. The pellet was resuspended in 10 vol. of buffer and recentrifuged. The final pellet, which corresponds to a total particulate fraction, was suspended in 200 vol. of buffer and used in the binding assays.

[^{3}H]Ketanserin binding

Four ml aliquots of total particulate fraction freshly prepared in Tris-HCl buffer, 50 mM pH 7.6, were incubated at 37 °C for 15 min with 2 nM [^{3}H]ketanserin (spec. act. 22.3 Ci/mmol, NEN, Boston) as previously described[14].

After incubation the [^{3}H]ligand–membrane complex was separated from the free ligand by rapid filtration through Whatman GF/B glass fibre filter discs. Filters were washed twice with 4 ml ice-cold Tris-HCl buffer, 50 mM pH 7.6, and then placed in plastic counting vials with 7 ml of Instagel II (Packard). After vigorous agitation they were counted for radioactivity in a Packard liquid scintillation spectrometer. Displaceable [^{3}H]ketanserin binding (this should be preferred to the term, specific binding), was defined as the difference between total binding and binding in the presence of 10^{-6} M methysergide. Protein content was measured by the Lowry et al. method.

RESULTS

Characterization of [^{3}H]ketanserin binding

[^{3}H]Ketanserin binding was performed in vitro using a total particulate fraction of human brain; various drugs were tested and the IC_{50} values determined. Table I shows that, as has been already reported for rat brain[14], 5-HT antagonists such as methysergide, spiperone, pipamperone and LSD were the most active compounds in displacing [^{3}H]ketanserin binding, whereas domperidone, prazosin, dexetimide and pyrilamine were found to be much less active or completely inactive. Among the agonists, only 5-HT competed at micromolar range with [^{3}H]ketanserin binding. Fig. 1 shows the inhibition curves of some 5-HT antagonists; interestingly ketanserin, spiperone and LSD displaced more [^{3}H]ketanserin binding sites than methysergide; the latter was adopted to determine non-displaceable binding (blank value) since methysergide had the lowest amount of displaceable binding sites with a plateau of maximal inhibition between 3×10^{-7} and 10^{-5} M.

Saturation experiments were performed by incubating human cortex membranes with increasing concentrations of [^{3}H]ketanserin. Fig. 2 shows that the displaceable binding was saturable from 1.5 nM while non-displaceable binding increased linearly. The Scatchard analysis gave a straight line and the K_d

TABLE I

IC_{50} values of various drugs for [^{3}H]ketanserin binding in human brain cortex and in rat frontal cortex

Drug	*IC_{50} (nM)*	
	Human	*Rat**
Pipamperone	1.1	2.6
Spiperone	3.6	1.7
(+)-Butaclamol	4.7	—
LSD	7.9	8.3
Ketanserin	8.9	1.3
Methysergide	20.4	3.1
Domperidone	94.4	263
(—)-Butaclamol	1,990	—
Pyrilamine	2,560	> 1,000
Serotonin	4,460	1,000
Prazosin	> 10,000	> 1,000
Dexetimide	> 10,000	> 1,000
Dopamine	> 10,000	> 10,000
Noradrenaline	> 10,000	> 10,000

* Taken from ref. 14.

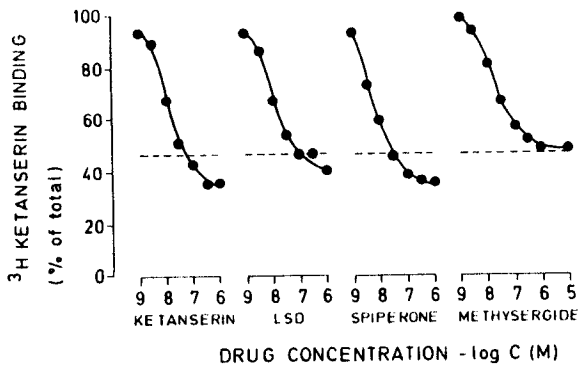

Fig. 1. Inhibition curves of [^{3}H]ketanserin binding by various drugs in total particulate fraction from human brain cortex. Non-displaceable binding (blank value) was defined by inhibition at 10^{-6} M methysergide.

and B_{max} values were 0.69 nM and 12.8 fmol/mg wet tissue.

Association and dissociation curves shown in Fig. 3 revealed a faster rate of association for [^{3}H]ketanserin than of dissociation by methysergide; specific binding was thus reversible. Table II summarizes all the kinetic parameters which were very similar to those previously found in rat frontal cortex[14].

Regional distribution

Displaceable [^{3}H]ketanserin binding was determined in 30 different regions coming from 8 human brains. In some cases, the same brain area was taken from two different hemispheres and measured separately. There was no significant difference in binding sites between the two sides. Table III shows that the cerebral cortex contains the highest density of [^{3}H]ketanserin binding sites. However, the distribution in the cortex was not identical in all the gyri; for instance, the pre- and post-central gyri revealed fewer binding sites than the other cortical areas. Dopaminergic areas like the caudate nucleus, the globus pallidus and the substantia nigra possessed a number of sites comparable to that found in the cerebellum or the hypothalamus. The thalamus, pons and medulla had only minor amounts of [^{3}H]ketanserin sites.

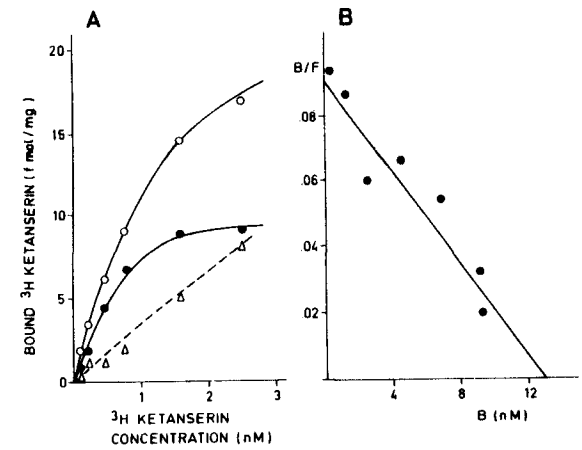

Fig. 2. Binding in a total particulate fraction from human brain cortex with increasing concentrations of [^{3}H]ketanserin. A: displaceable binding (●——●) was defined as the difference between total (○——○) and non-displaceable binding (△ - - - - △) occurring in the presence of 10^{-6} M methysergide. B: Scatchard plot of displaceable binding.

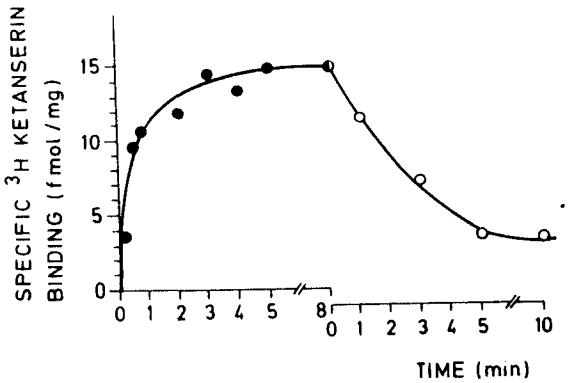

Fig. 3. Association (●——●) and dissociation (○——⊙) of displaceable [^{3}H]ketanserin in a total particulate fraction from human brain cortex. Samples were incubated for various times at 37 °C. The dissociation was started by adding methysergide in order to obtain a 10^{-6} M final concentration.

DISCUSSION

The present results provide the first regional distribution in human brain of serotonin S_2-receptors characterized in vitro by the [^{3}H]ketanserin binding assay. The high affinity of this binding in human brain homogenates is entirely consistent with an interac-

TABLE II

Kinetic parameters of [^{3}H]ketanserin binding in human brain cortex and in rat frontal cortex

Parameters	*Human*	*Rat**
K_d	0.69 nM*	0.42 nM
B_{max}	12.8 fmol/mg*	30.9 fmol/mg
Association half-life	± 30 s	—
Total association	3 min	3 min
Dissociation half-life	2.15 min	3.5 min
Total dissociation	5 min	10 min

* Taken from ref. 14.

TABLE III

Regional distribution of displaceable [³H]ketanserin binding in human brain

Values are means of experiments performed in duplicate ± S.E.M. or separate values. n = number of experiments.

Brain region	*Specific [³H]ketanserin binding (fmol/mg ± S.E.M.)*	*n*
Angular gyrus	17.4 (18.1–16.5)	2
Orbital gyrus	17.0 (17.3–16.7)	2
Supramarginal gyrus	16.9 ± 0.6	4
Inferior temporal gyrus	16.5 (17.7–15.2)	2
Middle temporal gyrus	15.8 (17.7–13.9)	2
Middle frontal gyrus	15.0 ± 0.9	4
Superior parietal lobule	12.7 ± 1.5	9
Inferior frontal gyrus	12.7 (13.3–12.0)	2
Superior temporal gyrus	12.5 ± 1.6	12
Cingulate gyrus	12.0 ± 0.8	5
Superior frontal gyrus	11.7 ± 1.7	16
Occipital lobe	11.2 ± 2.0	7
Precentral gyrus	9.1 ± 1.1	6
Postcentral gyrus	8.3 ± 1.3	12
Mamillary body	7.7 ± 1.6	2
Hippocampus	4.4 ± 0.7	4
Caudate nucleus	3.4 ± 1.1	7
Epiphysis	2.6	1
Globus pallidus	2.6 ± 0.6	6
Substantia nigra	2.4 ± 1.0	4
Geniculate body	2.4	1
Cerebellar cortex	2.2 ± 1.3	4
Uncus	2.0 ± 0.7	4
Hypothalamus	2.0	1
Thalamus	1.6 ± 1.2	3
Pons	1.3 (1.9–0.7)	2
Corpus callosum	0.6 ± 0.6	4
Medulla	0.5	1
Hypophysis	0.4	1
White matter	0.05 ± 0.04	4

tion at serotonin S_2-receptor sites; first, serotonin antagonists like methysergide, pipamperone and spiperone displaced [³H]ketanserin binding in the nanomolar range whereas drugs from other pharmacological groups (histamine H_1, adrenergic α_1, dopaminergic D_2 . . .) were much less active and even inactive. The IC_{50} values in human brain for these drugs were in good agreement with those previously reported in rat[9,14] and dog frontal cortex[9]. Secondly, among the agonists, only serotonin competed with [³H]ketanserin in the binding assay at a micromolar range; such a high concentration of biogenic amines is often necessary to displace [³H]ligands in binding studies[9,13,14]. Thirdly, the kinetic parameters found in the present work are in close agreement with those previously reported in rat frontal cortex (Table II); an important point was that [³H]ketanserin did not dissociate too quickly from the receptor site, a fact that can rule out possible release of labelled ligands from receptor sites during repeated washing of filters.

As shown in Fig. 1, special attention was paid to the measurement of non-displaceable or non-specific binding. In this regard, the choice of methysergide being used for the blank may be justified not only by the specificity of this drug but also by the fact that an inhibition plateau was obtained between 3×10^{-7} and 10^{-5} M. Methysergide and pipamperone show a lower amount of displaceable binding than ketanserin, LSD and spiperone (Fig. 1). The fact that the latter drugs displaced more [³H]ketanserin sites than methysergide or pipamperone, might suggest that these drugs also compete with non-specific displaceable binding sites. Therefore, methysergide and pipamperone may be considered as the most appropriate displacers in [³H]ketanserin binding.

The regional distribution of [³H]ketanserin binding revealed a prominent number of serotonin S_2-receptors in the cortex, which was about 10 times higher than in the basal ganglia, the thalamus and the hypothalamus. Moreover, in the cortex itself, the distribution was inhomogeneous; interestingly the pre- and post-central gyri which are known to be involved in sensory and motor activities contained much fewer S_2-receptor sites.

There is a striking difference between the regional distribution of serotonin S_2-receptors and the S_1-sites labelled with [³H]5-HT[8]; the latter binds to a large number of sites not only in the cortex but also in the hippocampus and the striatum. Moreover, the pineal gland, where there were few S_2-receptors, was reported to contain nearly as many S_1-sites as the cortex. This further strengthens the idea that the S_1- and S_2-sites are completely different entities.

The large number of serotonin S_2-receptors in the human cortex suggests that they may play an important role in this area of the brain; the involvement of these receptor sites in pathological states should be explored.

ACKNOWLEDGEMENTS

We thank D. Ashton for his help in preparing the manuscript. We are indebted to Prof. Brucher and Fievez for providing us the post-mortem brain samples. A part of this work was supported by I.W.O.N.L. J.M.M. is Aspirant of the Belgian FNRS.

REFERENCES

1 Bennett, J. L. and Aghajanian, G. K., D-LSD binding to brain homogenates: possible relationship to serotonin receptors, *Life Sci.*, 15 (1974) 1935–1944.

2 Bennett, J. P. and Snyder, S. H., Serotonin and lysergic acid diethylamide binding in rat brain membranes: relationship to postsynaptic serotonin receptors, *Molec. Pharmacol.*, 12 (1976) 373–389.

3 Bennett, J. P., Enna, S. J., Bylund, D. B., Gillin, J. G., Wyatt, R. J. and Snyder, S. H., Neurotransmitter receptors in frontal cortex of schizophrenics, *Arch. gen. Psychiat.*, 36 (1979) 927–934.

4 Cross, A. J., Interaction of [^{3}H]LSD with serotonin receptors in human brain, *Europ. J. Pharmacol.*, 82 (1982) 77–80.

5 Dahlström, A. and Fuxe, K., Evidence for the existence of monoamine containing neurons in the central nervous system, *Acta physiol. scand.*, 62, Suppl. 232 (1964) 1–55.

6 Fillion, G., Fillion, M. P., Spirakis, C., Bakers, J. M. and Jacob, J. J., 5-Hydroxytryptamine binding to synaptic membranes from rat brain, *Life Sci.*, 18 (1976) 65–74.

7 Enna, S. J., Bennett, J. P., Bylund, D. B., Snyder, S. H., Bird, E. D. and Iversen, L. L., Alterations of brain neurotransmitter receptor binding in Huntington's chorea, *Brain Research*, 116 (1976) 531–537.

8 Heltzel, J. A., Boehme, D. M. and Vogel, W. V., Serotonin binding to different regions of human brain, *Brain Research*, 204 (1981) 451–454.

9 Ilien, B., Gorissen, H. and Laduron, P. M., Characterization of solubilized serotonin (S_2) receptors in rat brain, *Molec. Pharmacol.*, 22 (1982) 243–249.

10 Ilien, B., Schotte, A. and Laduron, P. M., Solubilized serotonin receptors from rat and dog brain, *FEBS Lett.*, 138 (1982) 311–315.

11 Laduron, P. M. and Ilien, B., Solubilization of brain muscarinic dopaminergic and serotonergic receptors: a critical analysis, *Biochem. Pharmacol.*, 31 (1982) 2145–2151.

12 Leysen, J. E. and Laduron, P. M., A serotonergic component of neuroleptic receptors, *Arch. int. Pharmacodyn.*, 230 (1977) 337–339.

13 Leysen, J. E., Niemegeers, C. J. E., Tollenaere, J. P. and Laduron, P. M., Serotonergic component of neuroleptic receptors, *Nature (Lond.)*, 272 (1978) 161–171.

14 Leysen, J. E., Niemegeers, C. J. E., Van Nueten, J. M. and Laduron, P. M., ^{3}H-Ketanserin (R 41 468), a selective ligand for serotonin$_2$ receptor binding sites. Binding properties, brain distribution and functional role, *Molec. Pharmacol.*, 21 (1982) 301–314.

15 Nelson, D. L., Herbet, A., Bourgoin, S., Glowinski, J. and Hamon, M., Characteristics of central 5-HT receptors and their adaptive changes following intracerebral 5,7 dihydroxytryptamine in the rat, *Molec. Pharmacol.*, 14 (1978) 983–995.

16 Pedigo, N. W., Reisine, T. D., Fields, J. Z. and Yamamura, H. I., [^{3}H]Spiroperidol binding to two receptor sites in both the corpus striatum and frontal cortex of rat brain, *Europ. J. Pharmacol.*, 50 (1978) 451–453.

17 Peroutka, S. J. and Snyder, S. H., Multiple serotonin receptor differential binding of [^{3}H]-5-hydroxytryptamine, [^{3}H]-lysergic acid diethylamide and [^{3}H]-spiroperidol, *Molec. Pharmacol.*, 16 (1979) 687–699.

18 Peroutka, S. J., Lebovitz, R. M. and Snyder, S. H., Two distinct central serotonin receptors with different physiological functions, *Science*, 212 (1981) 827–829.

19 Withy, R. M., Mayer, R. J. and Strange, P. G., [^{3}H]Spiroperidol binding to brain neurotransmitter receptors, *FEBS Lett.*, 112 (1980) 293–295.