EVIDENCE FOR THE PRESENCE OF D₂ AND 5-HT₂ RECEPTORS IN THE HUMAN PREFRONTAL CORTEX

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Abstract—The D₂ receptor and two distinct affinity 5-HT₂ receptors in the human prefrontal cortex were labeled with ³H-spiperone. In the Scatchard analysis of stereoselective binding defined by (+)- and (–)-butaclamol, two high affinity binding sites were clearly demonstrated. Apparent K_D values of these higher and lower affinity sites determined in this manner were 0.028 and 0.64 nM, respectively. However, drug displacement studies of ³H-spiperone binding at these sites, using different concentrations of ³H-spiperone (0.05 nM for higher affinity sites and 0.5 nM for lower affinity sites), indicated that 70–80% of the higher affinity sites and almost all of the lower affinity sites showed characteristics of the 5-HT₂ receptor and that 20–30% of the higher affinity sites showed characteristics of the D₂ dopamine receptor. An additional saturation experiment related to the specific binding of ³H-spiperone to 5-HT₂ receptors confirmed the existence of two distinct populations of 5-HT₂ receptors that had different affinities for spiperone. Apparent K_D values for higher and lower affinity 5-HT₂ receptors were 0.091 and 1.27 nM, respectively. As the D₂ receptor is a possible site of antipsychotic action of neuroleptics, these receptors, especially the D₂ receptor in the human prefrontal cortex, seem to play an important role in psychotic disorders.

Binding assay techniques allow for a direct labeling of neurotransmitter receptors with radiolabeled agonists and antagonists in both the human and animal brain and provide a feasible approach for investigations on biochemical characteristics of neurotransmitter receptors. Using recently developed selective antagonists and agonists, subtypes of receptors such as D₁, D₂, D₃ and D₄ for the dopamine receptor (1) have been differentiated as well as 5-HT₁ and 5-HT₂ for the serotonin receptor (2–4). Elevation in the binding site of ³H-butyrophenones has been detected in the striatum and nucleus accumbens from schizophrenic patients (1, 5, 6). However, these brain areas do not

immediately seem related to some of the classical symptoms seen in schizophrenic patients. These symptoms such as the abnormality in thought, a spontaneity and emotional disturbances are more likely to be related to disorders of the prefrontal cortex than to those of the basal ganglia (7, 8). Although changes in the binding levels of ³H-LSD to the frontal cortex in schizophrenics (9, 10) and the identification of D₁ receptor subunits in the human frontal cortex (11) have been reported, the D₂ receptor which is a possible site for neuroleptic actions (12) and the 5-HT₂ receptor have not been identified in the human prefrontal cortex. Recently, a procedure was developed to

identify D_2 and $5-HT_2$ receptors separately and selectively using 3H -spiperone, and the density of each receptor in different regions of the rat brain (13) could be feasibly measured. These results prompted us to further investigate the D_2 and $5-HT_2$ receptors in the human prefrontal cortex. We have now identified and characterized the D_2 and $5-HT_2$ receptors in the prefrontal cortex of the autopsied human brain using specific antagonists for D_2 and $5-HT_2$ receptors, respectively.

MATERIALS AND METHODS

Human brains were obtained at autopsy from individuals who had died of causes unrelated to psychiatric or neurological diseases. All the subjects were male and the average age at death was 56 years. The interval between death and freezing of the brains at $-75^\circ C$ was 2 to 12 hr.

Crude membrane preparations from the prefrontal cortex (Areas 9, 10, 11 and 12, according to Brodmann) were prepared as follows: tissues were weighed, homogenized in 5 ml of ice cold 50 mM Tris/HCl buffer, pH 7.4, with a polytron (Brinkman PT-10; setting of 7, 20 sec) and centrifuged at 48,000 g for 10 min. The pellets were washed twice or five times by centrifugation and resuspension in the same Tris buffer and were then stored at $-75^\circ C$ until the time of binding assay.

Before use, homogenates were thawed and homogenized using the same method. Crude membrane preparations (0.47 to 0.65 mg protein/tube) were incubated with 3H -spiperone (21 Ci/mmol; RCC Amersham) in 2 ml of 50 mM Tris/HCl buffer, pH 7.4, containing 0.01% ascorbic acid for 10 min at $37^\circ C$, in triplicate. After incubation, these membranes were rapidly passed through Whatman GF/B glass fiber filters and rinsed three times with 8 ml of ice cold 50 mM Tris/HCl buffer. Radioactivity on the dried

filter was counted using Triton-toluene scintillant and a liquid scintillation spectrometer at efficiencies of 40–45%. Protein contents were determined by the method of Lowry et al. (14) using bovine serum albumin as a standard.

The sources of drugs were as follows: sulpiride, Fujisawa; butaclamol, Ayerst; haloperidol, Dainippon; ketanserin (tartrate of 3-(2-[4-(4-fluorobenzoyl)-1-piperidinyl]ethyl)-2,4 (1H, 3H)-quinazolinedione), Kyowa Hakko. Specific conditions for each experiment are given in the Results.

RESULTS

Stereoselective 3H -spiperone binding to the membrane from human prefrontal cortex: Membrane preparations washed twice or 5 times were incubated with various concentrations (0.02–2 nM) of 3H -spiperone in the presence of 1 μM (+)- or (–)-butaclamol. Stereoselective 3H -spiperone binding, defined as the difference in the amount of bound 3H -spiperone in the presence of 1 μM (–)- and (+)-butaclamol to the 5 times washed membrane preparations, was saturable and exhibited a biphasic pattern. As shown in Fig. 1, the Scatchard analysis showed the existence of two high affinity binding sites in the prefrontal cortex. These binding sites had apparent K_D values of 0.028 nM and 0.64 nM and apparent density values of 25 fmol/mg protein and 132 fmol/mg protein for the higher and lower affinity sites, respectively. In the twice washed membrane preparations, the saturation isotherms of stereoselective 3H -spiperone binding were monophasic indicating only the lower affinity sites with an apparent K_D of 0.69 nM and an apparent density of 87 fmol/mg protein (Fig. 1).

Characterization of 3H -spiperone binding sites by drug displacement experiments: Under these specified conditions we could consistently demonstrate two high affinity

Fig. 1. Representative Scatchard analysis (stereoselective ³H-spiperone binding; range was 0.02–2 nM) for human prefrontal cortex. B, bound and F, free. Five times (straight line) and twice (broken line) washed membrane preparations were incubated for 10 min at 37°C before filtration as described in Methods. Total binding and nonspecific binding were determined in the presence of 1 μM (–) and (+)-butaclamol, respectively; the difference is defined as stereoselective binding. All experimental determinations were made in triplicate. Each saturation experiment was repeated three times, giving results much the same as those presented in the figure.

Fig. 2. Displacement of ³H-spiperone binding by sulpiride (●—●), haloperidol (○—○), (+)-butaclamol (■—■) and ketanserin (□—□). Each point is the mean of triplicate determinations. Each displacement experiment was repeated three times, giving results much the same as those presented in the figure. Specific bindings were 72% and 73% of total bindings of 0.5 nM and 0.05 nM ³H-spiperone, respectively. The absolute amounts of total binding (cpm) per filter were: A, 200 and B, 1,900. A: displacement of 0.05 nM ³H-spiperone in human prefrontal cortex. B: displacement of 0.5 nM ³H-spiperone in human prefrontal cortex.

stereoselective ³H-spiperone binding sites in the human prefrontal cortex; therefore, the drug displacement experiments using sulpiride, haloperidol, (+)-butaclamol, and ketanserin were carried out with 0.05 nM and 0.5 nM ³H-spiperone in order to characterize the higher and lower affinity sites, respectively (Fig. 2A, B). Sulpiride, an antagonist which is 1,800 times more potent on D₂ than on 5-HT₂ receptors (15), was used to displace ³H-spiperone from D₂ receptors. The displacement curve of ³H-spiperone binding at 0.05 nM by sulpiride was clearly biphasic and displayed an intermediary plateau. The apparent IC₅₀ value for sulpiride sensitive sites inhibited by sulpiride

at low concentration in the prefrontal cortex (Fig. 2A) was about 10 μM and was much the same as that in the caudate nucleus (data not shown). Haloperidol which is 33 times more potent on D₂ receptors than on 5-HT₂ receptors (15, 16) gave a similar biphasic displacement curve (Fig. 2A). On the contrary, (+)-butaclamol which is only 3.8 times more potent on D₂ receptors than on 5-HT₂ receptors (16) displayed a monophasic

inhibition of ^3H -spiperone binding in the prefrontal cortex (Fig. 2A), with a much lesser potency than in the caudate nucleus (data not shown). Ketanserin, a recently developed antagonist for the 5-HT_2 receptor, which has a 100 times more potent affinity to 5-HT_2 receptors than to D_2 receptors (17) gave a biphasic displacement curve. As shown in Fig. 2A, ketanserin was most potent in inhibiting the binding of 0.05 nM ^3H -spiperone to the human prefrontal cortex. Ketanserin inhibited about 80% of the specific binding stereoselective binding at 10 nM in the prefrontal cortex (Fig. 2A), whereas only 20% of the specific binding was inhibited at this concentration in the caudate nucleus (data not shown). In addition, drug displacement experiments using 0.5 nM ^3H -spiperone were performed in an attempt to characterize the lower affinity sites (Fig. 2B). In this case, the sulpiride displacement curve was monophasic, and inhibition was not seen at concentrations lower than $10\text{ }\mu\text{M}$. Ketanserin inhibited ^3H -spiperone binding most potently; and at $0.1\text{ }\mu\text{M}$, a complete inhibition of the specific ^3H -spiperone binding occurred.

Specific ^3H -spiperone binding to 5-HT_2 receptors in the human prefrontal cortex:

The specific ^3H -spiperone binding to 5-HT_2 receptors was defined as the amount of bound ^3H -spiperone which is inhibited by the presence of $0.1\text{ }\mu\text{M}$ ketanserin in the presence of $10\text{ }\mu\text{M}$ sulpiride (to preclude the attachment of the ^3H -spiperone to D_2 receptors). The ^3H -spiperone ($0.02\text{--}2\text{ nM}$) binding to 5-HT_2 receptors in the prefrontal cortex showed biphasic saturation isotherms. Scatchard analysis clearly revealed the existence of two high affinity components of 5-HT_2 receptors (Fig. 3). The apparent K_D values of the higher and lower affinity components were 0.091 and 1.27 nM , respectively. The apparent density values for the higher affinity sites and total density were 15

Fig. 3. Representative Scatchard plot of specific ^3H -spiperone binding to 5-HT_2 receptors in the human prefrontal cortex. Saturation isotherms were obtained by placing ^3H -spiperone ($0.02\text{--}2\text{ nM}$) and five times washed membrane preparations in 50 mM Tris/HCl buffer, as described in Methods, and then incubating for 10 min at 37°C before filtration. Specific binding was measured relative to blanks containing $0.1\text{ }\mu\text{M}$ ketanserin in the presence of $10\text{ }\mu\text{M}$ sulpiride to preclude the attachment of the ^3H -spiperone to D_2 sites. All experimental determinations were made in triplicate. Each saturation experiment was repeated three times, giving results much the same as those presented in the figure.

fmol/mg protein and $124\text{ fmol/mg protein}$, respectively.

DISCUSSION

In the present experiment, we demonstrated the existence of two high affinity stereoselective ^3H -spiperone binding sites in the human prefrontal cortex. Extensively washed membrane preparations proved to be the primary requisite for observation of biphasic saturation isotherms, and these observations are consistent with the biphasic isotherms obtained in the case of the rat striatum (18). All these findings suggest the presence of endogenous inhibitors of ^3H -spiperone binding other than dopamine or serotonin because these amines are very weak displacers of ^3H -spiperone from D_2 or 5-HT_2 receptors (1, 4, 18). As butaclamol displaces stereoselectively the binding of ^3H -LSD from central serotonin receptors (19)

as well as the binding of ³H-spiperone from dopamine receptors, the two high affinity stereoselective ³H-spiperone binding sites in the prefrontal cortex appear to include these two components. It is thus essential to differentiate these two components of ³H-spiperone binding in the prefrontal cortex which receives both dopaminergic and serotonergic innervation (8, 20–24).

Furthermore, we attempted to differentiate subtypes of dopamine and serotonin receptors in the human frontal cortex using selective antagonists: sulpiride for the D₂ receptor and ketanserin (17) for the 5-HT₂ receptor. The drug displacement experiments by various antagonists using 0.05 and 0.5 nM ³H-spiperone characterized the higher and lower affinity ³H-spiperone binding sites as shown in Fig. 2. The displacement curves of 0.05 nM ³H-spiperone by various antagonists indicated that ³H-spiperone binding sites displaced by low concentrations of sulpiride or haloperidol are the dopaminergic D₂ component and occupy 20–30% of the total higher affinity sites. From the appearance of the drug displacement curve with sulpiride, the D₂ component in the human prefrontal cortex may have similar characteristics to those in the human (data not shown) and rat caudate nucleus (13).

Conversely, the 5-HT₂ component displaced by a low concentration of ketanserin seems to be a major component of the higher affinity sites. Our drug displacement experiments using 0.5 nM ³H-spiperone revealed that most of the lower affinity binding sites have the characteristics of the 5-HT₂ receptor. From the results obtained by the displacement experiments using different concentrations of ³H-spiperone, it was suggested that in the prefrontal cortex, there were two distinct components of 5-HT₂ receptors which have higher and lower affinities for ³H-spiperone. A further saturation experiment also demonstrated the existence of two high affinity 5-

HT₂ receptors in the prefrontal cortex. The apparent density values for specific ³H-spiperone binding to 5-HT₂ receptors were slightly smaller than those for the stereoselective ³H-spiperone binding sites. This comparison of the density values obtained by two saturation experiments indicated that most of the stereoselective ³H-spiperone binding sites are 5-HT₂ receptors and that the contribution of the D₂ component is not so great.

The present study provides the first evidence for the existence of D₂ dopamine and two 5-HT₂ receptors with different affinities in the human prefrontal cortex. As it is highly probable that the antipsychotic effects of neuroleptics are attributable to their ability to block D₂ receptors (12), the D₂ receptor in the prefrontal cortex may be a possible site for the therapeutic action of neuroleptics in schizophrenics. Abnormalities in two distinct 5-HT₂ receptors identified along with the D₂ receptor may be associated with psychotic disorders.

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