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 KD 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 KD 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 D₂ and 5-HT₂ 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 D₂ and 5-HT₂ receptors in the human prefrontal cortex. We have now identified and characterized the D₂ and 5-HT₂ receptors in the prefrontal cortex of the autopsied human brain using specific antagonists for D₂ and 5-HT₂ 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°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°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°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]-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 nM (+) or (-)-butaclamol. Stereoselective [³H]-spiperone binding, defined as the difference in the amount of bound [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 (+) or (-)-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 Kᵦ 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 Kᵦ 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
stereoselective $^3$H-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 $^3$H-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 D$_2$ than on 5-HT$_2$ receptors (15), was used to displace $^3$H-spiperone from D$_2$ receptors. The displacement curve of $^3$H-spiperone binding at 0.05 nM by sulpiride was clearly biphasic and displayed an intermediary plateau. The apparent IC$_{50}$ value for sulpiride sensitive sites inhibited by sulpiride at low concentration in the prefrontal cortex (Fig. 2A) was about 10 $\mu$M and was much the same as that in the caudate nucleus (data not shown). Haloperidol which is 33 times more potent on D$_2$ receptors than on 5-HT$_2$ receptors (15, 16) gave a similar biphasic displacement curve (Fig. 2A). On the contrary, (+)-butaclamol which is only 3.8 times more potent on D$_2$ receptors than on 5-HT$_2$ receptors (16) displayed a monophasic...
inhibition of $^3$H-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 $^3$H-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 $^3$H-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 μM. Ketanserin inhibited $^3$H-spiperone binding most potently; and at 0.1 μM, a complete inhibition of the specific $^3$H-spiperone binding occurred.

Specific $^3$H-spiperone binding to 5-HT$_2$ receptors in the human prefrontal cortex: The specific $^3$H-spiperone binding to 5-HT$_2$ receptors was defined as the amount of bound $^3$H-spiperone which is inhibited by the presence of 0.1 μM ketanserin in the presence of 10 μM sulpiride (to preclude the attachment of the $^3$H-spiperone to D$_2$ receptors). The $^3$H-spiperone (0.02–2 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 fmol/mg protein and 124 fmol/mg protein, respectively.

DISCUSSION

In the present experiment, we demonstrated the existence of two high affinity stereoselective $^3$H-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 $^3$H-spiperone binding other than dopamine or serotonin because these amines are very weak displacers of $^3$H-spiperone from D$_2$ or 5-HT$_2$ receptors (1, 4, 18). As butaclamol displaces stereoselectively the binding of $^3$H-LSD from central serotonin receptors (19).
as well as the binding of $^3$H-spiperone from dopamine receptors, the two high affinity stereoselective $^3$H-spiperone binding sites in the prefrontal cortex appear to include these two components. It is thus essential to differentiate these two components of $^3$H-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 D$_2$ receptor and ketanserin (17) for the 5-HT$_2$ receptor. The drug displacement experiments by various antagonists using 0.05 and 0.5 nM $^3$H-spiperone characterized the higher and lower affinity $^3$H-spiperone binding sites as shown in Fig. 2. The displacement curves of 0.05 nM $^3$H-spiperone by various antagonists indicated that $^3$H-spiperone binding sites displaced by low concentrations of sulpiride or haloperidol are the dopaminergic D$_2$ component and occupy 20–30% of the total higher affinity sites. From the appearance of the drug displacement curve with sulpiride, the D$_2$ 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-HT$_2$ 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 $^3$H-spiperone revealed that most of the lower affinity binding sites have the characteristics of the 5-HT$_2$ receptor. From the results obtained by the displacement experiments using different concentrations of $^3$H-spiperone, it was suggested that in the prefrontal cortex, there were two distinct components of 5-HT$_2$ receptors which have higher and lower affinities for $^3$H-spiperone. A further saturation experiment also demonstrated the existence of two high affinity 5-HT$_2$ receptors in the prefrontal cortex. The apparent density values for specific $^3$H-spiperone binding to 5-HT$_2$ receptors were slightly smaller than those for the stereoselective $^3$H-spiperone binding sites. This comparison of the density values obtained by two saturation experiments indicated that most of the stereoselective $^3$H-spiperone binding sites are 5-HT$_2$ receptors and that the contribution of the D$_2$ component is not so great.

The present study provides the first evidence for the existence of D$_2$ dopamine and two 5-HT$_2$ 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 D$_2$ receptors (12), the D$_2$ receptor in the prefrontal cortex may be a possible site for the therapeutic action of neuroleptics in schizophrenics. Abnormalities in two distinct 5-HT$_2$ receptors identified along with the D$_2$ receptor may be associated with psychotic disorders.

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REFERENCES

1) Seeman, P.: Brain dopamine receptors. Pharmacol. Rev. 32, 229–313 (1980)
2) Leysen, J.E., Niemegeers, C.J.E., Tollenaere, J.P., and Laduron, P.M.: Serotonergic component of neuroleptic receptors. Nature 272, 168–171 (1978)
3) Peroutka, S.J. and Snyder, S.H.: Two distinct serotonin receptors: regional variations in receptor binding in mammalian brain. Brain Res. 208, 339–347 (1981)
4) Seeman, P., Westman, K., Cosnina, D. and Warsh, J.J.: Serotonin receptors in hippocampus and frontal cortex. Eur. J. Pharmacol. 66, 179–191 (1980)
5) Lee, T. and Seeman, P.: Binding of 3H-neuroleptics and 3H-apomorphine in schizophrenic brains. Nature 274, 897–900 (1980)
6) Owen, F., Cross, A.J., Crow, T.J., Longden, A., Pulter, M. and Riley, G.J.: Increased dopamine-receptor sensitivity in schizophrenia. Lancet 29, 223–225 (1978)
7) Freeman, W.: Frontal lobotomy 1936–1956: A follow-up study of 3000 patients from one to twenty years. Am. J. Psychiatry 113, 877–886 (1957)
8) Hökfelt, T., Ljungdahl, A., Fuxe, K. and Johansson, O.: Dopamine nerve terminals in the rat limbic cortex: Aspects of the dopamine hypothesis of schizophrenia. Science 184, 177–179 (1974)
9) Bennett, J.P., Jr., Enna, S.J., Bylund, D.B., Gillin, J.C., Wyatt, R.J. and Snyder, S.H.: Neurotransmitter receptors in frontal cortex of schizophrenics. Arch. Gen. Psychiatry 36, 927–934 (1979)
10) Whitaker, P.M., Crow, T.J. and Ferrier, I.N.: Tritiated LSD binding in frontal cortex in schizophrenia. Arch. Gen. Psychiatry 38, 278–280 (1981)
11) Tanaka, C., Kuno, T. and Mizoi, Y.: Identification of the recognition binding subunit of the dopamine receptor in human brain. In Advances in Dopamine Research, Edited by Kohsaka, M., Shohmori, T., Tsukada, Y. and Woodruff, G.N., Advances in the Biosciences, Vol. 37, p. 267–272. Pergamon press, Oxford (1982)
12) Peroutka, S.J. and Snyder, S.H.: Relationship of neuroleptic drug effects at brain dopamine, serotonin, α-adrenergic, and histamine receptors to clinical potency. Am. J. Psychiatry 137, 1518–1522 (1980)
13) List, S.J. and Seeman, P.: Resolution of dopamine and serotonin receptor components of 3H-spiroperone binding to rat brain regions. Proc. Natl. Acad. Sci. U.S.A. 78, 2620–2624 (1981)
14) Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275 (1951)
15) Marchais, D., Tassin, J.P. and Bockaert, J.: Dopaminergic component of 3H-spiroperidol binding in the rat anterior cerebral cortex. Brain Res. 183, 235–240 (1980)
16) Quick, M., Iversen, L.L., Larder, A. and Mackay, A.V.P.: Use of ADTN to define specific 3H-spiroperone binding to receptors in brain. Nature 274, 513–514 (1978)
17) Leysen, J.E., Awouters, F., Kennis, L., Laduron, P.M., Vandenberk, J. and Janssen, P.A.J.: Receptor binding profile of R41 468, a novel antagonist at 5-HT₂ receptors. Life Sci. 28, 1015–1022 (1981)
18) Andorn, A.C. and Maguire, M.E.: 3H-spiroperidol binding in rat striatum: Two high affinity sites of differing selectivities. J. Neurochem. 35, 1105–1113 (1980)
19) Enna, S.J., Bennett, J.P., Jr., Burt, D.R., Creese, I. and Snyder, S.H.: Stereospecificity of interaction of neuroleptic drugs with neurotransmitters and correlation with clinical potency. Nature 263, 338–341 (1976)
20) Bergar, B., Thierry, A.M., Tassin, J.P. and Moynie, M.A.: Dopaminergic innervation of the rat prefrontal cortex: a fluorescence histochemical study. Brain Res. 106, 133–145 (1976)
21) Lindvall, O., Björklund, A., Moore, R.Y. and Stenevi, U.: Mesencephalic dopamine neurons projecting to neocortex. Brain Res. 81, 325–331 (1974)
22) Lindvall, O., Björklund, A. and Divac, I.: Organization of catecholamine neurons projecting to the frontal cortex in the rat. Brain Res. 142, 1–24 (1978)
23) Steinbusch, H.W.M.: Distribution of serotonin-immunoreactivity in the central nervous system of the rat cell bodies and terminals. Neuroscience 6, 557–618 (1981)
24) Thierry, A.M., Stinus, L., Blanc, G. and Glowinski J.: Some evidence for the existence of dopaminergic neurons in the rat cortex. Brain Res. 50, 230–234 (1973)