Dopamine receptors and schizophrenia

G. P. Reynolds

Department of Biomedical Science, The University of Sheffield, Sheffield S10 2TN, U.K.

**Introduction**

Dopamine systems have long been implicated in schizophrenia and its treatment. There is, however, little evidence for a primary abnormality of dopaminergic innervation in the disease, although subtle neuronal deficits, for which there is substantial evidence in the brain in schizophrenia, could conceivably affect dopaminergic neurotransmission in an indirect manner. However, dopamine receptors have attracted much research interest, primarily since the dopamine D2 receptor subtype has long been considered the common site of action of the classical antipsychotic drugs. In addition, there have been reports of abnormalities in the densities of these D2 and D2-like receptors in the brain in schizophrenia, and it is the possible roles of these subtypes of dopamine receptors in the aetiology and treatment of schizophrenia that will be reviewed here.

**D2 receptors and antipsychotic drugs**

Although the classical antipsychotic drugs may have effects at other neurotransmitter receptors, it is only at the D2 site that affinity for the receptor correlates well with the dosage of the drug used to control psychotic symptoms [1]. Positron emission tomography (PET) of D2 receptors in schizophrenic patients receiving such drugs has shown that their therapeutic efficacy is associated with an occupancy of these receptors at or above 70% [2]. One major problem with the drug treatment of schizophrenia is the induction of extrapyramidal motor side-effects such as parkinsonism; these are a direct consequence of D2 receptor blockade in the striatum, the brain region involved in control of automatic movement, and usually occur above about 80% receptor occupancy [2]. Thus, the desire for effective antipsychotic drugs without these, and other receptor-mediated, side-effects has been a major stimulus to research into schizophrenia and antipsychotic mechanisms. The particular efficacy of clozapine in the treatment of schizophrenia certainly indicates that other mechanisms may contribute to antipsychotic action. This ‘atypical’ antipsychotic drug has a very low propensity to induce extrapyramidal side-effects and will alleviate symptoms in up to 50% of ‘neuroleptic non-responders’, schizophrenic patients who do not respond adequately to treatment with classical antipsychotic drugs. Clozapine is unique in having a low (approx. 40%) in vivo occupancy of the D2 receptor at therapeutic doses [2]; although clozapine is known to bind with sub-micromolar affinity to a wide range of neurotransmitter receptor sites, its mechanism of antipsychotic action remains elusive [3,4].

Modern molecular biological techniques have demonstrated subtypes of the D2 receptor; it is found in a long (D2L) and a shorter (D2S) isoform, and a recent report finds that these two forms show differences in their affinities for antipsychotic drugs including clozapine [5]. It is notable that the D2S isoform, with a higher affinity for clozapine, is expressed in regions associated with a somewhat lower innervation by dopaminergic neurons. Whether this might impart some regional pharmacological selectivity has yet to be determined; however, it is notable in the light of the recent observation that clozapine has a higher affinity at D2-like receptors in the human frontal cortex than at striatal D2 sites [6]. Although these findings are of potential value in understanding mechanisms of action, there is no evidence that a substantial abnormality in the distribution of the two receptor subtypes occurs in schizophrenia.

**Novel D2-like receptors**

Recent molecular biological studies have identified mRNA for two proteins with structures typical of G-protein-linked receptors and with approximately 40% homology to the D2 receptor. These substantial similarities in structure were reflected in the new receptors’ pharmacology, indicating their function to be dopamine receptors. The first of the cloned D2-like receptors, D3, has a higher affinity for dopamine and other agonists than does D2, but a lower affinity for many antagonists [7]. However, as the receptor is expressed in certain limbic regions of the human brain, structures considered to be important in the development and treatment of psychosis, it has been suggested that blockade of these D3 sites may mediate antipsychotic effects.
while antagonist action at the D2 receptors of the striatum is responsible for extrapyramidal symptoms. The function of the D3 receptor is discussed more comprehensively elsewhere in this volume.

However, it is the other D2-like receptor that has generated the most interest as a possible site of antipsychotic action. D4 mRNA has a distribution in the brain that is very different from that of D2, being expressed mainly outside the striatum in regions that include the amygdala and frontal cortex [8,9]. These regions have a dopaminergic innervation in man that is much lower than either the striatum, where D2 is most concentrated, or the limbic regions where D3 is expressed. However, both the amygdala and frontal cortex are strongly implicated in the functional pathology of schizophrenia [10].

In the first report of its pharmacology, it was immediately apparent that the D4 receptor has some interesting properties that indicate its potential importance in the drug treatment of schizophrenia. Although most antipsychotic drugs have substantially lower affinities for D4 than for D2 receptors, the affinity of clozapine was reported to be 15 times greater at the D4 site [8]. This observation led to the suggestion that clozapine’s efficacy is due to D4 receptor blockade. Such an interpretation is open to criticism, particularly since the increased affinity of clozapine for the D4 receptor is not consistently reported; others have found clozapine’s affinity for human D4 in various expression systems to be only two- or threefold greater than the drug’s affinity at the D2 site [11-13].

D4 receptors in schizophrenia

The proposed involvement of D4-like receptors in schizophrenia goes beyond that role mediating the action of antipsychotic drugs; they may have an aetiological role. The first indication that D4 receptors may be abnormal in schizophrenia came from post-mortem studies that identified an increase in their density in the brains of schizophrenic patients [14]. Although initially considered to be unrelated to drug treatment, further studies failed to find an elevation in D4 sites in brain tissue from patients who had been free of antipsychotic treatment before death [15,16], supporting the interpretation that such treatment was responsible for the receptor increase. Certainly chronic treatment of animals with antipsychotic drugs leads to an up-regulation of D4 receptors [17]. We have observed that 6 weeks’ administration of haloperidol to rats can increase striatal and pallidal D2-like receptors by 63% and 95% respectively, similar to the changes observed in drug-treated schizophrenic patients [18]. However, some dispute over this interpretation still remains; an initial PET study of radioligand binding to D2 sites in vivo showed an increase in receptor density in drug-free patients [19], although further receptor-imaging studies, using different radioligands, have failed to find this increase [20,21].

The early post-mortem studies, and the PET studies mentioned above, were undertaken before the identification of the D4 and D4 receptors, and thus did not differentiate these various D2-like receptors in human brain. In the past 2 years attempts to remedy this have been made, yielding some fascinating, if confusing, results.

D4 receptors in schizophrenia

Perhaps the greatest recent stimulus to dopamine receptor research has been the report of a sixfold increase in D4 receptors in post-mortem brain tissue in schizophrenia [22]. Surprisingly, this was observed in the striatum, where only very low levels of D4 mRNA are found. The authors determined D4 receptors by subtracting saturable binding of [3H]raclopride to D2 and D3 sites from that of [3H]emonapride, defining D2, D1 and D4. This report also implies that the increase in D4 receptors is unrelated to the up-regulatory effects of antipsychotic drugs. However, there remains substantial evidence (discussed above) that the increase in D2-like receptors in post-mortem brain in schizophrenia is due to prior drug treatment.

There have been further attempts to determine brain D4 receptors in schizophrenia. An autoradiographic application of the differential radioligand-binding technique demonstrated an apparent D4 increase in schizophrenia [23], although it was reported that the D4 sites, even in control subjects, constituted approximately 50% of total D2-like receptors. This result suggests some methodological problem, probably related to the limitations in the use of raclopride to define all D2/D1 sites. An attempt to assess the D4 component of D2-like receptors by displacement of [3H]emonapride binding with raclopride was unable to identify any striatal D4 sites, and concluded that the elevation in striatal [3H]emonapride binding in schizophrenia is due to an excess of D2 or D3 receptors and seen only in patients receiving antipsychotic drugs [24]. In...
order to clarify these discrepancies, we have employed a new ligand, \([^{[125]I}]\)epidepride, which, like raclopride, has over 100-fold selectivity for the D\(_2\) and D\(_3\) sites over D\(_4\). We compared the saturable binding of this ligand with that of \([^{[3]H}]\)emonapride; both ligands defined a substantial (over 75\%) elevation in D\(_2\)-like receptors in the putamen of (drug-treated) schizophrenic subjects, with no evidence for D\(_4\) sites apparent [25]. We conclude that D\(_4\) receptors are not consistently detectable by conventional radioligand-binding techniques in human striatum, nor are they elevated in schizophrenia.

**Molecular genetics of D\(_2\)-like receptors in schizophrenia**

A further approach attempting to relate dopamine receptor abnormalities to schizophrenia has been the application of molecular genetics. The D\(_2\) receptor is coded for on chromosome 11, which has been implicated in genetic aspects of schizophrenia. However, the D\(_4\) region is not associated with the chromosome 11 linkage [26]. There has been one Japanese study of a point mutation with a higher frequency in patients with schizophrenia, particularly those with a family history, than in a control population (allele frequency 13.5\% and 1.8\% respectively) [27], although initial studies of the D\(_4\) receptor gene indicated no polymorphisms associated with the disease [28]. Interestingly, the D\(_4\) receptor gene demonstrates unusually frequent structural differences between individuals. A polymorphism of D\(_4\) has been identified [29] in which the length of the third intracellular loop, likely to be the site of G-protein interaction, varies according to the number and form of a 48-bp repeat sequence. Investigating these genetic differences in a group of clozapine-treated schizophrenic patients indicated that differences in clinical response to clozapine do not relate to D\(_4\) structure [30,31]. Other studies searching for an association of these and other D\(_4\) variants with schizophrenia have also proved negative [31–33], while linkage studies in large pedigrees have found no relationship to hereditary schizophrenia (e.g. [34]). Thus there appears to be no evidence for any association between D\(_4\) allelic forms and schizophrenia; even a deletion predicted to yield a non-functional receptor [35] in humans was not apparently associated with schizophrenia or any other major dysfunction.

Thus, there is as yet no reproducible evidence relating to a role for the D\(_4\) receptor in normal brain function, let alone in the aetiology of schizophrenia. Nevertheless, there remains substantial interest in this receptor as a potential site of antipsychotic action. We must await the development of selective D\(_4\) antagonists to identify the presence of these receptors in the brain and to determine whether they may indeed be valuable as sites of action in the treatment of schizophrenia. So far, however, the evidence does not provide much encouragement.

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