The dopamine D4 receptor in schizophrenia: an update[1]

INTRODUCTION

The dopamine D2 receptor as defined in classical pharmacological terms (not linked to adenylate cyclase) is historically the site of action of psychotropic drugs and the prime neurochemical candidate for schizophrenia. Molecular genetic studies of dopamine receptors have now shown that they belong to a genetically similar superfamily of receptors that are characterized by having seven transmembrane protein domains and are coupled to G-protein regulated second messenger systems. On the basis of sequence homology and pharmacological characteristics, they remain in two categories broadly similar to, and named after, traditional D1 and D2 receptors (Sibley & Monsma, 1992). The subfamily of D2 receptor is further divided on the basis of sequence into D3 and D4 receptors, which overall have the characteristics of D2 receptors but with interesting anatomical distributions and one or two crucial differences in pharmacology, such as the high affinity of clozapine for the D4 receptor (Van Tol *et al.* 1992). The overall D2 characteristics, plus the anomalous divergence from these characteristics, make the D4 receptor a highly attractive candidate for the dopamine receptor abnormality of schizophrenia. This editorial assesses progress to date in this rapidly evolving story.

BACKGROUND

D2 and D4 receptors in schizophrenia – resolution of some anomalies

Despite recent advances in the molecular genetics and epidemiology of schizophrenia, a dopamine receptor hypothesis overall endures as a strong candidate for a metabolic abnormality underlying some of the pathophysiology of schizophrenia. This theory is based on the ability of dopamine agonists and releasers to provoke psychosis (Randrup & Munkvad, 1967), the ability of antipsychotic drugs to block D2 receptors with a rank affinity that correlates with their clinical potency (Seeman *et al.* 1976) and the ability of geometrical isomers of flupenthixol to dissociate clinical efficacy on the basis of whether they possess D2 receptor blocking activity or not (Johnstone *et al.* 1978). More recently, dopamine D2 receptors have, in the main, been shown to be elevated in schizophrenic brain post-mortem (Mackay *et al.* 1978; Cross *et al.* 1981; Seeman, 1992). Most recently, attempts have been made to demonstrate the D2 receptor abnormality by functional neuroimaging techniques. Although only one study (Wong *et al.* 1986) shows bulk elevation of D2 receptors using N-methyl-spiperone and positron emission tomography; a number of other studies have shown a range of subtle abnormalities. Thus, Farde *et al.* (1990) noticed a greater degree of asymmetry in D2 receptors in patients. This has been confirmed by our own studies in a large group of never medicated patients where the asymmetry was strongest in male patients (Pilowsky *et al.* 1993). Finally, Martinot *et al.* (1991) demonstrated loss of the normal age dependent decline in D2 receptors in schizophrenic patients. On the whole, it is difficult to see how these minor anomalies relate to a simple D2 hypothesis and could be epiphenomena of other major changes in schizophrenia e.g. asymmetrical structural changes (Crow, 1995). Despite these advances, there are a number of inconsistencies in the D2 receptor hypothesis that have kept the elucidation of the role of D2 receptors in schizophrenia tantalizingly out of reach – inconsistencies which the D4 receptor

may have the capacity to resolve. For instance, increased density of D2 receptors in post-mortem samples from schizophrenics can, in a majority of studies, be due to D2 receptor supersensitivity induced by chronic antipsychotic treatment. It is also apparent that inconsistencies in drug response cannot be accounted for on the basis of a site of action at D2 receptors. For example, earlier PET and SPET studies have impressively demonstrated that good clinical response to clozapine occurs despite low D2 occupancy (Brucke *et al.* 1992; Farde *et al.* 1992; Pilowsky *et al.* 1992), strongly suggesting that clozapine action is not mediated by D2 receptor blockade. Conversely, up to 30% of patients remain unresponsive to typical antipsychotics and absence of response in treatment refractory patients is, nevertheless, associated with maximal D2 receptor blockade (Pilowsky *et al.* 1993).

Initial descriptions (Van Tol *et al.* 1992) suggested the D4 receptor was localized uniquely to frontal cortex, limbic areas and medial temporal lobe (all relevant localizations to the neuropsychology and neuropathology of schizophrenia (Kerwin, 1992). In addition, clozapine has a uniquely high affinity for the D4 receptor (Van Tol *et al.* 1991), suggesting this as a strong candidate for the locus of action of this drug.

Most recently, Van Tol *et al.* (1992) have shown that the D4 receptor occurs in at least five polymorphic forms whereby a 48 base-pair repeat sequence in the third intracytoplasmic loop occurs as a two- to eight-fold repeat. This is a functional polymorphism, expression systems for which display differing binding potential for clozapine. In general, such pharmacological functional polymorphisms are strong candidates for the pathophysiology of the disease relevant to the drug related polymorphism (Sobell *et al.* 1992). Excitement for the role of D4 receptors in schizophrenia was enhanced by the findings of Seeman *et al.* (1993*a*) using a radioligand method that subtracts the binding data of [^{3}H]raclopride D2, D3 selective) from [^{3}H]emonamide (D2, D3, D4 selective) demonstrated a six-fold elevation of this receptor in post-mortem schizophrenic brain.

Therefore, indirect post-mortem studies and the high degree of functional genetic variability strongly indicate the potential importance of D4 receptors in the aetiology of schizophrenia and response to antipsychotics. In the past 12 months many workers have attempted to confirm a direct role for D4 receptors in schizophrenia, in genetic, post-mortem, functional and pharmacological association studies and while the D4 receptor remains interesting, its complexity has surprised many and defied a simple testing of a linear relationship between the D4 receptor and schizophrenia.

GENETIC STUDIES OF D4 RECEPTORS AND SCHIZOPHRENIA

Family, twin and adoption studies leave no doubt that genetic factors are a major part of the aetiology of schizophrenia (McGuffin, 1988). The simplest and most direct explanation for a role of D4 in schizophrenia and antipsychotic drug response is that a genetic variant of it may be responsible for some aspect of the illness acting either as a single gene or as part of a polygenetic predisposition to the illness. The gene has been localized to chromosome 11p15.5 (Gerlenter *et al.* 1992) and a number of polymorphisms and variants have been isolated (see next section for references) allowing a role for the D4 gene in the aetiology of neuropsychiatric disorders to be tested by both linkage and allelic association studies. Without exception genetic studies have so far ruled out the D4 receptor gene as a major contributor to the development of schizophrenia. Linkage analysis studies have been performed using the polymorphic 48-bp repeat itself in British (Shaikh *et al.* 1994) Swedish (Barr *et al.* 1993), Italian (Macciardi *et al.* 1994) or French families (Campion *et al.* 1994), or other polymorphisms near the DRD4 locus in a small number of families in the USA (Coon *et al.* 1993). All of these provide evidence against a major gene at this site by either the LOD score method, using a variety of single gene models, or non-parametric methods such as affected sib-pair analysis. Similar findings have been reported for bipolar affective disorder (De bruyn *et al.* 1994) although a small positive lod score has been reported in an old order Amish family (Sidenberg *et al.* 1994). The question remains as to whether these and or other variants act as genes of small effect in a complex polygenic background, as a rare cause in certain families, or as modifiers of the phenotype.

Petronis *et al.* (1993), Shaikh *et al.* (1993) and Campion *et al.* (1994) have performed case/control allelic association study of unrelated schizophrenics and controls and found no significant association between alleles of the 48-bp repeat in exon III and schizophrenia. Allelic association between D4 and delusional disorder has been reported (Catalano *et al.* 1993) but this is as yet unreplicated.

In addition to the eight or more variants of the 48-bp repeat in exon III (Lichter *et al.* 1993; Shaikh *et al.* 1993) several new polymorphisms have been detected in other regions of the D4 gene. These are a G/C mononucleotide repeat with three common allelic forms in intron I (Petronis *et al.* 1994*a*), a SmaI PCR-RFLP in the 5' non-coding region (Petronis *et al.* 1994*b*) a 12-bp repeat in exon I (Catalano *et al.* 1993) a 13-bp deletion in exon I (Nothen *et al.* 1994) and a point mutation in the third cytoplasmic loop (Seeman *et al.* 1993*b*). To add to this complexity, extensive sequence variation occurs within the 48-bp repeats in exon III themselves, leading to at least 25 different haplotypes, some of which are very rare (Lichter *et al.* 1993). All of the variations described that fall within the coding region of the D4 gene alter the amino acid sequence, making it one of the most variable functional proteins ever described. This means that any two individuals are very unlikely to share an identical D4 protein.

When Van Tol *et al.* (1992) demonstrated that the 48-bp repeat in the third cytoplasmic loop displayed variations in sodium dependent clozapine binding, (the affinity for clozapine at the shorter 4-repeat is double that at the longer 7-repeat in the absence of sodium). It became possible to test the hypothesis that if the D4 receptor is a major site of clozapine, polymorphic differences may be reflected as variation in clinical response to the drug. The hypothesis is specifically that poor responders have an excess of longer repeats with lower affinities for clozapine. Three studies have now addressed this. In our own (Shaikh *et al.* 1993) we examined alleles of the 48-bp repeat in 41 responders and 23 non-responders to clozapine and found no direct relationship, indicating that the repeat variation does not have a simple influence on response. This finding is supported by Kennedy *et al.* (1993, 1994) who analysed the same polymorphism in 60 patients from the USA and Canada. In order to test for weaker genetic effects or the influence of rare alleles we have also examined a larger series of 200 patients (Shaikh *et al.* 1995) analysed by representing response as a continuous variable to minimize classification error, and again found no relationship between genotype and response. In a fourth study (Rao *et al.* 1994), responsivity to clozapine was classified *versus* fluphanazine, or placebo, instead of using an internal comparison. Although consistent with the earlier negative findings of Shaikh *et al.* (1993), it is not possible to draw any conclusions from this study because of the small number of patients used. However, a positive correlation between the D4 receptor and response has been found by Kennedy *et al.* (1994), analysing all known polymorphisms, including the exon III repeat, spaced throughout the gene. None of the polymorphisms showed an independent association with response, but combined analysis allowed prediction of 76% of the total variance and 84% of the variance in response alone. This has now been replicated in an independent sample (J. L. Kennedy, personal communication).

Thus, functional polymorphism, with respect to clozapine, does not reveal an obvious difference in response. This is evidence against this being a site of action of clozapine.

Do we need D4 at all?

The function of the receptor protein D4 in the brain remains obscure. However, a recent fascinating finding, which may help us understand its role, is the observation of an individual who completely lacks the D4 protein (Nothen *et al.* 1994). This is caused by the 13-bp deletion in exon I of the gene causing a natural nonsense mutation, resulting in a truncated, inactive protein. About 2% of the German population are heterozygotes and 1/2500 individuals homozygotes for this null mutation. Although relatively rare, the distribution of heterozygotes for the mutation does not vary between individuals with a range of psychiatric illnesses, including schizophrenia, and normal controls. Intriguingly, the single homozygous individual discovered to date has no major psychiatric illness but some somatic ailments including acoustic neurinoma, obesity and autonomic nervous system abnormalities (Nothen *et al.* 1994). These may not be related to the deletion, and series of such

individuals will need to be examined before definite conclusions can be made about the consequences of D4 protein loss.

The point mutation discovered in the third cytoplasmic loop of D4 (Seeman *et al.* 1994) is also likely to have a severely disruptive effect on the D4 receptor since it alters a valine to a glycine only one amino acid away from a serine that it known to be critical for attachment of dopamine to the receptor. This variant was found at high frequency (12·5 %) only in the Afro-Caribbean population, indicating that around 4 % will be homozygotes for this mutation. No association was found between heterozygosity for this mutation and schizophrenia. The high frequency of this mutation predicts that individuals homozygous for this mutation who are ostensibly normal will be common, thus providing further evidence that D4 is dispensible to psychiatric well-being.

Are there other examples of complete loss of neurotransmitter receptors proteins without phenotypic consequences? Mice homozygous for deletion of the $GABA_A$ receptors subunits $\alpha 5$ and $\gamma 3$ are phenotypically normal and do not even have neurological deficits (Culiat *et al.* 1994), whereas deletion of the $\beta 3$ subunit, located in the same chromosomal region, results in a neonatally fatal cleft palate syndrome in 95 % of individuals and neurological abnormalities in the animals that survive to term (Culiat *et al.* 1993).

Thus, it is becoming evident that some neurotransmitter receptor proteins are dispensible, and this begs the question of whether they have a role at all? Deletion may have more subtle behavioural or pharmacological consequences. For instance, deletion of protein with significant function may be compensated for by other pathways. If some neurotransmitter receptors have no significant function in the brain, they may instead represent an evolutionary dead end, originally arising through a gene duplication event from a primordal protein, and now caught midway between existence as a functional protein and an inactive pseudogene. Indeed, D4 may be a protein waiting for a function. The unprecedented genetic variability of the D4 protein, which appears to predate the divergence of modern humans in origin since all ethnic groups have similar variability (Lichter *et al.* 1993), may represent an accumulation of 'silent' mutations in a protein with no significant function.

Update on post-mortem studies

In an editorial accompanying the findings of elevated D4 receptors by Seeman *et al.* (1993*a*); Iversen (1993) commented 'If the finding can be confirmed the present paper will be a landmark on the way to a genuine improvement in our understanding of this enigmatic illness'. Much effort has now gone into this task. Recently, Murray *et al.* (1995) have replicated this finding in brains from drug-treated patients using quantitative receptor autoradiography but the elevation (two-fold) was more modest. Estimating very low abundance receptors by subtracting two estimates of very high abundance receptors is clearly prey to artefact. In addition, the absolute selectivities for dopamine receptors must be questioned. It is plausible that raclopride and emonamide may be acting, albeit at very low affinity, at other receptors giving a weak signal apparent after subtraction of raclopride from emonamide. With this in mind Reynolds & Mason (1994) attempted a replication using [³H]emonamide only and cold raclopride as a displacer and they were not able to replicate the finding. In the same brain they were able to detect elevated D2 receptors by this assay and the conditions of the assay would, in theory, easily detect a 40 % elevation of D4 receptors. However, Seeman *et al.* (1993*b*) had earlier argued that this approach produced differential displacement between controls and schizophrenics, making the subtractive approach the only means of comparison. There is, then, overall evidence in favour of a D4 elevation in schizophrenia. Two important facts remain to be answered. Is the putative elevation associated with an increase in mRNA encoding D4 receptors, and will selective ligands become available for a post-mortem binding study? An interesting study by Flamez *et al.* (1994) who used clozapine as a ligand could not detect D4 receptors in normal human brain tissue.

Further functional and binding studies on dopamine D4 receptors *in vitro*

The finding that clozapine has high affinity at D4 receptors (Van Tol *et al.* 1992) was the major

impetus in establishing this receptor as the prime target for clozapine's action. However, it would be interesting to know whether other active antipsychotics or, indeed, if inactive compounds, possess this and at what rank affinity. In addition, if one is ultimately to make a case for atypical antipsychotics acting at this site, it is important to have knowledge of the functional consequences of this. The binding literature is complicated. Some behaviourally inactive compounds such as (+)aporphines are highly selective and have high affinity for D4 receptors but their antipsychotic potential clinically, is unknown (Seeman & Van Tol, 1993). It is also the case that classical agents, like haloperidol, also have high affinity for D4 receptors (Lahti *et al.* 1993; Chabert *et al.* 1994). It will be important to determine selectivity ratios systematically. A higher affinity for haloperidol than clozapine does not devalue the possibility that clozapine may still occupy and act through this receptor *in vivo*. Thus, it may be that both haloperidol and clozapine mediate antipsychotic efficacy at D4 receptors and the additional selectivity and affinity of haloperidol at D2 receptors are responsible for the neurological side effects. Clearly, the next step is to develop selective D4 antagonists and perform clinical trials even if they are inactive in behavioural models.

Recently, there have been attempts to study D4 receptor functions *in vitro*. Two assays have been developed, one that shows increase guanine nucleotide exchange following agonist stimulation (as judged by uptake of 35S-γGTP (Chabert *et al.* 1994) and one that shows that the D4 receptor is negatively coupled to adenylate cyclase (i.e. stimulation reduces cAMP) (Cohen *et al.* 1992). Good agonists include quinpirole, bromocryptine and apomorphine. In these assays haloperidol is more potent than clozapine as a functional antagonist (Chabert *et al.* 1994). Again, it would be interesting to study the rank affinities of inactive compounds and antipsychotics of varying clinical potency.

CONCLUSION

Certainly, the D4 receptor is the potential candidate for being pathophysiologically abnormal and a site of action of atypical antipsychotics. Genetic studies exclude this as a major gene for schizophrenia. However, post-mortem studies now show a replicable elevation in D4 receptors albeit with indirect methods. Atypical antipsychotics do have high affinity for D4 receptors, as indeed do typical antipsychotics. Functional studies do not strongly differentiate them, but it is tempting to suggest that D4 may be a common basis for efficacy and D2 receptors the locus determining neurological side effects. Pharmacogenetic studies have not, however, demonstrated functional associates between genotype and drug response. Evidence is, therefore, accumulating for a role for D4 receptors. However, it is certainly not a gene predisposing to schizophrenia. The next steps will be to try to confirm post-mortem findings with gene expression studies and selective ligands, and to perform clinical trials with D4 selective blocking agents.

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