Pathology and drug action in schizophrenia: insights from molecular biology

Philip G. Strange

School of Animal and Microbial Sciences, University of Reading, Whiteknights, Reading RG6 6AJ, U.K.

Introduction

The application of molecular biology techniques to the study of the brain has provided major new insights into diseases of the brain and their treatment, as well as into normal brain function. This essay illustrates this impact of molecular biology by considering one brain disorder, schizophrenia, and its treatment.

Schizophrenia: the clinical picture

Schizophrenia is a severe disorder of personality that afflicts about 1% of the population [1]. Patients experience a variety of symptoms that have been divided into subgroups of positive symptoms (e.g. thought disorder, abnormal beliefs and experiences) and negative symptoms (e.g. poverty of speech, loss of emotional response, reduced motor function). This division may be helpful in relation to the way patients present, but more recently the syndrome of schizophrenia has been described as having three dimensions [2,3]. In this categorization there are the negative symptoms as before, but the positive symptoms are divided into further subgroups of psychotic and disorganized. It is thought that these three dimensions of symptoms may reflect different disease processes, emphasizing that schizophrenia may not be a homogeneous disorder.

Schizophrenia has a genetic basis, and evidence for this comes from the incidence of schizophrenia in families and in twin pairs. There is debate as to
A major goal of this work has been to verify ‘dopamine hypotheses’ of schizophrenia, which assert that the disorder is due to an elevation in dopamine function in the brain. Elevated dopamine function could occur at the level of dopamine receptors or at the level of dopamine itself. As outlined above, there is little consistent evidence that dopamine receptors are increased in the brain in schizophrenia. Studies on dopamine and its metabolites have been no more illuminating; changes have been described in some reports, but these have not been replicated [8]. More recently, however, it has been reported that the dopamine biosynthetic enzyme, dopa decarboxylase, is increased in schizophrenia [9]. Using in vivo imaging (PET) it has been shown that, in schizophrenia, there is an increased ability of the drug amphetamine to cause dopamine release [10]. Also, it has been shown that the impaired cortical activation seen in patients with schizophrenia when performing a verbal fluency task can be reversed by administration of the dopamine agonist apomorphine [11]. There is therefore continuing interest in ‘dopamine hypotheses’ of schizophrenia.

Figure 1. Multiple dopamine receptor subtypes

Work on receptors for the neurotransmitter dopamine using physiological, pharmacological and biochemical techniques suggested that there were two dopamine receptors (D₁ and D₂) with different biochemical and pharmacological properties and different functions. Molecular biological techniques, however, have defined five dopamine receptor sequences (D₁, D₂, D₃, D₄ and D₅). Each of these is predicted to be a G-protein-coupled receptor with seven membrane-spanning α-helical regions linked by intracellular and extracellular loops and folded together as shown in the diagram. The amino acid sequences of these receptors are related, but comparisons allow the receptors to be divided into two subgroups (D₁, D₅ and D₂, D₃, D₄). The pharmacological properties of the two subgroups resembled respectively the D₁ and D₂ receptors defined using biochemical and pharmacological techniques, and so the two subgroups are often referred to as D₁-like (D₁, D₅) and D₂-like (D₂, D₃, D₄). The complexity of the D₂-like receptors is increased by isoforms differing in the size (short/long) of the third intracellular loop for the D₂ and D₃ receptors and by polymorphisms of the D₄ receptor in the human population [37]. In this article dopamine receptor subtypes defined using molecular biological criteria are designated D₁–D₅, but are described as D₁-like or D₂-like when the subtype has been defined only by pharmacological properties.
of schizophrenia and the receptor genes; however, despite some reports claiming such a linkage, these have not been confirmed subsequently [17].

Some studies have been performed attempting to link the disorder to markers on different chromosomes (e.g. chromosomes 5 and 22). Although some of these reports were greeted with enthusiasm, again most have not been replicated. The most convincing linkage report so far is between a marker on chromosome 6 and the disorder, which has been observed in several independent studies [18]. Further extensive work will be required in order to understand the significance of this observation, but it provides the first clear breakthrough in understanding the genetics and hence the biology of schizophrenia.

Another area where genetic linkage analysis may be important is in susceptibility to drug treatment. For example, it could be that a mutation in a receptor gene might alter the susceptibility of an individual to the effects a drug. Indeed, it has been claimed that there is an association between the T102C allele of the 5-HT2A receptor and the lack of response to the drug clozapine. These findings have not, however, been replicated [13,14].

**Drug action in schizophrenia**

**Typical and atypical anti-psychotics**

A large number of drugs (anti-psychotic drugs) exist that have been or are used to treat schizophrenia (e.g. chlorpromazine, haloperidol), and the most prominent therapeutic effect of these drugs is to reduce the incidence of the positive symptoms of the disorder [1]. Some patients are, however, resistant to the therapeutic effects of the drugs. Some drugs (e.g. clozapine) have been reported to have effects on negative symptoms. In addition, many of the drugs induce motor side-effects (extrapyramidal side-effects), most notably a Parkinsonian-like syndrome in some patients after a short period of treatment and, after longer-term treatment, a hyperkinetic disorder, tardive dyskinesia.

Anti-psychotic drugs have been divided into two groups, the so-called typical and atypical anti-psychotics. Typical anti-psychotics (e.g. haloperidol) are defined as drugs that are therapeutically effective against the positive symptoms of schizophrenia and which also induce some of the side-effects outlined above. The atypical drugs (e.g. clozapine and risperidone) are defined as those drugs with therapeutic effects against the positive symptoms of schizophrenia but with a reduced tendency to induce motor side-effects. There has been some debate about this definition [19,20] as to whether the definition of atypical should include either efficacy against negative symptoms or efficacy in treatment-resistant patients. The simple definition is still widely used, and some feel that the differences between typical and atypical drugs are quantitative rather than qualitative.

It is of some interest to try to understand the basis of the actions of these drugs and the biochemical basis of the distinction between the typical drugs
rather loose definition based on overall clinical efficacy, so different drugs may be atypical for different mechanistic reasons.

Clozapine is an atypical anti-psychotic drug that has generated much interest owing to its therapeutic effects in patients resistant to other anti-psychotics and its effects on negative symptoms, and it is important to try to understand the mechanism of these effects. Much has been made of the higher affinity of this drug for the D_4 dopamine receptor compared with the D_2 receptor [23], and it has been argued that actions at D_4 receptors may contribute to the atypical actions of this drug. There has been some discussion about the exact affinity of clozapine for the D_4 dopamine receptor [22–24], but, based on carefully controlled experiments, an affinity of approx. 7 nM can be determined (Table 1) and the D_2/D_4 preference is about 5-fold [22]. The higher affinity of the D_4 receptor for clozapine could mean that it is preferentially occupied when clozapine is used therapeutically, which would render clozapine different mechanistically from many other anti-psychotics. It is, however, difficult to define the actual concentrations of drugs at receptor sites in the brain, so it is difficult to know if this D_2/D_4 preference is actually reflected in differential occupancies. It is more likely that actions of clozapine at other receptors (e.g. 5-HT receptors; see below) are responsible for the unusual actions of this drug, but there is considerable debate about this.

The recognition of the potential role of the D_3 and D_4 receptors as novel sites of anti-psychotic action has led to intensive efforts to synthesize selective

<table>
<thead>
<tr>
<th>Table 1. Dissociation constants of anti-psychotic drugs at dopamine and 5-HT receptor subtypes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Values for the D_2(short) and D_3 receptors were taken from [38], and were obtained in competition experiments versus [^3H]raclopride binding to the receptors expressed in CHO cells.[^3H]Raclopride is a radioligand that can be used under conditions that avoid experimental artefacts [22]. For the D_4 receptor the data were taken from [39], and were obtained using competition versus [^3H]spiperone binding corrected for potential artefacts [22]. The data for the D_4 receptor must, therefore, be taken as estimates of affinity. Data for the 5HT_2A receptor are from [40].</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dissociation constant (nM)</th>
<th>D_2</th>
<th>D_3</th>
<th>D_4</th>
<th>5HT_2A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpromazine</td>
<td>0.55</td>
<td>1.2</td>
<td>11.6</td>
<td>2</td>
</tr>
<tr>
<td>Clozapine</td>
<td>35</td>
<td>83</td>
<td>7</td>
<td>3.8</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>0.53</td>
<td>2.7</td>
<td>0.78</td>
<td>58</td>
</tr>
<tr>
<td>Raclopride</td>
<td>1</td>
<td>1.8</td>
<td>800</td>
<td>4400</td>
</tr>
<tr>
<td>Remoxipride</td>
<td>54</td>
<td>969</td>
<td>930</td>
<td>6400</td>
</tr>
<tr>
<td>Risperidone</td>
<td>1.3</td>
<td>6.7</td>
<td>2.3</td>
<td>0.20</td>
</tr>
<tr>
<td>Sertindole</td>
<td>0.38</td>
<td>1.63</td>
<td>–</td>
<td>0.29</td>
</tr>
<tr>
<td>(–)-Sulpiride</td>
<td>2.5</td>
<td>8</td>
<td>333</td>
<td>–</td>
</tr>
<tr>
<td>Thioridazine</td>
<td>1.2</td>
<td>2.3</td>
<td>–</td>
<td>1.3</td>
</tr>
</tbody>
</table>
pancy occurs quickly [29]. Occupancy of D$_1$-like receptors by many of the drugs was much lower (0–44%). Motor side-effects were seen in patients when D$_2$-like receptor occupancy exceeded ~80%. It was also found that haloperidol could be used successfully at lower doses to treat patients, and under these conditions occupancy of D$_2$-like receptors was only about 50%; motor side-effects were absent, which fits well with the threshold for occurrence of these side-effects suggested above [30].

The atypical drug clozapine has been studied using these techniques [7], and it has been found that at normal doses it occupies 38–63% of the D$_2$-like receptors in the striatum and 38–52% of the D$_1$-like receptors. These occupancies are consistent with the affinities of clozapine for the two subclasses of receptors when compared with the affinities of the typical drugs, although it is in fact very difficult to know the true concentrations of drugs at receptor sites in the brain. Clozapine, for example, is concentrated in the brain more than 20-fold over the serum, and other drugs are concentrated to different extents [31]. In one study, however, in vivo receptor occupancies in the striatum with increasing doses of clozapine were examined [29]; whereas 5-HT$_2$ receptors were occupied to a level of 96%, the maximum occupancy of D$_2$-like receptors was 61%, and for D$_1$-like receptors this figure was 48%. In complementary experiments raclopride was shown to occupy 97% of the D$_2$-like receptors. Therefore, even with apparently saturating concentrations of drug, clozapine is unable to occupy all the D$_2$-like receptors. These observations are difficult to explain, given that in in vitro assays clozapine and raclopride are fully competitive and seem to label the same set of D$_2$-like receptor sites.

It is beginning to be possible to examine extra-striatal D$_2$-like receptors using high-affinity probes such as [$^{123}$I]epidepride, and this has been used to examine D$_2$-like receptors in the temporal cortex of schizophrenics [32]. Surprisingly, clozapine was able to occupy ~90% of the D$_2$-like receptors in this brain region. These results were interpreted in terms of a limbic selectivity for the drug, but further controls are required to confirm these results. In particular, it will be important to define whether in cortical regions [$^{123}$I]epidepride is labelling other sites in addition to the D$_2$-like receptors (see [33]).

**Inverse agonism of anti-psychotic drugs**

It has been widely assumed that the mechanism of action of the anti-psychotic drugs is simply to act as antagonists of dopamine at its receptors (D$_2$-like). This notion has recently been challenged through the demonstration that a wide range of anti-psychotic drugs (typical and atypical) act as inverse agonists at D$_2$ receptors expressed in CHO cells [34]. Inverse agonism refers to the ability of these drugs to act in an opposite manner to agonists; whereas agonists increase the activity of a receptor system, inverse agonists suppress the activity of a receptor system. Similar, although less extensive, data describing inverse agonism were reported for D$_2$ receptors in pituitary cells [35]. These findings have
Summary

- Schizophrenia is a severe disorder of personality which has a genetic basis.
- Schizophrenia arises from a change in brain development.
- There is no strong evidence that disturbances in neurotransmitter systems are a primary cause.
- Anti-psychotic drugs act primarily through D₂ and D₃ dopamine receptors.
- The atypical drug clozapine may act through a number of different receptors, including D₂, D₃ and D₄ dopamine receptors.
- Anti-psychotic drugs are inverse agonists at D₂ dopamine receptors.

References