Expert Opinion

Central & Peripheral Nervous Systems

Targeting the dopamine D_2 receptor in schizophrenia

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After a 12-year search for the antipsychotic receptor, the binding site was discovered and labelled by [^{3}H]haloperidol in 1975. Of the various neurotransmitters, dopamine was the most potent in inhibiting the binding of [^{3}H]haloperidol, indicating that the antipsychotic receptor was a dopamine receptor, now named the dopamine D_2 receptor, a major targeting site in schizophrenia. All antipsychotic drugs, including traditional and newer antipsychotics, either bind to D_2 in direct relation to their clinical potencies or hinder normal dopamine neurotransmission, as in the case of partial dopamine agonists. In fact, the antipsychotic concentrations found in the plasma water of treated patients closely match the predicted therapeutic absolute concentrations, adjusted for the 60 – 75% D_2 occupancy needed for clinical efficacy. Antipsychotics that elicit low or no Parkinsonism or prolactinaemia are loosely attached to D_2 and rapidly dissociate from D_2, whereas those eliciting Parkinsonism stay tightly attached to D_2 for many hours. Because animal models of psychosis (amfetamine sensitisation, brain lesions) all show a marked elevation in the number of high-affinity states of D_2, the antipsychotics are thought to specifically target these D_2^{High} states in psychosis in general and schizophrenia in particular.

Keywords: antipsychotic, brain imaging, domperidone, dopamine receptor, psychosis, schizophrenia, supersensitivity

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1. Introduction

The discovery of dopamine receptors is closely associated with the discovery of antipsychotic drugs. The research in this field started with the synthesis of antihistamines after World War II, particularly with H Laborit using such compounds to enhance surgical analgesia. In patients receiving these various antihistamines, Laborit noticed a 'euphoric quietude', and that the patients were 'calm and somnolent, with a relaxed and detached expression.' Of this series of Rhône Poulenc compounds, RP4560, now known as chlorpromazine, was the most clinically acceptable.

Chlorpromazine was soon tested for various medical illnesses. Although Sigwald and Bouttier [1] were the first to use chlorpromazine as the sole drug for a psychotic patient, they did not report their findings until 1953, after a 1952 report by Delay *et al.* [2] that chlorpromazine alleviated hallucinations and stopped internal 'voices' in eight patients. An important feature of the action of chlorpromazine was that it was effective within three days. This relatively fast improvement, especially during the first week of antipsychotic treatment, has been observed many times, as summarised by Agid *et al.* [3].

The clinical success of chlorpromazine fostered the search to locate the therapeutic target and mode of action of chlorpromazine. The assumption then, as now, was that finding such a therapeutic target would open the avenue to uncovering the biochemical cause of psychosis and possibly schizophrenia.

2. Before the discovery of dopamine receptors

In searching for the therapeutic target and mechanism of action of chlorpromazine in the 1960s and 1970s, many types of physiological and biochemical experiments were carried out. A variety of possible therapeutic targets were explored for the mode of action of chlorpromazine, including its action on mitochondrial enzymes, sodium–potassium-ATPase and related enzymes, as well as its membrane-stabilising action, such as its strong potency to inhibit membrane action potentials, and to stabilise cellular and subcellular membranes from releasing their contents [4]. It also became clear in 1963 that all antipsychotics were surface active, readily explaining their hydrophobic affinity for biomembranes. Some of these non-receptor findings, such as the potent surface activities of the antipsychotics, showed an astonishingly excellent correlation with clinical antipsychotic potencies [5].

Because high doses of chlorpromazine and other antipsychotics (or 'neuroleptics', as they were then called) also elicited Parkinsonism as an unwanted side effect, basic scientists soon focused on the antipsychotic action on brain dopamine pathways. The reason for examining the brain dopamine pathway was based on the finding by Ehringer and Hornykiewicz [6] that the Parkinsonism of Parkinson's disease was associated with a major loss of brain dopamine. It was thought, therefore, that the unwanted side effect of chlorpromazine-induced Parkinsonism, as well as the antipsychotic action itself, might arise by antipsychotics interfering with dopamine neurotransmission. The working hypothesis was that if the brain target or targets for antipsychotics could be found, then it was possible that these targets could be overactive or underactive in psychosis or schizophrenia.

3. Therapeutic concentrations of antipsychotics

However, these early experiments in the 1960s revealed that the active concentrations *in vitro* of the antipsychotics were generally very high, between 20 nM and 100 nM [4]. Such concentrations, however, were far in excess of the nanomolar concentrations (e.g., 1 – 2 nM for haloperidol) that exist in the plasma water and in the spinal fluid in patients being successfully treated with these medications, as determined by plasma haloperidol measurements where the amount of haloperidol bound by the plasma proteins (92%) was taken into account [7]. It should be noted that an antipsychotic drug can exist in either a positively charged form or in a neutral uncharged form. The neutral form readily permeates cell membranes, and its concentration in the aqueous phase in the plasma is expected to be identical to the aqueous concentration of the antipsychotic in the spinal fluid in contact with the neurons.

4. Actions of antipsychotic drugs on neurons

Indirect evidence for the existence of distinct dopamine receptors on neurons and their sensitivity to antipsychotics came from *in vitro* and *in vivo* experiments, showing that dopamine agonists can excite or inhibit neurons in the nigrostriatal dopamine pathway. Moreover, other workers showed that direct application of dopamine on neurons also stimulated or inhibited snail neurons [8], and that haloperidol or fluphenazine could block these actions [9]. Here too, however, the antipsychotic concentrations used were far higher than those found in the plasma water or spinal fluid in patients; in fact, the concentrations used would be lethal to humans.

Additional work *in vivo* found that chlorpromazine and haloperidol increased the turnover of adrenaline and dopamine, as shown by the increased production of normetanephrine and methoxytyramine, respectively. To explain the increased production of these metabolites, it was suggested that 'the most likely [mechanism] appears to be that chlorpromazine and haloperidol block monoaminergic receptors in brain; as is well known, they block the effects of accumulated 5-hydroxytryptamine' [10]. In other words, these authors proposed that antipsychotics might block all three types of receptors for noradrenaline, dopamine and serotonin, but they did not identify which receptor was selectively blocked or how to identify or test any of these receptors directly *in vitro*. This study [10] in 1963 by Carlsson and Lindqvist is often mistakenly cited as discovering 'the dopamine receptor' and that antipsychotics selectively acted on this receptor. However, in 1964 N-E Andén, a student of A Carlsson, had a different view, and proposed that 'chlorpromazine and haloperidol delays the elimination of the (metabolites)' [11]. Moreover, seven years later, Andén *et al.* [12] reported that antipsychotics increased the turnover of both dopamine and noradrenaline, but they could not show that the antipsychotics were selective in blocking dopamine; for example, chlorpromazine enhanced the turnover of noradrenaline and dopamine equally. Therefore, it remained for *in vitro* radio-receptor assays to detect the dopamine receptor directly and to demonstrate antipsychotic selectivity for the dopamine receptor.

5. The dopamine D_1 receptor

With the advent of assays for adenylyl cyclase in the 1960s, it was found that dopamine stimulated adenylyl cyclase in the superior cervical ganglion [13]. This receptor was later named the dopamine D_1 receptor, selectively labelled by [^{3}H]SCH 23390, and subsequently cloned by three research groups in 1990.

The dissociation constants at D_1 for the antipsychotics are given in Table 1. There is no correlation between the antipsychotic clinical doses and the dissociation constants of the antipsychotic antagonists at D_1, as illustrated in Figure 1.

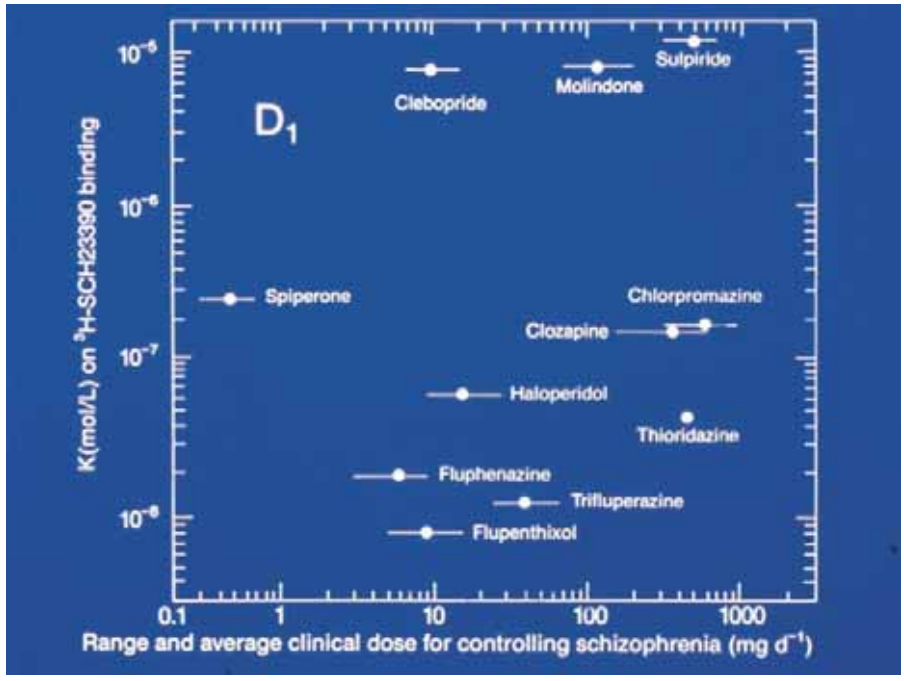

Figure 1. There is no correlation between the clinical antipsychotic doses and the antipsychotic dissociation constants (or concentrations) that inhibit the binding of a D_1 ligand ([^{3}H]SCH233900) at dopamine D_1 receptors in homogenised striatal tissue. The high concentrations inhibiting the D_1 receptor are far higher than those found clinically in the plasma water or spinal fluid.

Adapted from [14] with permission.

These data suggested that D_1 was not the major or common target for antipsychotics. Equally important, moreover, is the fact that the antipsychotic molarities at D_1 are between 10 nM and 10,000 nM, far in excess of the therapeutic concentrations in the spinal fluid of treated patients.

In addition to the lack of targeting D_1 receptors by clinical doses of the common antipsychotics, D_1-selective antagonists have not been found effective as antipsychotics.

6. Discovery of the antipsychotic dopamine D_2 receptor

In 1974 and 1975, in order to detect and discover the dopamine receptors on which the antipsychotics presumably acted, it was essential to label a receptor with a ligand, such as radioactive haloperidol, having an affinity (or dissociation constant) of ~ 1 nM, because, as indicated above, this was the haloperidol therapeutic concentration found in the spinal fluid or plasma water of treated patients. For this to occur, the specific activity of [^{3}H]haloperidol would have to be at least 10 Ci/mmol. Although [^{3}H]haloperidol donated in 1971 by Janssen Pharmaceutica only had a low specific activity of 0.07 Ci/mmole, I.R.E. Belgique custom synthesised [^{3}H]haloperidol (10.5 Ci/mmole) for Seeman's laboratory by June 1974.

Specific binding of this new [^{3}H]haloperidol to brain striatal tissue was readily detected in 1975 [15]. It was soon found that all the antipsychotics inhibited the binding of [^{3}H]haloperidol in direct relation to their clinical potencies [16], as shown in Figure 2.

The data in Figure 2, therefore, indicated that the 'antipsychotic receptor' had finally been successfully discovered. Equally important, of the endogenous compounds tested, dopamine was the most potent in inhibiting the binding of [^{3}H]haloperidol, indicating that the antipsychotic receptor was actually a dopamine receptor.

7. Nomenclature of dopamine receptors

The antipsychotic dopamine receptor labelled by [^{3}H]haloperidol was later termed the D_2 receptor [17]. It is important to note that the data for the binding of [^{3}H]haloperidol identifying the antipsychotic receptor [15] was very different from the pattern of [^{3}H]dopamine binding described by Burt *et al.* [18] and Snyder *et al.* [19]. For example, the binding of [^{3}H]haloperidol was inhibited by ~ 5000 nM dopamine, whereas that of [^{3}H]dopamine was inhibited by ~ 3 nM dopamine. For several years, this latter [^{3}H]dopamine binding site was termed the 'D_3site' [20], a term which is not to be confused with the later discovery of the dopamine D_3 receptor [21].

Table 1. *K* values (dissociation constants, nM)

Human clone	M_1	D_1	D_2	D_2	D_2	D_3	D_4	D_4
[³H] ligand used:	***QNB***	***Sch.***		***Raclo.***	***Spip.***	***Raclo.***		***Spip.***
[³H]Amisulpride			1.7	1.8	4.6			
Amoxapine	49		–	21	56			5.5
Aripiprazole				1.8				
Butaclamol-(+)				0.14	0.9			70
Chlorpromazine	3.4	17		1.2	4.6	1.4		9.6
[³H]Chlorpromazine			0.77				1.2	–
Clozapine	9.5	90	–	75	180	190	–	22
[³H]Clozapine			51				2	–
Clozapine-iso	14.4	120		15	60	21		21
Clozapine-desmethyl	17	73		180	300			120
Cyproheptadine				24				
Epidepride				0.04	0.06			
Flupentixol-*cis*		1.8		0.38	0.7	0.7		15
Flupentixol-*trans*				151				
Fluphenazine		2.6		0.55	1.2	0.17		30
Haloperidol	794	55		0.74	2.7	8.8		2
[³H]Haloperidol			0.4				0.85	–
Iloperidone (HP873)	109	5.6		5.4	10	20	–	9.6
Loxapine	117	18		9.2	22.7	7.2		8
Loxapine-iso	203	16		22	86	18		11
Melperone		148		152	375	315		720
Molindone		1558		4.9	15	44		3900
Olanzapine	2.1	9.2		7.4	21	14		15
[³H]Olanzapine			2.7				1.6	–
Perphenazine		4.5		0.27	0.47	0.23	–	32
Pimozide	470			1.4	0.95			
Prochlorperazine		7.7		1.7	4			89
Quetiapine	135	290		140	680	240		2000
[³H]Quetiapine			104					–
Raclopride			–	1.6	7.1	2.9		2400
[³H]Raclopride			1.9			1.6		–
Remoxipride	> 10 µM	4900		67	800	960		2400
Risperidone	> 10 µM	42		1.09	4	3.5		4.4
Risperidone-9-OH				1.6				
Sertindole	400	22	3	1.9	6.5	3	–	11
[³H]Sertindole			1.2				0.85	–

Dissociation constants (*K* values) for antipsychotics at cloned dopamine D_1, D_2, D_3 and D_4 receptors, and at the cholinergic muscarinic M_1 receptor.
Because [³H]raclopride is more loosely bound to D_2 than [³H]spiperone, the antipsychotic *K* values are consistently lower at D_2 when measured with [³H]raclopride.
Data from [30]. Blank boxes indicate not done. QNB: [³H]Quinuclidinylbenzilate (cholinergic muscarinic ligand); Raclo.: [³H]Raclopride; Sch.: [³H]Schering 23390; Spip.: [³H]Spiperone.

Table 1. *K* values (dissociation constants, nM) (continued)

Human clone	M_1	D_1	D_2	D_2	D_2	D_3	D_4	D_4
Spiperone			–	0.02	0.06		–	
[^{3}H]Spiperone			0.07			0.32	0.09	–
Sulpiride-S				9.9	8	10		1000
Trifluperazine		2.9		1.4	3.8	0.7		39
Ziprasidone	265	9		2.7	6	1.5		8

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Now that the various dopamine receptors have been cloned, the D_1-like group consists of D_1 and D_5, whereas the D_2-like group consists of D_2, D_3 and D_4. The D_2 receptor has three forms, D_2^{Short}, D_2^{Long}, and D_2^{Longer}.

8. Therapeutic concentrations of all antipsychotics occupy 60 – 75% of D_2

It is now known that therapeutic levels of all the antipsychotic drugs occupy 60 – 80% of brain D_2 receptors, as shown by Farde *et al.* [22] and confirmed by others [23] (see review in [24]). This range of 60 – 80% occupancy of D_2 for the therapeutic effect is not precise and rigid but is numerically derived from various series of patients and from many studies. In general, minimal D_2 occupancy for an antipsychotic effect would be 60 – 65%, although there are examples of patients responding favourably with lower D_2 occupancies (see later). Optimal antipsychotic action may generally require 65 – 70% D_2 occupancy. The side effects of hyperprolactinaemia and Parkinsonism generally arise above 78% and 80 – 82%, respectively, but here too, there are considerable variations from patient to patient. D_2 occupancies with aripiprazole can attain apparent D_2 occupancies exceeding 90%, even in the absence of Parkinsonism. However, in the case of aripiprazole, a partial agonist at D_2, the high D_2 occupancies may be associated with some internalisation of D_2 into the cytoplasm of the neurons, because dopamine agonists are known to cause D_2 receptor internalisation.

A current active area of clinical research on dopamine receptors is to measure the occupancy of D_2 receptors in both the striatum and outside the striatum in individuals taking antipsychotic medications. Some researchers find that the same D_2 occupancy occurs in both striatal and limbic regions, while others find a higher occupancy in the limbic regions [25,26]. Interestingly, Kessler *et al.* have found that olanzapine-treated patients have a significantly lower D_2 occupancy of 40.2% in the combined region of the substantia nigra and the ventral tegmental area, in contrast to the D_2 occupancy of 59.3% in the striatum of haloperidol-treated patients [27].

Of the three D_2-like receptors (D_2, D_3 and D_4), only the D_2 receptor itself is blocked by antipsychotic drugs in direct relation to their clinical antipsychotic potencies, as illustrated in Figure 2. No other receptor, including any other dopamine receptor, or any of the serotonin receptors or glutamate receptors, exhibit such a correlation.

Although this well-established finding in Figure 2 may be criticised as simply a relation between the D_2-blocking concentrations and the clinical doses at which extrapyramidal signs first appear, it is important to note that the absolute concentrations of antipsychotics which block D_2 receptors in the brain are precisely identical to the therapeutic concentrations found in the spinal fluid or plasma water of patients whose psychotic symptoms are successfully controlled by antipsychotics. These two sets of identical concentrations are here presented for the first time in Table 2 and Figure 3, as detailed in the following paragraphs with many references to the literature.

In fact, the therapeutic concentration in the plasma water or the spinal fluid of any antipsychotic can be reasonably predicted from the dissociation constant, or *K* value, at D_2 and by knowing that 60 – 75% of the D_2 receptors need to be occupied for optimum clinical antipsychotic action (D_2 occupancies > 78 or 79% are usually associated with hyperprolactinaemia or Parkinsonian signs [28]).

The predicted therapeutic concentrations of antipsychotics are worked out in Table 2, knowing only the *K* value and the fact that 60 – 75% occupancy is therapeutic. The antipsychotic concentrations found in the plasma water of patients is in good agreement with the predicted concentrations, as shown in Table 2 and in Figure 3.

The relations in Figures 2 and 3 remain a cornerstone of the dopamine hypothesis of psychosis or schizophrenia, still the major contender for an explanatory theory of schizophrenia causation.

The data in Table 2 and Figure 3 have not been corrected for the effect of endogenous dopamine that competes with the antipsychotic for D_2. Endogenous dopamine acts to raise the antipsychotic dose needed to block D_2, as detailed elsewhere [30]. It should also be noted that the interaction between the drug, the ligand, the endogenous dopamine, and the receptor, all complicate the interpretation of data for the dissociation constants of the various drugs for the receptor. Nevertheless, despite such

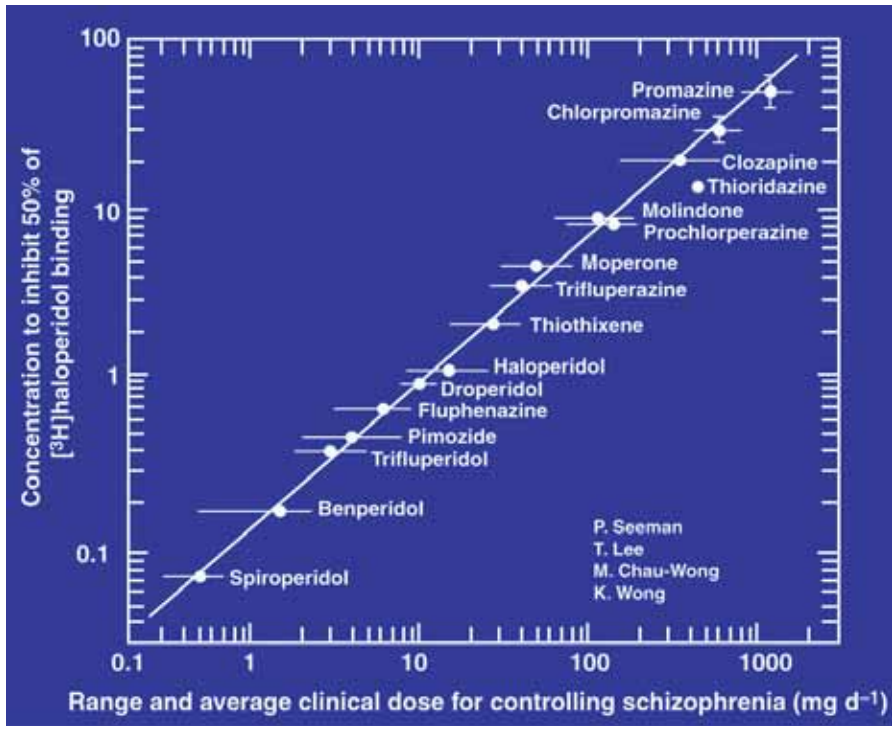

Figure 2. The clinical antipsychotic doses correlate with the concentrations that 50% inhibit the specific binding of [^{3}H]haloperidol in homogenised caudate nucleus tissue (calf). These concentrations are similar to those found in the plasma water or spinal fluid in antipsychotic-treated patients.
Redrawn from data in [15,16], with permission.

complications, and despite variations in the *in vitro* composition of buffers and salts between different laboratories, the dissociation constants of various antipsychotics at the D_2 receptor are now in general agreement.

9. 'Fast-off-D_2' theory of atypical antipsychotic action

As noted above, clinically effective doses of antipsychotic drugs occupy between 60 and 75% of brain dopamine D_2 receptors in patients, as measured by positron emission tomography or single-photon emission tomography in the human striatum.

Clozapine and quetiapine, however, have consistently been apparent exceptions. For example, in patients taking therapeutically effective antipsychotic doses of clozapine, the drug only occupies between 0 and ~ 50% of brain dopamine D_2 receptors 6 – 12 h after the oral dose, as measured by a variety of radioligands using either positron tomography or single photon tomography (reviewed in [24,29,30]).

However, the apparently low occupancy of D_2 by clozapine and quetiapine is readily explained by the fact that these two antipsychotics rapidly dissociate from D_2 [24,30]. This also holds for remoxipride and amisulpride (see Table 3 and the reference therein), two atypical drugs not used clinically in Canada. For example, [^{3}H]clozapine, [^{3}H]quetiapine, [^{3}H]remoxipride and [^{3}H]amisulpride dissociate from human cloned D_2 receptors at least one hundred times faster than [^{3}H]haloperidol or [^{3}H]chlorpromazine.

Using methods similar to those reported earlier for the human cloned dopamine D_2^{Long} receptor in CHO cells [24], and using drug concentrations found in the spinal fluid of patients, the times for 50% dissociation from D_2 are in Table 3.

These *in vitro* data in Table 3 match those found clinically for clozapine, quetiapine and haloperidol in schizophrenia patients and volunteers. For example, it has been found by positron emission tomography (PET), using [^{11}C]raclopride, that the human brain (striatum) occupancy of D_2 by quetiapine and clozapine rapidly falls off within 24 h, in contrast to that for haloperidol which maintains its D_2 occupancy constant over 24 h. The maximum occupancy of D_2 by either clozapine or quetiapine occurs at 2 h and is in the therapeutic range of 65 – 72% (summarised in [30]). The D_2 occupancy by clozapine falls off by 50% in about 18 h, whereas that for quetiapine falls off by 50% in about 8 h (summarised in [30]).

Thus, the rapid release of clozapine and quetiapine from D_2 and their replacement by endogenous dopamine would readily account for the low D_2 receptor occupancy shown by

Table 2. Therapeutic concentrations of antipsychotic drugs in the spinal fluid or plasma water.

Drug	Average therapeutic concentration (ng/ml plasma)	% of drug free in plasma	Therapeutic free concentration in plasma water (nM)	Dissociation constant (K_i) in D_2^{Long} (nM) [30]	Concentration (nM) in spinal fluid to occupy 60 – 75% of D_2 = K_i x (1.5 – 3)
Aripiprazole	240 – 440 [37]	~1%	5 – 10	1.8	2.7 – 5.4
Chlorpromazine	22 – 134 [48-50]	2.1% [51,52]	4.6 – 8.8	1.2	1.8 – 3.6
Clozapine	162 – 504 [32,34]	8.2% [33,101]	40 – 126	75	113 – 225
Clozapine-demethyl	90 – 277*	9.7% [101]	28 – 86	180	
Clozapine-like range:			40 – 170‡		
Flupentixol-cis	2.2 [53]	35.5% [54]	1.4 – 2.2	0.38	0.6 – 1.1
Haloperidol	**	8.5%**	1 – 3**	0.74	1.1 – 2.2
Olanzapine	23 – 120 [29,34]	7% [31]	5 – 24	7.4	11 – 22
Perphenazine	6.5 nM	9**	0.4 – 0.8	0.27	0.4 – 0.8
Quetiapine	300 – 800 [36]§	17% [35]	130 – 354	140	210 – 420
Raclopride	41 – 100 nM**	4.8%**	2 – 4.8	1.6 – 1.9	2.6 – 5
Remoxipride	3600 nM**	5.5%**	198	67	100 – 201
Risperidone	11 [47]	10% [46]	2.6	1.1	1.7 – 3.3
9-OH-risperidone	24 [47]	22.6% [46]	12¶	1.6	
Thioridazine	**	0.15%**	3.4 – 3.7	1.1	1.7 – 3.3
Trifluoperazine	18 – 97 [55]	1.8% [56]	0.8 – 4	1.4	2.1 – 4.2

The antipsychotic therapeutic concentrations in the plasma water (or spinal fluid) can be predicted from the dissociation constants, K_i or K_d (in column 5), and adjusted for the therapeutic D_2 occupancy of 60 – 75% (as shown at the top of column 6). The observed therapeutic concentrations in the plasma water of patients maintained on antipsychotics were derived from the observed values (usually in ng/ml plasma), corrected for % drug bound to plasma proteins, and are shown in column 4. *Clozapine-demethyl concentration in plasma is 55 ± 7% that of clozapine [38-44], except for the value of 135% [45]. ‡Net clozapine-like concentration is sum of clozapine plus half that for demethyl-clozapine, because demethyl-clozapine has about half the affinity for D_2. §At 1 – 2 hour peak. ¶Net risperidone-like activity is sum of risperidone concentration and 9-OH-risperidone, where the latter is reduced by 0.69 because of lower affinity for D_2. **References in [32].

these atypical antipsychotics, especially because the brain scans are taken 6 – 12 h after the last oral dose in patients on maintenance doses.

It is important to emphasise that the rapid release of clozapine and quetiapine is a molecular event which occurs quickly regardless of the clinical dose used. In other words, even though high doses of clozapine and quetiapine may be used in the patient, these drugs continue to go on and off the D_2 receptor rapidly, allowing extensive and frequent access of endogenous dopamine to the receptor.

Hence, it appears that some antipsychotics such as clozapine and quetiapine occupy D_2 receptors transiently during the day. As just mentioned, PET imaging of patients with schizophrenia reveals that the D_2 receptor occupancies by clozapine and quetiapine wear off quickly after an oral dose, and patients may show no occupancy whatsoever within 48 h of the last dose, in contrast to typical antipsychotics which may continue to occupy D_2 receptors for days. This may explain why psychotic relapses of patients on clozapine and quetiapine occur soon after withdrawal of the antipsychotic (reviewed in [24]), much earlier than after withdrawal of conventional antipsychotic drugs, such as haloperidol or chlorpromazine.

The data for the rapidly dissociating antipsychotics (amoxapine, aripiprazole, clozapine, perlapine, quetiapine, remoxipride and paliperidone) are compatible with the low or absent amount of extrapyramidal signs (EPS) in patients. The extent of risperidone-associated EPS may depend on the proportions of risperidone and its metabolite, paliperidone, in the patient. Olanzapine has a slow off-rate from D_2, compatible with its dose-dependent incidence of EPS; however, the potent anticholinergic action of olanzapine (its dissociation constant of 2.1 nM matches that of benzatropine, Cogentin®, at the muscarinic receptor) provides an effective anti-EPS mechanism.

Atypical antipsychotics, therefore, are helpful to patients by transiently occupying D_2 and then rapidly dissociating to allow dopamine neurotransmission, as illustrated in Figure 4. This keeps prolactin levels normal, spares cognition and obviates EPS.

9.1 Clinical implications of the 'Fast-off-D_2' theory of atypical antipsychotic action

As outlined above, the 'fast-off-D_2' theory of atypical antipsychotic action is that the atypicals have low affinities for D_2, and are loosely bound to, and rapidly released from, D_2.

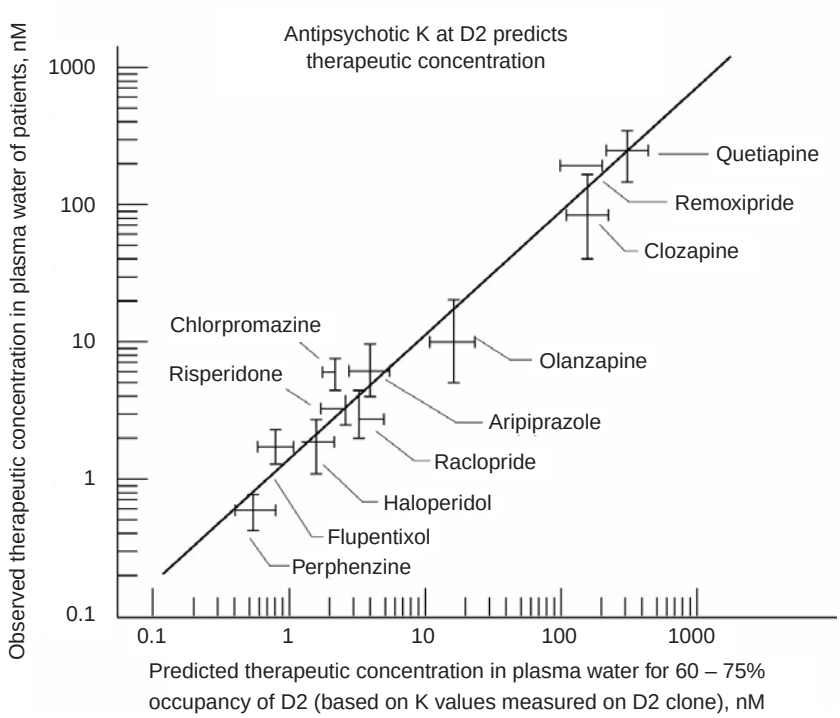

Figure 3. The observed therapeutic antipsychotic concentrations in the spinal fluid or in the plasma water in treated patients are essentially identical to the predicted antipsychotic concentrations that occupy ~ 60 – 75% of the D_2 receptors *in vitro*. The concentrations in the plasma water were obtained by correcting for the amount bound to the plasma proteins. For example, the concentrations to occupy 75% of D_2 were calculated as being three times higher than the dissociation constant at D_2 (Table 1).

A critical aspect of the theory is that the atypical antipsychotics bind more loosely to D_2 than does dopamine itself, whereas the traditional, typical antipsychotics bind more tightly than dopamine, the dissociation constant for dopamine being 1.7 nM at the functional high-affinity state of D_2, as listed in Table 3.

Table 3 illustrates a general demarcation between typicals and atypicals. That is, the typical antipsychotics generally have *K* values lower than the *K* for dopamine (at the high-affinity state of the D_2 receptor), whereas the atypicals have *K* values higher than that for dopamine.

Although risperidone appears to be an exception to this generalisation, risperidone is the weakest 'atypical' antipsychotic, eliciting dose-dependent extrapyramidal signs in 60 – 70% of patients taking ≥ 6 mg per day. In the temporal cortex of humans, risperidone remains attached to D_2 for a very long time, requiring at least 60 h for its occupancy to fall by 50% [58].

Clearly, the separation between typicals and atypicals in Table 3 is not sharp and precise because antipsychotic drugs with *K* values between 2 nM and 10 nM, including loxapine, often reveal dose-dependent extrapyramidal signs. Thus, the demarcation between typical and atypical antipsychotics is not a sharp divide but rather a continuous one. Antipsychotics become increasingly more atypical as their binding to the D_2 receptor becomes more loose and they are released more quickly.

Although almost all atypical antipsychotics have loose binding, with dissociation constants looser than 1.8 nM, they can still elicit dose-dependent Parkinsonism. For example, olanzapine, with a dissociation constant of 7.4 nM, is known to be associated with a dose-dependent incidence of extrapyramidal signs in some patients, especially at higher doses. If the binding is extremely 'loose,' as with clozapine, remoxipride, quetiapine, and melperone, essentially no EPS occurs.

Drugs that are too 'loose' or have far too low an affinity for D_2 receptors cease to exhibit any antipsychotic activity at all. Moreover, although the degree of occupancy of atypicals at D_2 receptors has a direct influence on EPS, the potent anticholinergic action of olanzapine and clozapine provides an additional anti-EPS mechanism. Olanzapine at 20 mg/day, for example, occupies up to 79% of the cholinergic muscarinic receptors in humans [59]. Furthermore, in the case of thioridazine, its anticholinergic properties keep it relatively free of eliciting EPS.

Table 4 summarises a few clinical distinctions between the typical antipsychotics which are tightly bound to D_2 and the

Table 3. Fast-off-D_2 antipsychotics generally elicit no or low Parkinsonism.

	Time for 50% offset from D_2 [57]	K_i or K_d (nM)	Parkinsonism
Remoxipride (5 nM)	13 s	67	Absent
Clozapine (200 nM)	15 s	75	Absent
Quetiapine (200 nM)	16 s	140	Absent or low
Perlapine (140 nM)	24 s	138	Absent or low
S-(-)-Amisulpride (4 nM)	42 s	1.7*	Absent or low
Aripiprazole (10 nM)	52 s	1.8‡	Low
9-Hydroxy-risperidone (2 nM)§	60 s	1.6	Low
Amoxapine (40 nM)	66 s	21	Low
Loxapine (20 nM)	16 min	9.2	Dose-dependent
Olanzapine (5 nM)	17 min	7.4	Dose-dependent
Dopamine at D_2^{High} state		1.7	
Raclopride (4 nM)	23 min	1.6	Dose-dependent
Risperidone (2 nM)	27 min	1.09	Dose-dependent
Chlorpromazine (1.5 nM)	30 min	1.2	Common
Haloperidol (2 nM)	38 min	0.4 – 0.74	Common, dose-dependent

The times for 50% offset from the cloned D_2^{Long} receptor are shown in column 2. The K_i or K_d values are from **Table 1**. The K_i values are the dissociation constants or inhibition concentrations which inhibited the binding of the [^{3}H]ligand by 50%, corrected for the concentration of the [^{3}H]ligand; the K_d values are the dissociation constants obtained by means of saturation data, using the titrated form of the drug, as indicated. The antipsychotics with *K* values lower than that for dopamine (at D_2^{High}) desorb slowly from D_2 and are consistently associated with Parkinsonism. Antipsychotics with *K* values > 20 nM dissociate quickly and elicit little or no Parkinsonism. Antipsychotics with intermediate values (loxapine and olanzapine) elicit dose-dependent Parkinsonism. Data from [30,57]. *Amisulpride has low and slow permeation into brain. ‡Aripiprazole is a partial agonist. §Paliperidone.

atypical antipsychotics which are loosely bound to D_2. The required antipsychotic dose (in milligrams) will be low for tightly bound drugs, but high for loosely bound drugs. The typicals, being tightly bound to D_2, will elicit EPS and elevated prolactin, whereas the atypicals, being loosely bound and rapidly released from D_2, will not elicit these side effects, or at least to a markedly lesser extent. Finally, because the typicals remain attached to D_2 and readily accumulate in brain tissue, the typicals will eventually lead to tardive dyskinesia. The atypicals, however, are much less fat-soluble, and because they are readily released from D_2 and from the brain tissue, the risk of causing tardive dyskinesia is much reduced or absent.

9.2 'Fast-off-D_2' theory predicts low antipsychotic dose treatment of L-DOPA psychosis

The treatment of patients with psychosis in Parkinson's disease (as a consequence of L-DOPA treatment) is best done with a very loose binding antipsychotic, such as clozapine or quetiapine, to allow dopamine neurotransmission required for motor function to continue.

It is well known in neurology that L-DOPA psychosis in a Parkinson's diseased patient is best treated with a dose of clozapine which is ~ 10% the dose normally used for psychosis in schizophrenia. The 'fast-off-D_2' hypothesis readily and quantitatively predicts this. In Parkinson's disease, where 90 – 95% of the dopamine content is absent, the synaptic release of dopamine is depressed by over 90%, effectively reducing the amount of endogenous dopamine that would otherwise compete with the administered antipsychotic, as previously analysed [30].

Thus, although a daily dose of 500 mg clozapine might be suitable for treating schizophrenia psychosis, a dose of 50 mg (or less) would be more than adequate to treat L-DOPA psychosis. It is important to note that this analysis [30] holds for competition between endogenous dopamine and a loosely bound antipsychotic. A tightly bound antipsychotic, such as haloperidol, would not readily permit endogenous dopamine to replace it competitively.

9.3 'Fast-off-D_2' theory predicts difference between clozapine and isoclozapine

As reported by Kapur *et al.* [60], the single most powerful predictor of atypicality is the low affinity to, and fast dissociation from, the D_2 receptor, and not high affinity to any other receptor. This hypothesis is supported by their findings that clozapine and isoclozapine have identical potencies on many cloned receptors, including muscarinic M_1, dopamine D_1, dopamine D_4, serotonin-1A, and serotonin-2A receptors, but differ fivefold in their potency only on D_2 receptors. Thus, in several tests of atypicality (early activation of certain genes, catalepsy in animals and prolactin elevation), clozapine behaves like an

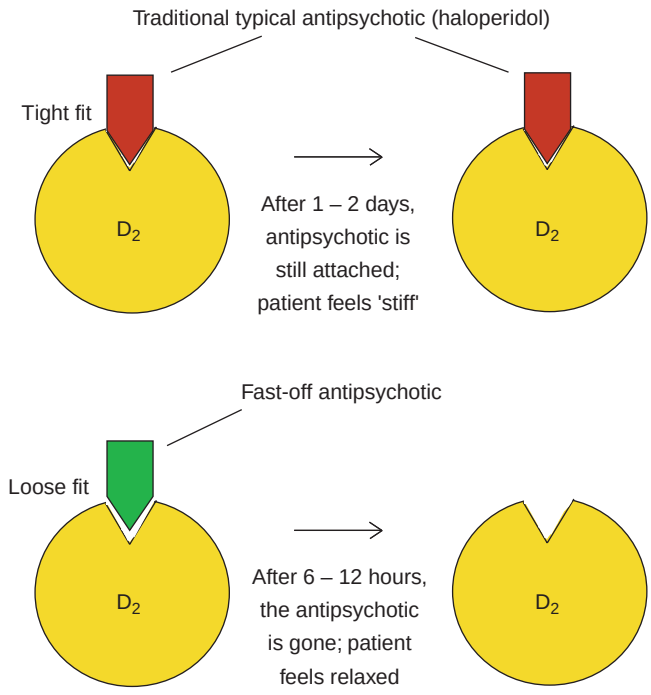

Figure 4. Antipsychotics that are tightly bound to D_2 markedly reduce dopamine neurotransmission and elicit Parkinsonism and prolactinaemia. Antipsychotics that are loosely bound to D_2 desorb quickly from D_2 and elicit little or no Parkinsonism or prolactinaemia.

Table 4. Aspects of 'tight' and 'loose' antipsychotic binding at dopamine D_2 receptors.

	'Tight'	'Loose'
Dose	Low	High
Extrapyramidal signs	Yes	No
Prolactin	High	Normal
Tardive dyskinesia	High risk	Low risk

atypical antipsychotic. Isoclozapine, however, behaves like a conventional antipsychotic.

10. Are serotonin receptors a clinical target for atypical antipsychotics?

Although it has often been suggested that the blockade of serotonin-2A receptors may contribute to antipsychotic action and may alleviate the Parkinsonism caused by D_2 blockade [62], the following findings do not support this principle.

10.1 Remoxipride does not block 5-HT_2 receptors

Remoxipride is a highly effective atypical antipsychotic drug with no extrapyramidal signs in humans, as well as no catalepsy in rats at doses 30 times higher than the antihyperactivity dose [61] and no hyperprolactinaemia, yet does not block serotonin-2A receptors [30,32]. For example, the dissociation constant of remoxipride at serotonin-2A receptors is at least 100-fold higher than that at the therapeutic D_2 receptor [30,32], indicating that remoxipride does not occupy serotonin-2A receptors at clinically therapeutic doses. As indicated in Table 3, remoxipride has the highest fast-off-D_2 rate of all antipsychotics.

10.2 Amisulpride does not block 5-HT_2 receptors

Amisulpride is a highly effective antipsychotic which is atypical and which does not occupy any serotonin-2A receptors in humans at doses up to 1200 mg per day [63]. However, because amisulpride has a very low permeability across the blood–brain barrier, it must be given at very high doses; 600 – 800 mg/day for antipsychotic action. Therefore, despite the rapid dissociation of 42 seconds, amisulpride from D_2 receptors (Table 3) of the anterior pituitary gland, the pituitary is outside the blood–brain barrier and is fully exposed to the massive concentration of amisulpride in the plasma, thereby resulting in a maintained block of D_2 with hyperprolactinaemia [64]. However, despite the elevated prolactin, amisulpride has no effect on serotonin receptors but provides good antipsychotic action without Parkinsonism. It is the rapid dissociation of 42 seconds (Table 3) which assists in minimising or preventing Parkinsonism, in keeping with the fast-off basis for clozapine and quetiapine.

10.3 Serotonin receptor blockade enhances catalepsy

Instead of reducing catalepsy, as predicted by the serotonin-blockade hypothesis, selective serotonin-2A receptor blockade with the drug M100,907 enhances the catalepsy of submaximal doses of the D_2 block by raclopride [65].

10.4 Antipsychotic catalepsy doses are not related to D_2/5-HT_2 ratio

There is no correlation between the antipsychotic catalepsy doses and the ratio of the antipsychotic dissociation constants at D_2 and at serotonin-2A receptors [66,67].

10.5 Antipsychotic D_2/5-HT_2 ratio does not identify atypical antipsychotics

Using the ratio of antipsychotic dissociation constants obtained in this laboratory on human cloned D_2 and serotonin-2A receptors (Table 1 and [30]), there is no obvious demarcation between typical and atypical antipsychotics.

10.6 Serotonin receptor block does not alleviate extrapyramidal signs

A high degree of serotonin-2A receptor occupancy (95%) by risperidone (6 mg/day) does not prevent EPS in a series of patients [68]. Moreover, although loxapine and olanzapine have 5-HT-$_{2A}$/D_2 receptor affinity ratios similar to that of clozapine, Kalkman *et al.* found that clozapine inhibited the cataleptic response to loxapine or olanzapine. These results provide a strong argument against the idea that 5-HT-$_{2A}$ blockade is the explanation for the low EPS profile of clozapine [69].

10.7 Serotonin receptor block does not alter threshold for D_2 block

Using [^{11}C]raclopride for imaging brain D_2 receptors and [^{11}C]setoperone for imaging brain serotonin-2A receptors, Kapur *et al.* [23] have found that the high occupancy of serotonin-2A receptors by olanzapine or by risperidone did not alter the D_2 occupancy required for the antipsychotic effect or the D_2 occupancy at which extrapyramidal signs occur. The threshold doses for antipsychotic action consistently occupy 65% of brain D_2 receptors in patients, and the threshold doses for extrapyramidal signs consistently occupy 80% of brain D_2 receptors in patients, whether or not the serotonin-2A receptors were occupied.

It is not clear, therefore, what clinical benefit, if any, is provided by the blockade of serotonin receptors. Although low doses of cyproheptadine have been used [70] to block serotonin-2A receptors and supplement antipsychotic administration, it should be noted that cyproheptadine has a D_2 blocking action. It has a *K* value of 24 nM at D_2 receptors, compared with 75 nM for clozapine and 21 nM for amoxapine (Table 1). Amoxapine, although marketed as an antidepressant, has antipsychotic properties.

10.8 Chlorpromazine blocks 5-HT_2 receptors but elicits Parkinsonism

Chlorpromazine, the first typical antipsychotic, at 500 mg/day blocks 65% of serotonin-2A receptors. This 'high level of serotonin-2A block suggests that the distinct clinical profiles of chlorpromazine and clozapine are unrelated to serotonin-2A receptor blockade' [63].

10.9 Block of serotonin receptors not needed for antipsychotic action

It has been reported that the block of serotonin-2A receptors 'is not a prerequisite for the antipsychotic effect' [68,69]. In fact, full block of serotonin-2A receptors occurs at subtherapeutic doses of risperidone, olanzapine and clozapine, indicating that serotonin-2A blockade has little or no antipsychotic action (but see [70]).

13.10 Agonism of serotonin-1A receptors reduces catalepsy

Although it has long been known that the stimulation of serotonin-1A receptors can alleviate catalepsy in animals caused by D_2 blockade [71,72], only recently have drugs been found which simultaneously block D_2, but stimulate serotonin-1A receptors [73].

14. Additional evidence for D_2 as the therapeutic target for antipsychotics

The D_2 receptor continues to be the main therapeutic target for antipsychotic action, whether it is targeted by antagonists or partial agonists, such as aripiprazole (Figure 2). In fact, as stated by Su *et al.* [74], 'no drug has yet been identified with antipsychotic action without a significant affinity for the D_2 receptor'.

In addition, over and above the pharmacological role of D_2 as an antipsychotic target, there are findings indicating that various aspects of D_2 contribute to psychosis in general, or schizophrenia, in particular.

For example, Hirvonen *et al.* [75] have found that the number of D_2 receptors is significantly elevated in healthy identical co-twins of individuals with schizophrenia. These findings suggest that an elevation of D_2 may be a necessary requirement for schizophrenia, but may not be not sufficient to elicit the syndrome.

Furthermore, the D_2 receptor has a polymorphism at position 311 where the serine is replaced by cysteine. In schizophrenia, it has been found in 27 samples, comprising 3707 patients and 5363 control subjects, that the serine311cysteine polymorphism was significantly associated with schizophrenia [76].

Although measurements on nonmedicated schizophrenia patients with [^{11}C]methylspiperone revealed elevations in striatal D_2 density, whereas measurements with [^{11}C]raclopride did not show such elevations (reviewed in [30]), the number of D_2 receptors in the caudate-putamen is significantly elevated in untreated schizophrenia [77].

15. No evidence for D_3 as an antipsychotic target

A detailed analysis along the lines shown in Table 2 and Figure 2, using the *K* values for D_3 in Table 1, shows that D_3 is occupied by some, but not all antipsychotics, as is the case with D_2. This analysis, for example, readily shows that D_3 is not extensively occupied at clinical concentrations of remoxipride, clozapine, spiperone, quetiapine, molindone, melperone and haloperidol [67].

Although BP 897 is a partial agonist at D_3 with a selectivity for D_3 of ~ 100-fold higher than that for D_2, BP 897 (at 10 mg/day) did not appear effective against schizophrenia symptoms [78]. Other drugs moderately selective for D_3, such as S33138 and A437,203, are currently being tested on schizophrenia patients. The highly D_3-selective drug, FAUC 365, has not yet been tested in this disease. It is possible that the D_3-selective compounds may be helpful in treating drug abuse [79-82].

16. No evidence for D_4 as an antipsychotic target

Interestingly, clozapine has a higher affinity at D_4 than at D_2, as shown in Table 1. Nevertheless, despite the selectivity of clozapine for D_4, clozapine occupies the necessary 60 – 70% occupancy of brain D_2 receptors at clinical doses (~ 400 mg/day), compatible with the idea that D_2 is the therapeutic target for clozapine, as with all the other antipsychotics. It may be noted that iso-clozapine causes catalepsy, in contrast to clozapine, which does not elicit catalepsy. Both drugs have identical affinity for D_4, but isoclozapine has higher affinity for D_2 (see Table 2) and, therefore, causes catalepsy.

Although the gene expression of D_4 was found to be elevated in the frontal cortex of schizophrenia tissues, selective D_4 antagonists, such as sonepiprazole and L-745,870, did not have any antipsychotic action.

17. D_2^{High} as the antipsychotic target

The D_2 receptor, similar to many other G-protein-linked receptors, has a state of high affinity and a state of low affinity for dopamine. The binding of dopamine to D_2 occurs in two concentration ranges. Low nanomolar concentrations of dopamine bind to the high-affinity state of the receptor (D_2^{High}) whereas high micromolar concentrations bind to the low-affinity state of the receptor (D_{2Low}).

D_2^{High} is the functional state in the anterior pituitary, on which dopamine and other dopamine-like drugs (bromocriptine) act to inhibit the release of prolactin. D_2^{High} is presumably also the primary functional form of D_2 in the nervous system, although little work has been done to prove this point.

Although it has been reported that 90% of the D_2 receptors in brain slices are in the D_2^{High} state, the proportion of D_2 receptors in the high-affinity state in homogenised striatum *in vitro* is generally between 15% and 20%. The D_2^{High} state quickly converts in a matter of seconds or minutes into the D_{2Low} state by guanine nucleotide.

Extensive reviews by Lieberman *et al.* [83], and by Curran *et al.* [84] show that up to 70% of schizophrenia patients are supersensitive to either methylphenidate or amfetamine at doses which do not affect control humans. These findings are in agreement with the dopamine hypothesis of psychosis or schizophrenia first outlined by J Van Rossum in 1967 [85]:

'The hypothesis that neuroleptic drugs may act by blocking dopamine receptors in the brain has been substantiated by preliminary experiments with a few selective and potent neuroleptic drugs. There is an urgent need for a simple isolated tissue that selectively responds to dopamine so that less specific neuroleptic drugs can also be studied and the hypothesis further tested.... When the hypothesis of dopamine blockade by neuroleptic agents can be further substantiated it may have fargoing consequences for the pathophysiology of schizophrenia. Overstimulation of dopamine receptors could then be part of the aetiology.'

A wide variety of brain alterations in animals (lesions, birth injury by C-section and anoxia, amfetamine or phencyclidine treatment, knockouts of a variety of receptors) all lead to the final common finding of behavioural dopamine supersensitivity and elevated proportions of D_2 receptors in the D_2^{High} state in the striatum [86].

For example, repeated administration of amfetamine to animals or humans leads to behavioural dopamine supersensitivity. Although the density of D_2 receptors in the striatum does not change in such animals, it is remarkable that the density of D_2^{High} receptors increases dramatically by several-fold [86].

A similar situation occurs in animals that receive hippocampal lesions neonatally. Such animals as adults reveal behavioural dopamine supersensitivity, and the striatum contains a marked increase in the proportion of D_2 receptors in the high-affinity state.

All dopamine-supersensitive animals examined to date reveal elevated D_2^{High} receptors. Because antipsychotics such as haloperidol successfully block the symptoms of psychosis found in amfetamine psychosis, phencyclidine psychosis, and brain damage psychosis in humans, and because these conditions in rats are associated with markedly elevated D_2^{High} states, it is reasonable to conclude that the main target of antipsychotic action is not only D_2, but the high-affinity state of D_2.

Therefore, the molecular control of the high-affinity state of D_2 is emerging as a central problem in this field. At present, there is uncertainty as to whether this high-affinity state of D_2 is controlled through G_o or one of the G_i proteins, because this varies from cell to cell.

18. Non-dopamine receptor targets

Although the major emphasis of this review has focused on the dopamine D_2 receptor as the main therapeutic target in

treating psychosis in general and schizophrenia in particular, there is a constant search for new targets that may either contribute to the therapeutic action or that may alleviate some of the side effects of D_2 blockade.

For example, a major difficulty emerging with the more recently developed antipsychotics, the so-called atypical antipsychotics such as olanzapine, quetiapine or clozapine, is that they lead to considerable weight gain with a significant risk for diabetes. Although the cause of such weight gain is not known, it is likely related to the blockade of histamine H_1 receptors [87]. Olanzapine, for example, has a potent dissociation constant of 0.087 nM at the histamine H_1 receptor, in contrast to its dissociation constant of 7 – 20 nM at the therapeutic D_2 receptor [30,87]. Currently, there is active research on developing D_2 antagonists that have less affinity for the histamine H_1 receptor in order to minimise the risk of weight gain [88].

Although serotonin-2A receptors do not appear to contribute to either the therapeutic aspect of antipsychotics or significantly alter the side effect profile, as discussed above, there are now at least fourteen types of serotonin receptors that have been identified. Some of these, such as the serotonin-6 and serotonin-7 receptors [90,91], have been suggested to be targets for some of the newer antipsychotics. There is no consensus on this matter, however. Clozapine, for example, has a high affinity for the serotonin-6 and serotonin-7 receptors, with a dissociation constant of 4 – 8 nM at both receptors [89,90], in contrast to the necessary therapeutic concentration of 113 – 225 nM in the patient's spinal fluid or free in the plasma water (Table 2). Clozapine, in other words, has very high affinity for the serotonin-6 and -7 receptors, but the extremely high occupation of these receptors does not appear to be relevant to the therapeutic antipsychotic action. The serotonin-6 and -7 receptors are also unlikely to be a common target for antipsychotics in general because older antipsychotics have very high dissociation constants, such as > 5000 nM for haloperidol, 1250 nM for melperone, 1595 nM for spiperone, and > 5000 nM for trifluperidol [89].

19. Expert opinion and future outlook

Of the five dopamine receptors and their variants, the D_2 receptor and its properties continue to be the most actively investigated because D_2 is the main clinical target for antipsychotics and for the dopamine agonist treatment of Parkinson's disease. The D_1 receptor, however, also has an important clinical role in treating Parkinson's disease because the stimulation of D_1 synergises with the stimulation of D_2, possibly via D_1/D_2 heterodimers or cell–cell interactions.

Probably the most central question in determining the basis of psychosis or schizophrenia is to determine the molecular basis of dopamine supersensitivity and to determine which proteins or genes regulate the maintenance of D_2 receptors in their high-affinity state. The discovery of such genes or proteins which foster the conversion of D_2 into more high-affinity states will provide new and possibly more accurate targets for new antipsychotics.

The clinician can apply some of the principles in this review to the treatment of individual patients. Atypical agents, being newer and still protected by patent, are much more expensive than the older drugs. Because they do not elicit EPS and do not elevate prolactin levels does not mean that they are free of serious side effects. One could argue that the side effects associated with some of the atypical drugs (agranulocytosis, obesity, diabetes, ophthalmological problems, cardiovascular problems, sexual problems, obsessive-compulsive symptoms, convulsions, insomnia) are more serious than EPS, high prolactin and even tardive dyskinesia. Low dose, extended dosing regimens of typical drugs may be best suited for a specific patient. Patients known to be non-adherent to regular medication may do better on those drugs that are more tightly bound to the D_2 receptor, where risk of relapse through a short period of non-compliance is a lesser risk.

On the other hand, patients with a history of neuroleptic malignant syndrome or tardive dyskinesia are best treated with drugs that are readily displaced so that, should the syndrome return, the drug is quickly out of their brain. In psychotic patients, high stress levels, accompanied by high endogenous dopamine release, will necessitate higher doses of antipsychotic drug. Periods of low stress will require lower doses. Psychotic patients who may temporarily benefit from high prolactin levels (those who do not want to conceive or, conversely, postpartum women whose milk is insufficient for breastfeeding) may preferentially be prescribed typical antipsychotics. Those with beginning signs of tardive dyskinesia, on the other hand, should be discontinued from the typical antipsychotics and prescribed the newer drugs instead. Knowing how drugs work greatly expands the clinician's repertoire of strategies, allowing optimisation of drug regimens for individualised treatment.

Finally, in order to diagnose and categorise early stages of psychosis, as well as to follow and treat the course of the illness, it is now possible to image the D_2^{High} states directly in patients [91,92].

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