The Dopamine Receptors

The Receptors

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Preface

As sites of action for drugs used to treat schizophrenia and Parkinson's disease, dopamine receptors are among the most validated drug targets for neuropsychiatric disorders. Dopamine receptors are also drug targets or potential targets for other disorders such as substance abuse, depression, Tourette's syndrome, and attention deficit hyperactivity disorder. When chapters were being written for the first edition of "The Dopamine Receptors," published in 1997, researchers were still coming to grips with the discovery of novel dopamine receptor subtypes whose existence had not been predicted by pharmacological analysis of native tissue. Although we are still far from a complete understanding of the roles of each of the dopamine receptor subtypes, the decade since the publication of the first edition has seen the creation and characterization of mice deficient in each of the subtypes and the development of increasingly subtype-selective agonists and antagonists. Many of the chapters in this second edition rely heavily on new knowledge gained from these tools, but the use of knockout mice and subtype-selective drugs continues to be such a dominant theme in dopamine receptor research that these topics are also discussed in stand-alone chapters. The field of G protein-coupled receptors has advanced significantly since the publication of the first edition, with a model of GPCR signaling based on linear, compartmentalized pathways having been replaced by a more complex, richer model in which neurotransmitter effects are mediated by a signalplex composed of numerous signaling proteins, including multiple GPCRs, other types of receptors, such as ionotropic receptors, accessory and scaffolding proteins, and effectors. Again, although many chapter topics are affected by this more complex model, key aspects of the model are specifically addressed in new chapters on dopamine receptor-interacting proteins and on dopamine receptor oligomerization.

My goal has been to produce a book that will serve as a reference work on the dopamine receptors while also highlighting the areas of research that are most active today. To achieve this goal, I encouraged contributors to write chapters that set a broad area of research in its historical context and that look forward to new research opportunities. I hope that readers will agree with me that the authors have achieved that goal.

Portland, Oregon
March, 2009

Kim A. Neve

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Chapter 1
Historical Overview: Introduction to the Dopamine Receptors

Philip Seeman

Abstract A long-term search for the mechanism of action of antipsychotic drugs was motivated by a search for the cause of schizophrenia. The research between 1963 and 1975 led to the discovery of the antipsychotic receptor, now known as the dopamine D_2 receptor, the target for all antipsychotic medications. There are now five known dopamine receptors, all cloned. Although no appropriate animal model or brain biomarker exists for schizophrenia, it is known that the many factors and genes associated with schizophrenia invariably elevate the high-affinity state of the D_2 receptor or D_2^{High} by 100–900% in animals, resulting in dopamine supersensitivity. These factors include brain lesions; sensitization by amphetamine, phencyclidine, cocaine, or corticosterone; birth injury; social isolation; and more than 15 gene deletions in the pathways for the neurotransmission mediated by receptors for glutamate (NMDA), dopamine, GABA, acetylcholine, and norepinephrine. The elevation of D_2^{High} receptors may be the unifying mechanism for the various causes of schizophrenia.

Keywords Neuroleptic · Antipsychotic receptor · D_2^{High} receptor · Membrane stabilization · [^{3}H]haloperidol · Van Rossum hypothesis of schizophrenia · Dopamine supersensitivity · [^{3}H]domperidone

1.1 Introduction

The background to dopamine receptors is intimately associated with the history of antipsychotic drugs. The research in this field started with the development of antihistamines after the Second World War, with H. Laborit using these compounds to

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This chapter is dedicated to the memory of Hyman Niznik and Hubert H.M. Van Tol, pioneers in dopamine receptors.

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enhance analgesia [1]. In individuals receiving one of these series of medications, Laborit noticed a "euphoric quietude"; the patients were "calm and somnolent, with a relaxed and detached expression." Compound 4560 (now named chlorpromazine) was the most potent of the Rhone Poulenc compounds in the series.

Chlorpromazine was soon tested by many French physicians for various diseases. While Sigwald and Bouttier [2] were the first to use chlorpromazine as the only medication for a psychotic individual, they did not report their observations until 1953. The 1952 report by Delay et al. [3] showed that within 3 days [4, 5] chlorpromazine reduced hallucinations and stopped internal "voices" in eight patients, a significantly dramatic finding.

With the "neuroleptic" or antipsychotic action of chlorpromazine capturing the attention of the psychiatric community, the specific target of action for chlorpromazine became a research objective for basic scientists. The working assumption then, and still is the case now, was that the discovery of such a target might open the pathway to uncovering the biochemical cause of psychosis and possibly schizophrenia.

1.2 Membrane Stabilization by Antipsychotics

With the introduction of chlorpromazine to psychotic patients in state and provincial hospitals in North America in the late 1950s and early 1960s, the number of patients hospitalized with schizophrenia became markedly reduced. The basic science premise gradually emerged – if the target sites for antipsychotics could be found, then perhaps these sites were overactive in psychosis or schizophrenia. In the 1960s, however, no one agreed on what schizophrenia was. Inclusion criteria varied so much that it was impossible to decide which patients to study, let alone what to study. But everyone agreed that chlorpromazine and the many other new antipsychotic drugs, most of which were phenothiazines, alleviated the symptoms of schizophrenia, however defined.

But where in the nervous system does one start to look for an antipsychotic target? Moreover, were there many types of antipsychotic targets to identify?

With the advent of the electron microscope, the 1960s was an active decade of discovery of subcellular particles and cell membranes. In those days, therefore, it seemed reasonable to start by examining the actions of antipsychotics on cell membranes. In particular, did antipsychotics readily locate to cell surfaces and cell membranes and thereby alter membrane structure and function? Did antipsychotics target mitochondria, the structure of which was being rapidly revealed by electron microscopy?

In my own research in 1963, it was important to determine whether antipsychotics permeated cell membranes and whether the drugs were membrane active. I started with an artificial lipid film floating on water, and measured the film pressure with a 1 cm square of sand-blasted aluminum hanging into the bath (Wilhelmy method; [6]). Upon the addition of an antipsychotic to the water below the film, the

aluminum plate immediately rose, showing that the film pressure had been altered by the antipsychotic. This indicated that the antipsychotic molecules had entered into the single layer of lipid molecules floating on the water surface, expanding the intermolecular spaces between the lipid molecules. Therefore, could it be that cell membrane lipids were targets for antipsychotics?

To my surprise, however, when I omitted the lipid molecules, the addition of the antipsychotic still altered the surface pressure of the water surface. In other words, I had accidentally discovered that antipsychotics were surface active [7].

These surface-active potencies showed an excellent correlation with clinical antipsychotic potencies. However, I later realized that the antipsychotic concentrations were all in the micromolar range, a concentration subsequently found to be far in excess of that which was clinically effective in the plasma water or spinal fluid in patients taking antipsychotic medications.

Although all the antipsychotics were surface active and readily acted on artificial lipid films, it was essential to determine whether antipsychotics had similar membrane actions on human red blood cell membranes. In fact, this did occur, and it was found that low concentrations of antipsychotics readily expanded red blood cell membranes by ~0.1–1% and, in doing so, exerted an anti-hemolytic action by allowing the cells to become slightly larger and stabilized before hemolysis occurred [8–11].

This membrane stabilization by antipsychotics was also associated with electrical stabilization of the membrane. That is, it soon became clear that the antipsychotics were potent anesthetics, blocking nerve impulses at antipsychotic concentrations of between 20 nM and 1,000 nM (Fig. 1.1, top correlation line) [10, 12]. However, here too, these membrane-stabilizing concentrations were still in excess of those found clinically in the spinal fluid of treated patients (see following section). The driving criterion throughout this research was to find a target that was sensitive to the antipsychotic concentrations found in the spinal fluid of psychotic patients on maintenance doses of antipsychotic medications.

1.3 Therapeutic Concentrations of Antipsychotics

Although antipsychotics stabilize a variety of cellular and subcellular membranes [10], these antipsychotic concentrations are generally between 20 nM and 100 nM. The therapeutic molarities, however, were not known until the data on haloperidol were analyzed. In the case of haloperidol, for example, only 8% of haloperidol was free and not bound to plasma proteins [13]. Therefore, the active free concentration of haloperidol in the patient plasma water or in the spinal fluid would be between 1 nM and 2 nM [14, 15, 16]. Based on the standard pharmacological principle that the non-protonated form of tertiary amines readily permeates cell membranes [8], this concentration in the aqueous phase in the plasma is expected to be identical to the aqueous concentration of haloperidol in the spinal fluid.

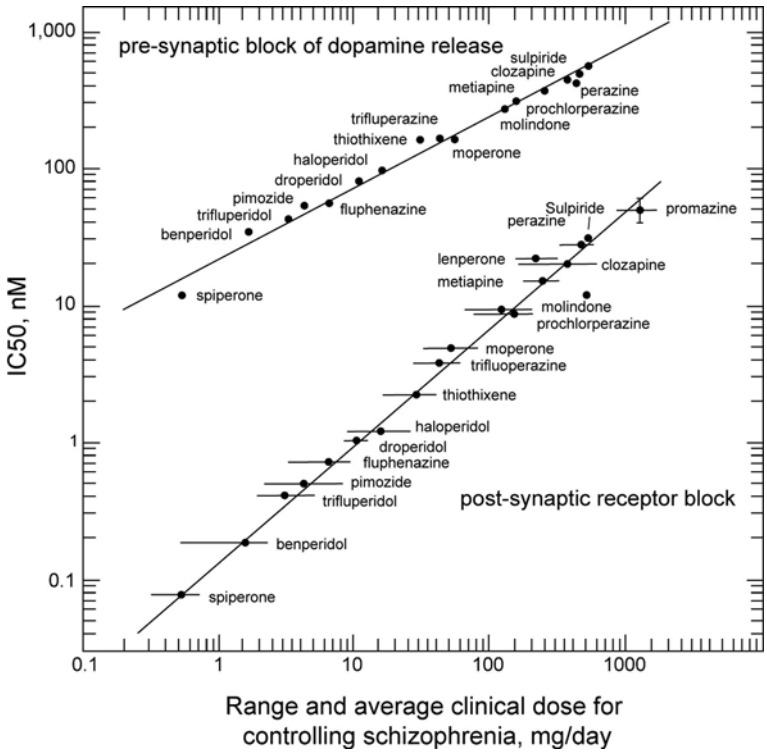

Fig. 1.1 All antipsychotic drugs inhibit the binding of [^{3}H]haloperidol to dopamine D_2 receptors (in calf striatal homogenate) in direct relation to the clinical antipsychotic potencies (*lower line*) [17,18,20]. The *upper line* indicates that antipsychotics also block the stimulated release of [^{3}H]dopamine (from rat striatal slices) at concentrations which correlate with their clinical potencies [12]; however, the antipsychotic concentrations required for this presynaptic action are much higher than those that inhibit [^{3}H]haloperidol binding to the D_2 receptors (*lower line*) or those which are found in the spinal fluid of patients being treated with antipsychotics [14] (re-drawn and adapted from [82] with permission)

1.4 Discovery of the Antipsychotic Dopamine Receptor

These latter calculations were critical for the discovery of the antipsychotic dopamine receptor [17, 18, 19]. That is, in order to detect or label a receptor with a dissociation constant of ∼1 nM for radioactive haloperidol, the specific activity of [^{3}H]haloperidol would have to be at least 10 Ci/mmol. However, the [^{3}H]haloperidol samples from Janssen Pharmaceutica (Belgium) kindly provided to the author's laboratory by Dr. J.J.P. Heykants in 1971 and by Dr. Jo Brugmans in 1972 had a specific activity of only 0.032–0.071 Ci/mmol, too low to detect specific binding for a site with an expected dissociation constant of ∼1 nM. Although New England Nuclear Corp. (Boston, MA) custom tritiated haloperidol for the author's laboratory, the specific activity was only ∼0.1 Ci/mmol.

Finally, after my extensive correspondence with Dr. Paul A.J. Janssen and Dr. J. Heykants, they asked I.R.E. Belgique (National Institut Voor Radio-Elementen, Fleurus, Belgium; Mr. M. Winand) to custom synthesize [^{3}H]haloperidol for the author's laboratory. I.R.E. Belgique soon thereafter provided us with relatively high specific activity [^{3}H]haloperidol (10.5 Ci/mmol) by June 1974.

This [^{3}H]haloperidol readily enabled us to detect the specific binding of [^{3}H]haloperidol to brain striatal tissue. Our laboratory submitted an abstract describing this to the Society for Neuroscience before the annual May 1975 deadline [17]. This report listed the following important IC_{50} values to inhibit the binding of [^{3}H]haloperidol: 2 nM for haloperidol, 20 nM for chlorpromazine, 3 nM for (+)butaclamol, and 10,000 nM for (–)butaclamol. The stereoselective action of butaclamol and the good correlation between the IC_{50} values and the clinical doses indicated that we had successfully identified the antipsychotic receptor. Moreover, of all the endogenous compounds tested, dopamine was the most potent in inhibiting the binding of [^{3}H]haloperidol, thus indicating that the antipsychotic receptor was a dopamine receptor.

The data of Seeman et al. [17] were confirmed by more extensive publications [18, 20, 21, 22], showing a clear correlation between the clinical potencies and the antipsychotic dissociation constants (Fig. 1.1, bottom correlation line).

At the CINP (Collegium Internationale Neuro-Psychopharmacologicum) meeting held in Paris in July 1975, during the evening courtyard reception at the City Hall of Paris, I rushed up to Dr. Paul Janssen and showed him the chart correlating the average clinical antipsychotic doses with the in vitro antipsychotic potencies. He laughed and said that averaging the clinical doses for each antipsychotic was like averaging all the religions of the world. Nevertheless, the correlation remains a cornerstone of the dopamine hypothesis of schizophrenia, still the major contender for an explanatory theory of schizophrenia causation.

1.5 Nomenclature of Dopamine Receptors

The receptor labeled by [^{3}H]haloperidol was later named the D2 receptor [23]. It is important to note that the data for the binding of [^{3}H]haloperidol identifying the antipsychotic receptor [17, 18] differed from the pattern of [^{3}H]dopamine binding described by Burt et al. [24] and Snyder et al. [25]. For example, the binding of [^{3}H]haloperidol was inhibited by ~10,000 nM dopamine, while that of [^{3}H]dopamine was inhibited by ~7 nM dopamine. For several years, this latter [^{3}H]dopamine binding site was termed the "D3 site" [26, 27], a term which is not to be confused with the discovery of the D_3 dopamine receptor [28]. As summarized in Table 1.1, there are now five different dopamine receptors that have been cloned.

At the same 1975 CINP meeting where I showed the correlation chart to Dr. Janssen, I happened to meet Dr. Sol Snyder in the lobby of the convention hotel and told him that I had custom prepared [^{3}H]haloperidol and that it was now available. The pattern of [^{3}H]haloperidol binding later published by Snyder et al.

[25] and by Burt et al. [24] agreed with my findings. The paper by Snyder et al. [25] kindly cited my paper of November, 1975, describing the [^{3}H]haloperidol-labeled antipsychotic receptor [18]. In addition, the publication of Burt et al. [24] kindly acknowledged the receipt of the drug samples of (+)- and (–)-butaclamol from our laboratory so that they could demonstrate stereoselective binding of [^{3}H]haloperidol.

Table 1.1 Key findings related to dopamine receptors

Year	Key findings related to dopamine receptors	Authors	References
1952	Analgesia and "euphoric quietude" with RP 4560	Laborit (Lacomme et al.)	[1]
1952–1953	Chlorpromazine (RP 4560) has effective antipsychotic action	Delay et al.; Sigwald and Bouttier	[2, 3]
1960	Very low amount of dopamine in Parkinson's diseased brain	Ehringer and Hornykiewicz	[29]
1963	Two antipsychotics increase normetanephrine and methoxytyramine	Carlsson and Lindqvist	[30]
1964	Three antipsychotics increase HVA and DOPAC; elimination delayed?	Andén et al.	[31]
1965	Dopamine can excite or inhibit neurons	Bloom et al.	[83]
1966	Dopamine hypothesis of schizophrenia outlined	Van Rossum	[33]
1971	Dopamine stimulates adenylate cyclase	Kebabian and Greengard	[38]
1971	Haloperidol measured in patient's plasma (see 1977 below)	Zingales et al.	[15]
1974	2.5 nM haloperidol blocks tritiated dopamine receptors	Seeman et al.	[19]
1974	Haloperidol blocks excitation in Helix	Struyker Boudier et al.	[84]
1975	Tritiated haloperidol labels dopamine receptors	Seeman et al.	[17, 18]
1975	Antipsychotic doses correlate with blockade of dopamine receptors	Seeman et al.	[18, 20]
1976	Sulpiride resolves two dopamine sites; no effect on adenylate cyclase	Roufogalis et al.	[42]
1976	Two dopamine receptors proposed: inhibitory and excitatory	Cools; Van Rossum	[35]

Table 1.1 (continued)

Year	Key findings related to dopamine receptors	Authors	References
1977	Dopamine stimulates adenylate cyclase in parathyroid	Brown et al.	[39]
1977	92% of plasma haloperidol bound, indicating 2 nM free in water	Forsman and Öhman	[13]
1978	Two dopamine receptors: coupled and uncoupled to adenylate cyclase	Spano et al.; Garau et al.	[36, 37]
1978	Presynaptic action of apomorphine reduces release of dopamine	Starke et al.	[53]
1978	Elevated D2 in postmortem schizophrenia brain	Lee et al.	[59]
1979	Names of D1 and D2 used	Kebabian and Calne	[23]
1979	Dopamine inhibits adenylate cyclase in ant pituitary	De Camilli et al.	[43]
1983	Identical antipsychotic Ki values at striatum and limbic D2 receptors	Seeman and Ulpian	[85]
1984	Kd values of D2 ligands depend on final tissue concentration	Seeman et al.	[56]
1984	D_2^{High} and D_{2Low} affinity states of D2 receptors	Wreggett and Seeman	[55]
1985	D_2^{High} is functional state of D2	McDonald et al.; George et al.	[51, 52]
1986	Elevated D2 measured in living schizophrenia patients	Wong et al.	[68]
1986	Labeling of D2 receptors in living humans by positron emission tomography	Farde et al.	[86]
1988	Antipsychotics occupy 60–80% of D2 in living schizophrenia patients	Farde et al.	[70]
1988–1989	Cloning of the rat D_{2Short} and D_{2Long} receptors	Bunzow et al.; Giros et al.	[46, 48]
1989	Cloning of the human D_{2Short} and D_{2Long} receptors	Grandy et al.	[47]
1989	90% of D2 receptors are in D_2^{High} state in brain slices	Richfield et al.	[54]
1989	Endogenous dopamine lowers radio-raclopride binding; relevant to PET	Seeman et al.	[81]
1990–1991	Dopamine D1 and D5 receptors cloned	Sunahara; Zhou et al.	[40,41,87]

Table 1.1 (continued)

Year	Key findings related to dopamine receptors	Authors	References
1990	Dopamine D3 receptor cloned	Sokoloff et al.	[28]
1991	Dopamine D4 receptor cloned	Van Tol et al.	[50]
1992	Block of D2 >80% by antipsychotics associated with Parkinsonism	Farde et al.	[69]
1992	Synaptic dopamine at rest is ∼2 nM, ∼100–200 nM during firing	Kawagoe et al.	[88]
1995	Drug Ki depends on fat solubility of ligand	Seeman and Van Tol	[57]
1996	Amphetamine-induced release of dopamine is higher in schizophrenia	Laruelle et al.	[80]
1998	D_{2Short} receptors located mostly in nigral neurones	Khan et al.	[89]
1999	Therapeutic doses of antipsychotics block 60–80% D2	Kapur et al.	[71]
1999	Isoleucine at position 154 in D2 causes myoclonus dystonia	Klein et al.	[90]
1999	Rapid release of clozapine and quetiapine from D2 receptors	Seeman et al.	[74]
2000	New $D_{2Longer}$ receptor	Seeman et al.	[49]
2003	Antipsychotics occupy more D2 in limbic areas than striatum	Bressan et al.	[75]
2005	Dopamine supersensitivity correlates with elevated D_2^{High} states	Seeman et al.	[91]
2005	Dopamine receptor contribution to action of PCP, LSD, and ketamine	Seeman et al.	[92]
2005	Higher D2 density in healthy identical twins of schizophrenia patients	Hirvonen et al.	[66]
2006	Markedly elevated D_2^{High} receptors in all animal models of psychosis	Seeman et al.	[93, 94]

1.6 Antipsychotic Accelerated Turnover of Dopamine

In 1960 Ehringer and Hornykiewicz [29] discovered that the content of dopamine was extremely low in the postmortem brains of patients who died with Parkinson's disease. This discovery immediately suggested that the well-known Parkinsonism

caused by antipsychotics was probably associated in some way with interference of dopamine neurotransmission by the antipsychotics. However, there were many possible molecular modes of interference, including presynaptic and postsynaptic mechanisms.

The finding of Ehringer and Hornykiewicz naturally stimulated brain research on dopamine. Carlsson and Lindqvist [30] soon reported that chlorpromazine and haloperidol increased the production of normetanephrine and methoxytyramine, metabolites of epinephrine and dopamine, respectively. To explain the increased production of these metabolites, these authors 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 . . . ."

In other words, these authors proposed that antipsychotics blocked 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. The paper by Carlsson and Lindqvist [30] is often mistakenly cited as discovering the principle that antipsychotic drugs selectively block dopamine receptors. A year later, even the students of the Carlsson laboratory, Andén et al. [31], limited their speculation to proposing that "chlorpromazine and haloperidol delays the elimination of the (metabolites). . .," a hypothesis no longer held. Moreover, even after 7 years, although Andén et al. [32] reported that antipsychotics increased the turnover of both dopamine and noradrenaline, 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 radioreceptor assays to detect the dopamine receptor directly and to demonstrate antipsychotic selectivity for the dopamine receptor.

In fact, when the antipsychotic dopamine receptor was discovered [18, 20], there was a peak surge in the rate of citations of the paper by Carlsson and Lindqvist [30], a peak stimulated by the actual discovery of the dopamine receptor method, as shown in Fig. 1.2. This figure also shows that there was approximately a 12-year interval between the onset of dopamine research and the research on dopamine receptors, indicating that the two fields were stimulated by separate developments.

1.7 The Dopamine Hypothesis of Schizophrenia, and Dopamine Receptors in the Human Brain

As already noted, the paper by Carlsson and Lindqvist [30] is often mistakenly cited as the origin of the dopamine hypothesis of schizophrenia. However, the dopamine hypothesis of schizophrenia was first outlined in 1967 by Van Rossum [33] (see [34]) as follows:

"The hypothesis that neuroleptic drugs may act by blocking dopamine receptors in the brain has been substantiated by preliminary experiments with a few

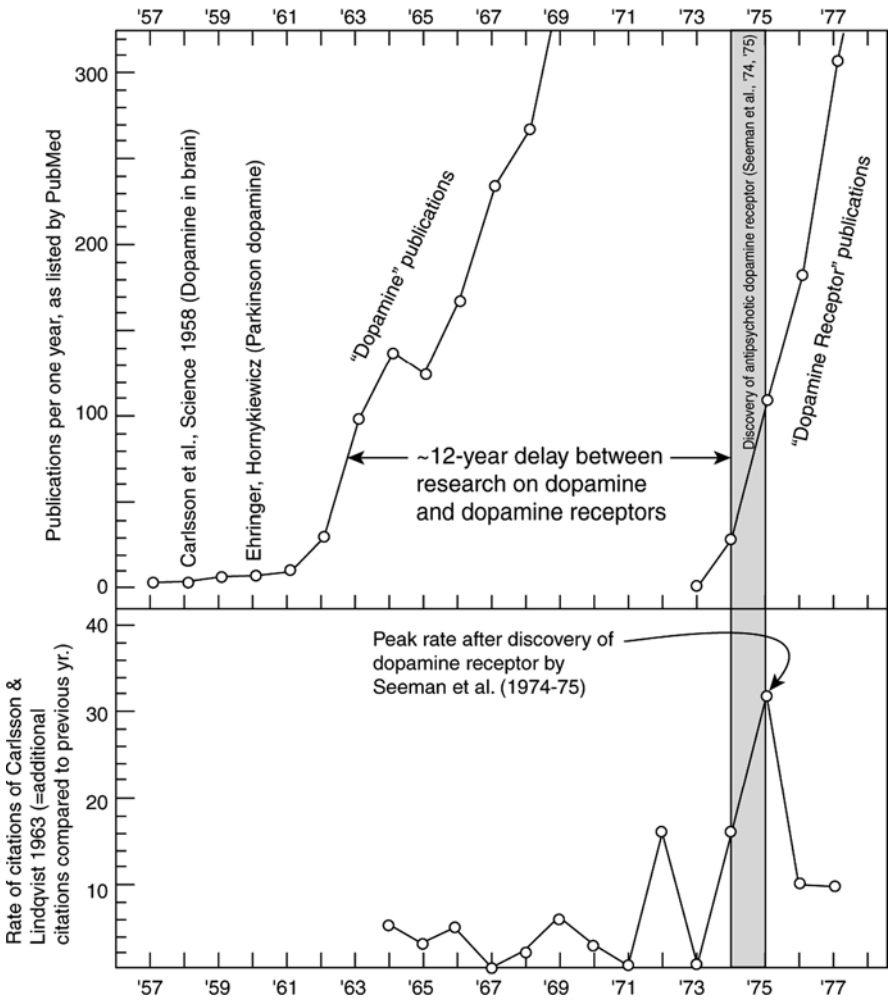

Fig. 1.2 *Top*: Annual number of publications on "dopamine" and on "dopamine receptors," as listed by PubMed online. Dopamine was found in brain tissue by Montagu [95] in Weil-Malherbe's laboratory [96, 97] and by Carlsson et al. [98]. There is a 12-year interval between the two sets of publications, suggesting that the two onsets of publications were stimulated by separate other publications. *Bottom*: Annual rate of citations (Web of Science, Thomson Scientific, Philadelphia, PA) of the article by Carlsson and Lindqvist [30], describing the increased production of normetanephrine and methoxytyramine by chlorpromazine or haloperidol. The citation rate of this 1963 article peaked in 1975 when the dopamine receptors were discovered [17, 18, 19] (from [82] with permission)

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. Over-stimulation of dopamine receptors could then be part of the etiology."

With the discovery of the antipsychotic dopamine receptor in vitro, it became possible to measure the densities and properties of these receptors directly not only in animal brain tissues but also in the postmortem human brain and, at a later time, in living humans by means of positron emission tomography. Many, but not all, of these findings directly or indirectly support the dopamine hypothesis of schizophrenia.

1.8 Key Advances Related to Dopamine Receptors

Many of the significant advances in dopamine receptors and the dopamine hypothesis of psychosis or schizophrenia are listed in Table 1.1. Between 1976 and 1979, it became clear that there were two main groups of dopamine receptors, D1 and D2 [23, 35, 36, 37]. The D1-like group of receptors were associated with dopamine-stimulated adenylate cyclase [38, 39], but were not selectively labeled by [^{3}H]haloperidol. The antipsychotic potencies at these D1 receptors did not correlate with clinical antipsychotic potency [26]. The D1-like receptors now consist of the cloned D_1 and D_5 receptors [40, 41].

The D2-like receptors did not stimulate adenylate cyclase and are now known to inhibit adenylate cyclase [42, 36, 37, 43, 44, 45]. The D2-like group now includes the cloned D_{2Short} [46, 47], D_{2Long} [48], $D_{2Longer}$ [49], D_3 [28], and D_4 dopamine receptors [50].

Moreover, each of these receptors has a state of high affinity and a state of low affinity for dopamine, with D_2^{High} being the functional state in the anterior pituitary [51, 52], in nigral dopamine terminals (presynaptic receptors [53]), and presumably in the nervous system itself. Although this latter point has not been unequivocably established, Richfield et al. [54] have found that 90% of the D_2 receptors in brain slices are in the D_2^{High} state. The D_2^{High} state can be quickly converted into the D_{2Low} state by guanine nucleotide [55].

The differences in findings on dopamine receptors between laboratories are explained by technically different methods and ligands. For example, the dissociation constant of a ligand at the D_2 receptor can vary enormously, depending on the final concentration of the tissue [56]. Moreover, fat-soluble ligands, such as [^{125}I]iodosulpride, [^{3}H]nemonapride, and [^{3}H]spiperone, invariably yield higher dissociation constants than less fat-soluble ligands (such as [^{3}H]raclopride) for competing drugs [21, 57]. This technical effect also occurs with positron emission tomography ligands [58].

Although the density of D_2 receptors in postmortem human schizophrenia tissues is elevated [26, 59, 60–62], some of this elevation may have resulted from the antipsychotic administered during the lifetime of the patient. An example of this elevation is shown in Fig. 1.3, where it may be seen that the postmortem tissues from half of the patients who died with schizophrenia revealed elevated densities of

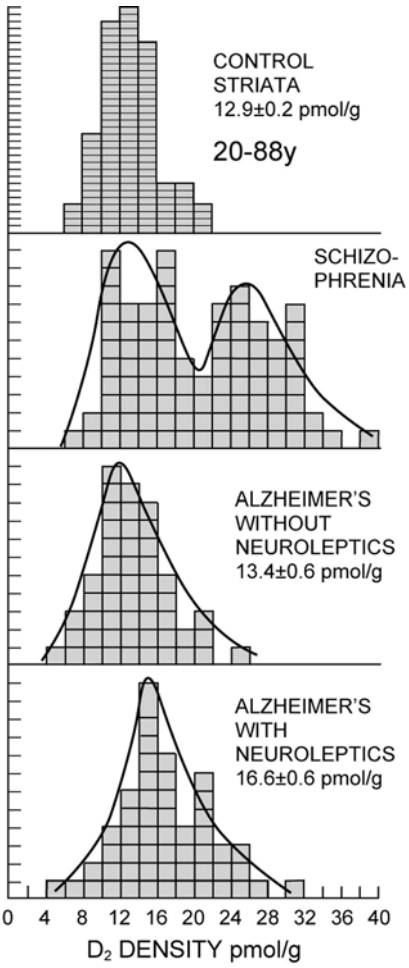

Fig. 1.3 Elevation of dopamine D_2 receptors in postmortem caudate–putamen tissues from patients who had died with schizophrenia. Each *box* indicates the D_2 density measured by saturation analysis with [^{3}H]spiperone (Scatchard method for Bmax; centrifugation method) [62]. The D_2 densities in the postmortem striata from schizophrenia patients exhibit a bimodal pattern, with half the values being two or three times the normal density. Most of the schizophrenia patients had been treated with antipsychotics during their lifetime. Although the Alzheimer patient tissues also revealed a small elevation of D_2 densities, the magnitude and pattern were different than that for schizophrenia (re-drawn and adapted from [82] with permission)

[^{3}H]spiperone-labeled D2-like receptors in the caudate–putamen tissue. The other half of the postmortem schizophrenia tissues were normal in D_2 density even though most of the patients were known to have also been treated with antipsychotics during their lifetime.

It is often surprising to encounter people who are resistant to advances in science. For example, I vividly recall one British psychiatrist standing up and shouting at me from the audience: "Post-mortem dopamine receptors? Do you actually expect me to believe that these dead receptors come to life and bind your radioactive material?" I answered that the same type of question was raised a century ago when people seriously questioned whether ferments could be isolated and still have activity, but that we can now buy crystallized enzymes for a few dollars and that these ferments are fully active. And, of course, thanks to many of the contributors to the present

book on "The Dopamine Receptors," one can now purchase frozen clones of the five different dopamine receptors.

1.9 Is D_2^{High} the Unifying Mechanism for Schizophrenia?

Throughout the years between 1963 and the present, the overall strategy has been to identify the main target of antipsychotic medications and then to determine whether these antipsychotic targets are overactive in schizophrenia or in animal models of psychosis. Has this strategy worked? The answer is yes. First, the primary target for antipsychotics, the dopamine D_2 receptor, has been identified, and, second, many avenues indicate that D_2^{High} (the high-affinity state of the D_2 receptor) may be the unifying mechanism for schizophrenia.

In particular, the following facts on dopamine receptors validate the 45-year search for a basic unifying mechanism for schizophrenia:

1. All antipsychotic drugs, including the newer dopamine partial agonists such as aripiprazole [22] or OSU 6162 [63], block dopamine D_2 receptors in direct relation to their clinical potency. Even the glutamate-type antipsychotic [64] has a significant dopamine partial agonist action on D_2 receptors [65].
2. The brain imaging by Hirvonen et al. [66] shows that the D_2 density is elevated in healthy identical co-twins of patients who have schizophrenia. This finding suggests that the elevation of D_2 receptors is necessary for psychosis. At the same time, however, the findings of Hirvonen et al. also illustrate that in addition to elevated D_2 receptors there is likely another factor precipitating the psychotic symptoms. This additional factor may well be that a certain proportion of D_2 receptors must convert into the high-affinity state.
   At the same time, the elevation of D_2 is becoming recognized as a valuable biomarker for prognosis and outcome in first-episode psychosis [67]. Earlier work had shown that the density of D_2 receptors labeled by [^{11}C]methylspiperone was elevated in drug-naive schizophrenia patients [68]. However, no such elevation of D_2 receptors was found in schizophrenia patients when [^{11}C]raclopride was used (Refs in [69]).
3. It has been consistently found that psychotic symptoms are alleviated when 65% to 75% of the brain D_2 receptors (as measured in the striatum) are occupied by antipsychotics [70, 69]. It is now considered unlikely that the blockade of serotonin-2 receptors assists in alleviating psychosis and affecting D_2 occupancy [71, 72, 73]. The antipsychotic occupancy of D_2 may or may not be higher in limbic regions [21, 74, 75, 76, 77].
4. In contrast to traditional antipsychotics such as chlorpromazine and haloperidol that can elicit Parkinsonism, clozapine and quetiapine do not produce Parkinsonism, consistent with the fact that clozapine and quetiapine dissociate rapidly from the D_2 receptor [21].
5. The psychotic symptoms in schizophrenia increase or intensify when the individual is challenged with psychostimulants at doses that have little effect in

control subjects. As reviewed by Lieberman et al. [78], 74–78% of patients with schizophrenia become worse with new or intensified psychotic symptoms after being given amphetamine or methylphenidate. Psychotic symptoms can also be elicited in this way in control subjects, but only in 0–26%.

6. In a meta-analysis of 27 studies (3,707 schizophrenia patients and 5,363 control subjects), Glatt and Jönsson [79] have found that the Ser311Cys polymorphism in the D_2 receptor was significantly associated with schizophrenia ($P = 0.002$–0.007), indicating that this polymorphism in D_2 may contribute a significant and reliable risk for the illness.
7. Amphetamine-induced release of endogenous dopamine in humans is a possible marker of psychosis [80], using the principle worked out in animals [81].
8. Although no appropriate animal model or brain biomarker exists for schizophrenia, it is known that the many factors and genes associated with schizophrenia invariably elevate dopamine D_2^{High} receptors by 100–900% in animals, resulting in dopamine supersensitivity. These factors include brain lesions; sensitization by amphetamine, phencyclidine, cocaine, or corticosterone; birth injury; social isolation; and more than 15 gene deletions in the pathways for the neurotransmission mediated by receptors for glutamate (NMDA), dopamine, GABA, acetylcholine, and norepinephrine. A list of these psychosis-precipitating factors is given in Table 1.2, along with the magnitude of the elevations that these factors elicit in the proportion of D_2^{High} receptors in the striata of mice or rats. The total density of D_2 generally does not change.

Table 1.2 Increase in D_2^{High} receptors in dopamine supersensitive animal models for psychosis

Percentage of increase in proportion of D_2^{High}	Treatment	References
	Sensitization by	
250%	Amphetamine	[93, 94]
180%	Phencyclidine	[91]
160%	Cocaine	[99]
125%	Caffeine	[100]
50%	Quinpirole	[94]
210%	Corticosterone	[91]
	Lesions of	
270%	Neonatal hippocampus	[91]
160%	Neonatal hippocampus	[94]
130%	Cholinergic lesion in cortex	[94]
100%	Entorhinal hippocampus	[101]
	Knockout of gene for	
200–900%	D4 receptor	[91]
60–340%	GRK6	[91, 94]
232%	Alpha-Adrenoceptor-1b	[102]
225%	$GABA_{B1}$	H. Mohler and P. Seeman (unpublished)
200%	Dopamine-beta-hydroxylase	[91, 94]

Table 1.2 (continued)

Percentage of increase in proportion of D_2^{High}	Treatment	References
160%	Trace amine-1 receptor	[103]
135%	RGS9-2	[91, 94]
133%	Nurr77	L.E. Trudeau, P. Seeman (unpublished)
129%	Postsynaptic density 95	J.-M. Beaulieu, P. Seeman (unpublished)
120%	Tyrosine hydroxylase (no dopamine)	[91]
90%	COMT	[91]
60–80%	Vesicular monoamine transporter	[104]
48%	RII beta (protein kinase A)	[91, 94]
39%	Dopamine transporter	[104]
	Other	
130–460%	Cesarian birth with anoxia (rat)	[91, 94]
228%	Rats socially isolated from birth	[105]
100%	Reserpine-treated rats	[91, 94]
	Animals not showing supersensitivity	
–7%	Dopamine D1 receptor knockout mice	[91, 94]
19%	Glycogen synthase kinase 3 knockout mice	[91, 94]
–75%	Adenosine A2A receptor knockout mice	[91, 94]
20%	mGluR5 knockout mice	[91, 94]

Abbreviations: COMT, catechol-O-methyl transferase; GABAB1, the B1 subtype of G protein-coupled receptors for GABA; GRK6, G protein-coupled receptor kinase 6; mGluR5, metabotropic glutamate receptor 5; Nurr77, orphan nuclear receptor 77; RII beta, the IIβ form of the regulatory subunit of cyclic AMP-dependent protein kinase; RGS9-2, regulator of G protein signaling 9-2

Because antipsychotic drugs directly block D_2 receptors, it is not surprising that antipsychotics also cause an increase in the proportion of D_2^{High} receptors. In fact, it has long been known that administration of antipsychotic drugs can induce dopamine supersensitivity and antipsychotic tolerance in animals. These effects are also found in humans and presumably are the basis for supersensitivity psychosis or rebound psychosis upon drug withdrawal. Although D_2^{High} receptors become elevated after long-term antipsychotics, these elevated D_2^{High} states readily reverse, unlike the essentially permanently elevated D_2^{High} states in the other animal models of psychosis mentioned above.

The strategy, the objective, and the questions on dopamine receptors still remain. What is the molecular pathway for antipsychotic action via the dopamine receptors? Are any of these steps specifically altered in schizophrenia? What is the intracellular biochemical mechanism of converting D_{2Low} into D_2^{High}?

At present, the most promising direction in this field is to examine the molecular basis of dopamine supersensitivity, because up to 70% of patients are supersensitive to either methylphenidate or amphetamine at doses that do not affect control humans. Moreover, as shown in Table 1.2, a wide variety of brain alterations (lesions, drug treatment, receptor knockouts) all lead to the final common target of elevated proportions of D_2 receptors in the D_2^{High} state. 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 Go or one of the Gi proteins, because this varies from cell to cell.

It is currently proposed that there are multiple pathways in the various types of psychosis that all converge to elevate the D_2^{High} state in specific brain regions and that this elevation elicits psychosis. This proposition is supported by the dopamine supersensitivity that is a common feature of schizophrenia and that also occurs in many types of genetically altered, drug-altered, and lesion-altered animals. Dopamine supersensitivity, in turn, correlates with D_2^{High} states. The finding that all antipsychotics, traditional and recent ones, act on D_2 receptors further supports the proposition.

Altogether, the dawn of the neurotransmitter era has proven to be an exciting chapter in neuropsychopharmacology. The art of psychiatry is becoming a science. It has been a privilege to participate in these developments. I thank my fellow students for making it possible.

References

1. Lacomme M, Laborit H, Le Lorier G, et al. [Obstetric analgesia potentiated by associated intravenous dolosal with RP 4560.] Bull Féd Soc Gynecol Obstet Lang Fr 1952;4:558–62.
2. Sigwald J, Bouttier D. 3-Chloro-10-(3'-dimethylaminopropyl)-phenothiazine hydrochloride in current neuro-psychiatry. Ann Méd Interne (Paris) 1953;54:150–82.
3. Delay J, Deniker P, Harl J-M. Traitement des états d'excitation et d'agitation par une méthode médicamenteuse dérivée de l'hibernithérapie. [Therapeutic method derived from hiberno-therapy in excitation and agitation states.] Ann. Méd-Psychol. (Paris) 1952;110:267–73.
4. Agid O, Kapur S, Arenovich T, et al. Delayed-onset hypothesis of antipsychotic action: a hypothesis tested and rejected. Arch Gen Psychiatry 2003;60:1228–35.
5. Kapur S, Arenovich T, Agid O, et al. Evidence for onset of antipsychotic effects within the first 24 hours of treatment. Am J Psychiatry 2005;162:939–46.
6. Wilhelmy L. Ann Physik Lpz 1863;119:177.
7. Seeman P, Bialy HS. The surface activity of tranquilizers. Biochem Pharmacol 1963;12:1181–91.
8. Seeman P. Erythrocyte membrane stabilization by local anesthetics and tranquilizers. Biochem Pharmacol 1966a;15;1753–66.
9. Seeman P. Membrane stabilization by drugs: tranquilizers, steroids and anesthetics. Int Rev Neurobiol 1966b;9:145–221.

10. Seeman, P. The membrane actions of anesthetics and tranquilizers. Pharmacol Rev 1972;24:583–655.
11. Seeman P, Weinstein J. Erythrocyte membrane stabilization by tranquilizers and antihistamines. Biochem Pharmacol 1966;15:1737–52
12. Seeman, P, Lee T. Antipsychotic drugs: Direct correlation between clinical potency and presynaptic action on dopamine neurones. Science 1975;188:1217–9.
13. Forsman A, Öhman R. Studies on serum protein binding of haloperidol. Curr Ther Res Clin Exp 1977;21:245–55.
14. Seeman, P. Dopamine receptor sequences. Therapeutic levels of neuroleptics occupy D_2, clozapine occupies D_4. Neuropsychopharmacology 1992;7:261–84.
15. Zingales IA. A gas chromatographic method for the determination of haloperidol in human plasma. J Chromatogr 1971;54:15–24.
16. Seeman, P. Therapeutic receptor-blocking concentrations of neuroleptics. Int Clin Psychopharmacol 1995;10 (Suppl 3):5–13.
17. Seeman P, Wong M, Tedesco J. Tranquilizer receptors in rat striatum. Soc Neurosci Abstr 1975;1:405.
18. Seeman P, Chau-Wong M, Tedesco J, et al. Brain receptors for antipsychotic drugs and dopamine: direct binding assays. Proc Natl Acad Sci USA 1975;72:4376–80.
19. Seeman P, Wong M, Lee T. Dopamine receptor-block and nigral fiber impulse-blockade by major tranquilizers. Fed Proc 1974;33:246.
20. Seeman P, Lee T, Chau-Wong M, et al. Antipsychotic drug doses and neuroleptic/dopamine receptors. Nature (Lond.) 1976;261:717–9.
21. Seeman, P. Atypical antipsychotics: mechanism of action. Can J Psychiat 2002;47:27–38.
22. Seeman P. Targeting the dopamine D2 receptor in schizophrenia. Expert Opin Ther Targets 2006;10:515–31.
23. Kebabian JW, Calne DB. Multiple receptors for dopamine. Nature 1979;277:93–6.
24. Burt DR, Creese I, Snyder SH. Properties of [^{3}H]haloperidol and [^{3}H]dopamine binding associated with dopamine receptors in calf brain membranes. Mol Pharmacol 1976;12: 800–12.
25. Snyder SH, Creese I, Burt DR. The brain's dopamine receptor: labeling with [^{3}H]dopamine and [^{3}H]haloperidol. Psychopharmacol Commun 1975;1:663–73.
26. Seeman, P. Brain dopamine receptors. Pharmacol Rev 1980;32:229–313.
27. Seeman, P. Nomenclature of central and peripheral dopaminergic sites and receptors. Biochem Pharmacol 1982;31:2563–8.
28. Sokoloff P, Giros B, Martres MP, et al. Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. Nature 1990;347:146–51.
29. Ehringer H, Hornykiewicz O. Distribution of noradrenaline and dopamine (3- hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system. Klin Wochenschr 1960;38:1236–9.
30. Carlsson A, Lindqvist M. Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. Acta Pharmacol Toxicol (Copenh) 1963;20:140–4.
31. Andén N-E, Roos B-E, Werdinius B, 1964. Effects of chlorpromazine, haloperidol and reserpine on the levels of phenolic acids in rabbit corpus striatum. Life Sci 1964;3:149–58.
32. Andén N-E, Butcher SG, Corrodi H, et al. Receptor activity and turnover of dopamine and noradrenaline after neuroleptics. Eur J Pharmacol 1970;11:303–14.
33. Van Rossum JM. The significance of dopamine-receptor blockade for the action of neuroleptic drugs. In: Brill H, Cole JO, Deniker P, Hippius H, Bradley PB, eds. Neuro-Psycho-Pharmacology, Proceedings of the Fifth International Congress of the Collegium Internationale Neuro-Psycho-pharmacologicum, March 1966, Excerpta Medica Foundation, Amsterdam, 1967;321–9.
34. Baumeister AA, Francis JL. Historical development of the dopamine hypothesis of schizophrenia. J History Neurosci 2002;11:265–77.

35. Cools AR, Van Rossum JM. Excitation-mediating and inhibition-mediating dopamine-receptors: a new concept towards a better understanding of electrophysiological, biochemical, pharmacological, functional and clinical data. Psychopharmacologia 1976;45: 243–54.
36. Spano PF, Govoni S, Trabucchi M. Studies on the pharmacological properties of dopamine receptors in various areas of the central nervous system. Adv Biochem Psychopharmacol 1978;19:155–65.
37. Garau L, Govoni S, Stefanini E, et al. Dopamine receptors: pharmacological and anatomical evidences indicate that two distinct dopamine receptor populations are present in rat striatum. Life Sci 1978;23:1745–50.
38. Kebabian JW, Greengard P. Dopamine-sensitive adenyl cyclase: possible role in synaptic transmission. Science 1971;174:1346–9.
39. Brown EM, Carroll RJ, Aurbach GD. Dopaminergic stimulation of cyclic AMP accumulation and parathyroid hormone release from dispersed bovine parathyroid cells. Proc Natl Acad Sci USA 1977;74:4210–3.
40. Sunahara RK, Niznik HB, Weiner DM, et al. Human dopamine D_1 receptor encoded by an intronless gene on chromosome 5. Nature 1990;347:80–3.
41. Sunahara RK, Guan H-C, O'Dowd B, et al. Cloning of the gene for a human dopamine D5 receptor with higher affinity for dopamine than D_1. Nature 1991;350:614–9.
42. Roufogalis BD, Thornton M, Wade DN. Specificity of the dopamine sensitive adenylate cyclase for antipsychotic antagonists. Life Sci 1976;19:927–34.
43. De Camilli P, Macconi D, Spada A. Dopamine inhibits adenylate cyclase in human prolactin-secreting pituitary adenomas. Nature 1979;278:252–4.
44. Meunier H, Labrie F. The dopamine receptor in the intermediate lobe of the rat pituitary gland is negatively coupled to adenylate cyclase. Life Sci 1982;30:963–8.
45. Onali P, Olianas MC, Gessa GL. Characterization of dopamine receptors mediating inhibition of adenylate cyclase activity in rat striatum. Mol Pharmacol 1985;28:138–45.
46. Bunzow JR, Van Tol HH, Grandy DK, et al. Cloning and expression of a rat D_2 dopamine receptor cDNA. Nature 1988;336:783–7.
47. Grandy DK, Marchionni MA, Makam H, et al. Cloning of the cDNA and gene for a human D_2 dopamine receptor. Proc Natl Acad Sci USA 1989;86:9762–976
48. Giros B, Sokoloff P, Martres MP, et al. Alternative splicing directs the expression of two D_2 dopamine receptor isoforms. Nature 1989;342:923–6.
49. Seeman P, Nam D, Ulpian C, et al. A new dopamine receptor, D2Longer, with a unique TG splice site, in human brain. Mol Brain Res 2000;76:132–41.
50. Van Tol HHM, Bunzow JR, Guan H-C, et al. Cloning of the gene for a human dopamine D_4 receptor with high affinity for the antipsychotic clozapine. Nature 1991;350:610–14.
51. McDonald WM, Sibley DR, Kilpatrick BF, et al. Dopaminergic inhibition of adenylate cyclase correlates with high affinity agonist binding to anterior pituitary D2 dopamine receptors. Mol Cell Endocrinol 1984;36:201–9.
52. George SR, Watanabe M, Di Paolo T, et al. The functional state of the dopamine receptor in the anterior pituitary is in the high affinity form. Endocrinology 1985;117:690–7.
53. Starke K, Reimann W, Zumstein A, et al. Effect of dopamine receptor agonists and antagonists on release of dopamine in the rabbit caudate nucleus in vitro. Naunyn Schmiedebergs Arch Pharmacol 1978;305:27–36.
54. Richfield EK, Young AB, Penney JB. Properties of D2 dopamine receptor autoradiography: high percentage of high-affinity agonist sites and increased nucleotide sensitivity in tissue sections. Brain Res 1986;383:121–8.
55. Wreggett KA, Seeman P. Agonist high- and low-affinity states of the D2 dopamine receptor in calf brain: partial conversion by guanine nucleotide. Mol Pharmacol 1984;25:10–17.
56. Seeman P, Ulpian C, Wreggett KA, et al. Dopamine receptor parameters detected by ^{3}H-spiperone depend on tissue concentration: analysis and examples. J Neurochem 1984;43:221–35.

57. Seeman P, Van Tol HHM. Deriving the therapeutic concentrations for clozapine and haloperidol: The apparent dissociation constant of a neuroleptic at the dopamine D2 or D4 receptor varies with the affinity of the competing radioligand. Eur J Pharmacol – Mol Pharmacol Section 1995;291:59–66.
58. Hagberg G, Gefvert O, Bergström M, et al. N-[^{11}C]methylspiperone PET, in contrast to [^{11}C]raclopride, fails to detect D_2 receptor occupancy by an atypical neuroleptic. Psychiatry Res 1998;82:147–60.
59. Lee T, Seeman P, Tourtellotte WW, et al. Binding of ^{3}H-neuroleptics and ^{3}H-apomorphine in schizophrenic brains. Nature 1978;274:897–900.
60. Lee T, Seeman P. Elevation of brain neuroleptic/dopamine receptors in schizophrenia. Am J Psychiat 1980;137:191–7.
61. Seeman, P. Dopamine receptors and the dopamine hypothesis of schizophrenia. Synapse 1987;1:133–52.
62. Seeman P, Ulpian C, Bergeron C, et al. Bimodal distribution of dopamine receptor densities in brains of schizophrenics. Science 1984;225:728–31.
63. Seeman P, Guan H-C. Dopamine partial agonist action of (-)OSU6162 is consistent with dopamine hyperactivity in psychosis. Eur J Pharmacol 2007;557:151–3.
64. Patil ST, Zhang L, Martenyi F, et al. Activation of mGlu2/3 receptors as a new approach to treat schizophrenia: a randomized Phase 2 clinical trial. Nat Med 2007;13:1102–10.
65. Seeman P, Caruso C, Lasaga M. Dopamine partial agonist actions of the glutamate receptor agonists LY 354,740 and LY 379,268. Synapse 2008;62:154–8.
66. Hirvonen J, van Erp TG, Huttunen J, et al. 2005. Increased caudate dopamine D_2 receptor availability as a genetic marker for schizophrenia. Arch Gen Psychiatry 2005;62:371–8.
67. Corripio I, Perez V, Catafau AM, Mena E, Carrio I, Alvarez E: Striatal D2 receptor binding as a marker of prognosis and outcome in untreated first-episode psychosis. Neuroimage 2006;29:662–6.
68. Wong DF, Wagner HN Jr, Tune LE, et al. Positron emission tomography reveals elevated D_2 dopamine receptors in drug-naive schizophrenics. Science 1986;234:1558–63.
69. Farde L, Nordstrom AL, Wiesel F-A, et al. Positron emission tomographic analysis of central D_1 and D_2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. Relation to extrapyramidal side effects. Arch Gen Psychiatry 1992;49:538–44.
70. Farde L, Wiesel F-A, Halldin C, et al. Central D_2-dopamine receptor occupancy in schizophrenic patients treated with antipsychotic drugs. Arch Gen Psychiatry 1988;45:71–6.
71. Kapur S, Zipursky RB, Remington G. Clinical and theoretical implications of 5-HT_2 and D_2 receptor occupancy of clozapine, risperidone, and olanzapine in schizophrenia. Am J Psychiatry 1999;156:286–93.
72. Nyberg S, Farde L. The relevance of serotonergic mechanisms in the treatment of schizophrenia has not been confirmed. J Psychopharmacol 1997;11:13–4.
73. Knable MB, Heinz A, Raedler T, et al. Extrapyramidal side effects with risperidone and haloperidol at comparable D_2 receptor occupancy levels. Psychiatry Res 1997;75:91–101.
74. Seeman P, Tallerico T. Rapid release of antipsychotic drugs from dopamine D2 receptors: an explanation for low receptor occupancy and early clinical relapse upon drug withdrawal of clozapine or quetiapine. Am J Psychiat 1999;156:876–84.
75. Bressan RA, Erlandsson K, Jones HM, et al. Is regionally selective D_2/D_3 dopamine occupancy sufficient for atypical antipsychotic effect? An in vivo quantitative [^{123}I]epidepride SPEC study of amisulpride-treated patients. Am J Psychiat 2003;160:1413–20.
76. Talvik M, Nordström AL, Nyberg S, et al. No support for regional selectivity in clozapine-treated patients: a PET study with [^{11}C]raclopride and [^{11}C]FLB 457. Am J Psychiat 2001;158:926–30.
77. Kapur S, Seeman P. Does fast dissociation from the dopamine D_2 receptor explain the action of atypical antipsychotics? - A new hypothesis. Am J Psychiat 2001;158:360–9.
78. Lieberman JA, Kane JM, Alvir J. Provocative tests with psychostimulant drugs in schizophrenia. Psychopharmacology 1987;91:415–33.