Rapid Release of Antipsychotic Drugs From Dopamine D_2 Receptors: An Explanation for Low Receptor Occupancy and Early Clinical Relapse Upon Withdrawal of Clozapine or Quetiapine

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Objective: In an attempt to understand the basis of early relapse after antipsychotic withdrawal, the objective of this study was to determine whether the low occupancy of dopamine D_2 receptors by clozapine and by quetiapine, as seen by brain imaging, could arise from a rapid release of some of the D_2-bound clozapine or quetiapine by the brain imaging compounds and by the action of a physiological concentration of dopamine. **Method:** Human cloned D_2 receptors were first pre-equilibrated with the [^{3}H]antipsychotic drug, after which raclopride, iodobenzamide, or dopamine (at the physiological concentration in the synapse) was added, and the time course of release of the [^{3}H]antipsychotic from the D_2 receptor was measured. **Results:** Within 5 minutes, low concentrations of raclopride and iodobenzamide displaced appreciable amounts of [^{3}H]clozapine and [^{3}H]quetiapine from the D_2 receptors but, during the course of 1 hour, did not displace any of the other antipsychotic [[^{3}H]ligands. [^{3}H]Clozapine and [^{3}H]quetiapine, moreover, were displaced by dopamine (100 nM) at least 100 times faster than the other antipsychotic [^{3}H]ligands. **Conclusions:** Clozapine and quetiapine are loosely bound to the D_2 receptor, and the injected radioactive ligand at its peak concentration may displace some of the D_2-bound antipsychotic drug, resulting in apparently low D_2 occupancies. Therefore, under clinical brain imaging conditions with [^{11}C]raclopride, D_2 occupancies by clozapine and by quetiapine may be higher than currently estimated. These considerations may result in high levels of the D_2 receptors being occupied by therapeutic doses of clozapine or quetiapine. The rapid release of clozapine and quetiapine from D_2 receptors by endogenous dopamine may contribute to low D_2 receptor occupancy and to early clinical relapse upon withdrawal of these medications.

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In the treatment of patients with schizophrenia, withdrawal of clozapine often results in early clinical relapse with psychotic symptoms within 1–14 days (1–11). A similar clinical situation appears to occur with quetiapine (12). Ordinarily, the rate of relapse in patients who have had antipsychotic drugs withdrawn is approximately 6%–10% per month for those who have been taking traditional antipsychotics such as phenothiazines, haloperidol, loxapine, and flupenthixol (13, 14). The relapse rate for those taking clozapine or quetiapine, however, is approximately five times higher (7, 9, 12). The rapid relapse after clozapine withdrawal has been variously attributed to clozapine-induced supersensitivity of the receptors for dopamine (1, 2), acetylcholine (4, 8), or serotonin (9). The research in the present study, however, illustrates that, more than any of the other antipsychotic drugs, clozapine and quetiapine are rapidly displaced from dopamine D_2 receptors by competing compounds, including endogenous dopamine—a factor that may contribute to the early relapse of patients who have had these drugs withdrawn.

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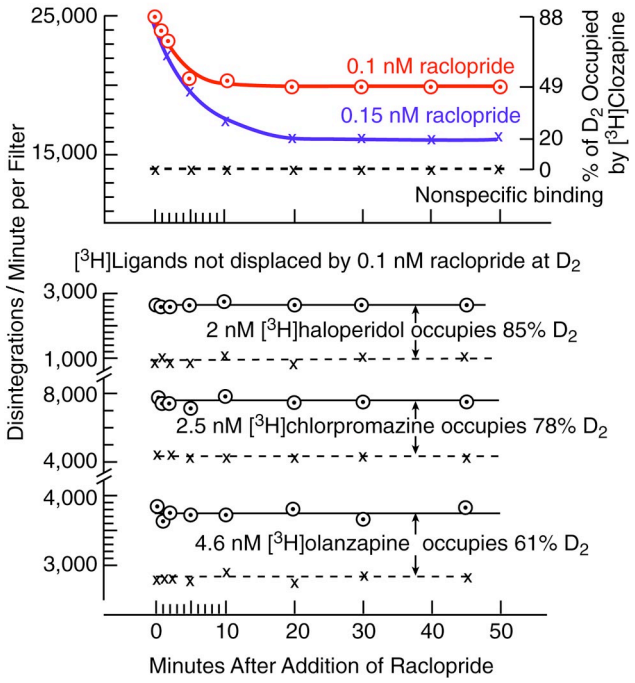
A reported feature of clozapine is that therapeutic doses apparently occupy low levels of dopamine D_2 receptors, generally between 20% and 50%, as monitored by positron emission tomography (PET) (15–18) or by single photon emission computed tomography (SPECT) (19–28). This is in contrast to all other antipsychotic drugs, which, when given at equally effective therapeutic doses, occupy between 60% and 80% of dopamine D_2 receptors, as monitored by PET (29–44) or by SPECT (19–28, 45–47). This feature of low D_2 occupancy by clozapine is also seen with quetiapine, therapeutic doses of which occupy 25%–45% of D_2 receptors (48–52).

The reports of consistently low occupancy of D_2 receptors by clozapine has, therefore, appeared to "support the hypothesis that the mechanism of action of clozapine involves other receptor systems than the D_2 subtype" (16; see also reference 44). If this were true, it would have major consequences for the search for the causes of psychosis and for the design of future antipsychotic medications. The research in the present study, however, shows that the low occupancy of D_2 receptors by clozapine or by quetiapine may be partly due to rapid displacement of some of the clozapine or quetiapine from the receptors by endogenous dopamine and by the injected [^{11}C]raclopride or [^{123}I]iodobenzamide ([^{123}I]IBZM) used in imaging the brain dopamine D_2 receptors.

FIGURE 1. Rapid Displacement by Raclopride of 310 nM [^{3}H]Clozapine From Human Cloned Dopamine D_{2long} Receptors (top)[a]



[a] Receptors had been pre-equilibrated for 1 hour with the [^{3}H]ligand. Raclopride did not displace D_2-bound [^{3}H]haloperidol, [^{3}H]-chlorpromazine, or [^{3}H]olanzapine. Nonspecific binding was defined as that in the presence of 10 μM *S*-sulpiride.

METHOD

The experiments in this study used human cloned dopamine D_{2long} receptors in *Spodoptera frugiperda* insect cells (Biosignal, Inc., Montreal) or human cloned dopamine D_{2short} receptors in mouse L cells (Biosignal, Inc.). The two types of tissue yielded identical results.

Displacement Method

The displacement of antipsychotic [^{3}H]ligands from dopamine D_2 receptors by raclopride, by iodobenzamide, or by 100 nM dopamine was done as follows. The frozen pellet of cloned D_2 receptors was resuspended in 10 ml of buffer (50 mM Tris-HCl, pH 7.4; 1 mM EDTA; 5 mM KCl; 1.5 mM $CaCl_2$; 4 mM $MgCl_2$; and 120 mM NaCl) at a final concentration of 10 μg protein/ml. The final suspension contained one of the following antipsychotic [^{3}H]ligands with or without additional nonradioactive drug added, to match the molarities of antipsychotic drug found in the spinal fluid or in the plasma water of patients: 10 nM [^{3}H]clozapine (84 Ci/mmol; New England Nuclear Corp., Boston) and 300 nM clozapine; 10 nM [^{3}H]quetiapine (14 Ci/mmol; custom-prepared by New England Nuclear Corp.) and 200 nM quetiapine; 2 nM [^{3}H]haloperidol (12 Ci/mmol; New England Nuclear Corp.); 2.5 nM [^{3}H]chlorpromazine (25 Ci/mmol; New England Nuclear Corp.); 10 nM [^{3}H]remoxipride (46 Ci/mmol; prepared in 1991 by Astra Arcus AB, Södertälje, Sweden); 4.6 nM [^{3}H]olanzapine (81 Ci/mmol; Lilly Research Laboratories, Indianapolis); 2.4 nM [^{3}H]raclopride (79 Ci/mmol; New England Nuclear Corp.); or 4.4 nM [^{3}H]sertindole (47 Ci/mmol; H. Lundbeck A/S, Copenhagen-Valby). The final suspension remained at room temperature for 1 hour to permit the [^{3}H]antipsychotic to bind to the dopamine D_2 receptors. An aliquot of 0.1 ml, containing raclopride, iodobenzamide, or dopamine, was then added to the 10-ml suspension. After rapid mixing, aliquots of 1 ml were quickly removed from the suspension at various times and immediately filtered under vacuum through presoaked glass fiber filters (Whatman GF/B; Whatman Products, Clifton, N.J.) with the use of a Millipore filter manifold. After the filters were washed rapidly with 5 ml of buffer, they were placed in scintillation minivials (Packard, Chicago) and monitored for tritium 6 hours later in a Packard 4660 scintillation spectrometer at 55% efficiency. Nonspecific binding of each antipsychotic [^{3}H]ligand was done in the presence of 10 μM *S*-sulpiride.

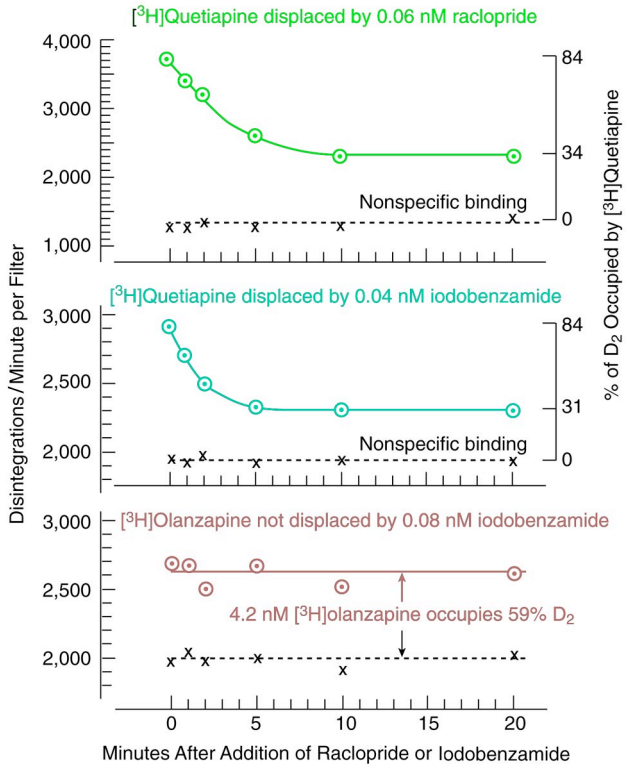
Competition Method

Raclopride and iodobenzamide were also tested for their ability to compete against [^{3}H]clozapine or [^{3}H]quetiapine by using the traditional competition equilibrium method (53, 54) as follows. Each incubation tube received 0.50 ml of buffer with varying concentrations of raclopride or iodobenzamide, 0.25 ml of [^{3}H]clozapine (final concentration of 10 nM in the presence of a final concentration of 300 nM clozapine) or [^{3}H]quetiapine (final concentration of 10 nM in the presence of a final concentration of 200 nM quetiapine), and 0.25 ml of tissue (10 μg protein per tube). After the tubes were incubated at room temperature for 2 hours, the incubates were filtered by means of a 12-well cell harvester (Titertek, Skatron, Lier, Norway) and buffer-presoaked glass fiber filter mats (number 7034, Skatron, Sterling, Va.). After filtering of the incubates, the filter mat was rinsed with 7.5 ml buffer for 15 seconds. The filters were pressed out, placed in scintillation minivials (Packard), and monitored for tritium as described above. Nonspecific binding was defined as the binding that occurred in the presence of 10 μM *S*-sulpiride.

RESULTS

Previous work (53, 54) had shown that clozapine and quetiapine have low affinity for D_2 receptors—lower than any other antipsychotic drug—suggesting

FIGURE 2. Rapid Displacement by Raclopride and by Iodobenzamide of 210 nM (Including up to 410 nM) [^{3}H]Quetiapine From Human Cloned Dopamine D_{2long} Receptors (top and middle)[a]



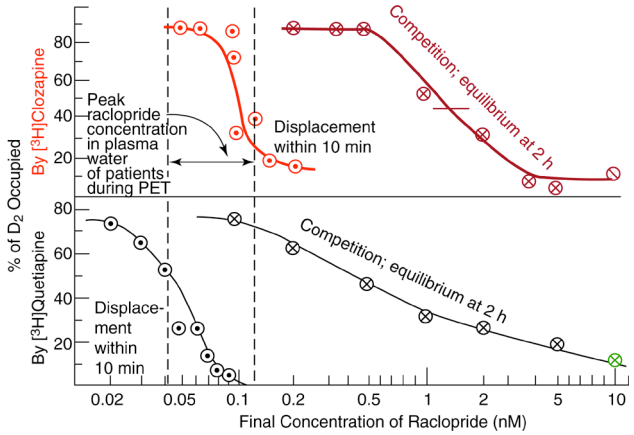
[a] Receptors had been pre-equilibrated for 1 hour with the [^{3}H]ligand. Raclopride did not displace D_2-bound [^{3}H]olanzapine or any of the other antipsychotic [^{3}H]ligands. Nonspecific binding was defined as that in the presence of 10 μM *S*-sulpiride.

that the low affinity and loose binding of these drugs at D_2 may cause them to be readily displaced from D_2 by other drugs, including endogenous dopamine. This was directly examined, therefore, by using [^{3}H]clozapine, [^{3}H]quetiapine, raclopride, and dopamine.

Raclopride Displacement of [^{3}H]Clozapine and [^{3}H]Quetiapine

Raclopride readily displaced 310 nM [^{3}H]clozapine within 5 minutes, but it did not displace D_2-bound [^{3}H]haloperidol (2 nM), [^{3}H]chlorpromazine (2.5 nM), or [^{3}H]olanzapine (4.6 nM), as shown in figure 1. The molarities chosen for each of these [^{3}H]ligands are those which are found in the spinal fluid or in the plasma water of patients receiving therapeutic maintenance doses of antipsychotic drugs (53, 54). The percent occupancy of D_2 by each [^{3}H]ligand was calculated as C/(C + K), where C was the [^{3}H]ligand concentration, and K was the real binding constant (independent of the radioligand used [53, 54]) for the antipsychotic drug (44 nM for clozapine, 78 nM for quetiapine, 0.375 nM for haloperidol, 0.7 nM for chlorpromazine, and 2.9 nM for olanzapine [53, 54]). Raclopride did not displace [^{3}H]sertindole, [^{3}H]raclopride, or [^{3}H]remoxipride (data not shown).

FIGURE 3. Displacement by Raclopride Concentrations Between 0.07 and 0.15 nM of D_2-Bound [^{3}H]Clozapine (310 nM) Within 10 Minutes[a]



[a] Lower concentrations of raclopride (0.03–0.09 nM) displaced D_2-bound [^{3}H]quetiapine (210 nM) within 10 minutes. Much higher concentrations of raclopride were needed to compete with these ligands when the conventional competition method was used. The dashed vertical lines indicate the raclopride concentration range occurring in the plasma water of patients undergoing positron emission tomography.

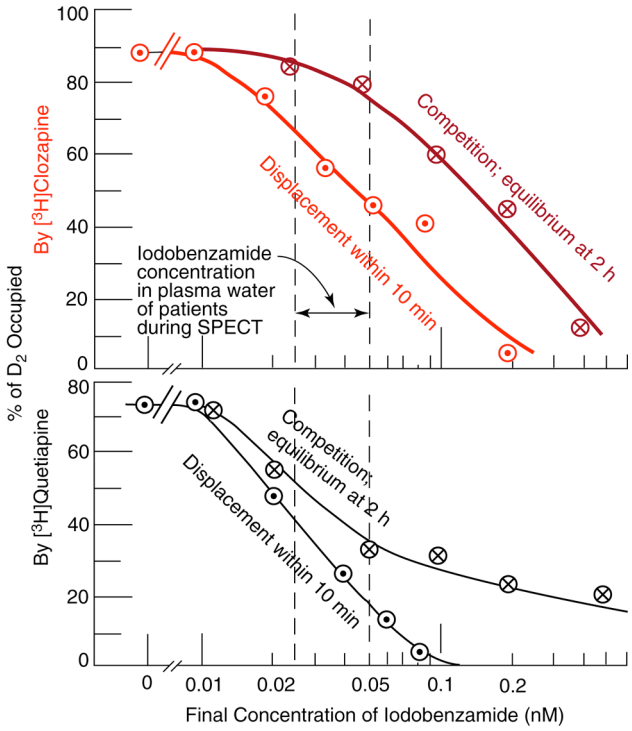
Within minutes, raclopride also readily displaced 210 nM [^{3}H]quetiapine (figure 2, top), including up to 410 nM (data not shown).

A full range of raclopride concentrations was tested on D_2-bound [^{3}H]clozapine and [^{3}H]quetiapine. Raclopride concentrations between 0.07 and 0.15 nM displaced D_2-bound [^{3}H]clozapine (310 nM) within 10 minutes (figure 3). Lower concentrations of raclopride (0.03– 0.09 nM) displaced D_2-bound [^{3}H]quetiapine (210 nM) within 10 minutes. (In order to simulate the PET procedure, [^{3}H]raclopride was added to D_2 receptors that had been pre-incubated with either clozapine or haloperidol at concentrations that bound to the same proportion of D_2; the amount of [^{3}H]raclopride binding, however, was consistently higher in the presence of clozapine [data not shown], suggesting that clozapine, not haloperidol, was partly replaced by [^{3}H]raclopride.) Much higher concentrations of raclopride were needed to compete with these ligands when the conventional competition equilibrium method was used (where the tissue was added to tubes containing the ligand and the competing drug) (figure 3).

Iodobenzamide Displacement of [^{3}H]Clozapine and [^{3}H]Quetiapine

A similar situation occurred with iodobenzamide, which displaced D_2-bound [^{3}H]clozapine and D_2-bound [^{3}H]quetiapine within minutes (figure 2, middle), but which did not displace any of the other [^{3}H]ligands (e.g., [^{3}H]olanzapine, figure 2, bottom). A full range of iodobenzamide concentrations was tested on D_2-bound [^{3}H]clozapine and on D_2-bound [^{3}H]quetiapine. Iodobenzamide concentrations between 0.02 and 0.1 nM displaced D_2-bound [^{3}H]clozapine (310 nM) as well as

FIGURE 4. Displacement by Iodobenzamide Concentrations Between 0.02 and 0.1 nM of D_2-Bound [^{3}H]Clozapine (310 nM) and D_2-Bound [^{3}H]Quetiapine (210 nM) Within 10 Minutes[a]



[a] Somewhat higher concentrations of iodobenzamide were needed to compete with these ligands when the conventional competition method was used. The dashed vertical lines indicate the iodobenzamide concentration range occurring in the plasma water of patients undergoing single photon emission computed tomography.

D_2-bound [^{3}H]quetiapine (210 nM) within 10 minutes (figure 4). Somewhat higher concentrations of iodobenzamide were needed to compete with these ligands when the conventional competition method was used.

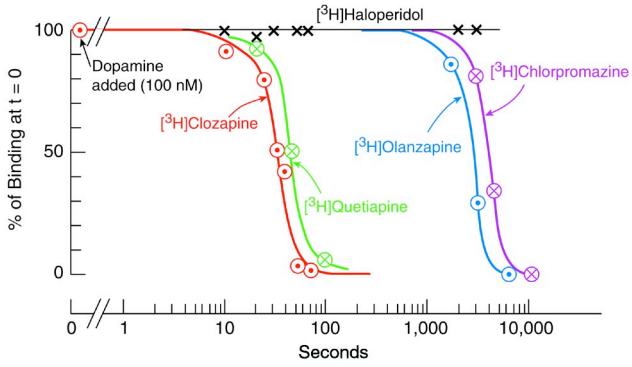
Dopamine Displacement of [^{3}H]Clozapine and [^{3}H]Quetiapine

A physiological concentration of dopamine, 100 nM, mimicking the average concentration of dopamine in the synapse (see Discussion), displaced D_2-bound [^{3}H]clozapine or [^{3}H]quetiapine about 100 times more quickly than [^{3}H]chlorpromazine, [^{3}H]haloperidol, or [^{3}H]olanzapine (figure 5).

DISCUSSION

The main finding was that low concentrations of raclopride, iodobenzamide, or dopamine could (within minutes) displace some of the [^{3}H]clozapine or [^{3}H]quetiapine prebound to D_2 receptors (figures 3–5), in contrast to [^{3}H]haloperidol and other [^{3}H]antipsychotic drugs. This finding is relevant and applicable to clinical brain imaging as follows. The amount of [^{11}C]raclopride injected into human volunteers for determining

FIGURE 5. Rapid Displacement of [^{3}H]Clozapine and [^{3}H]Quetiapine by Dopamine at D_2 Receptors[a]



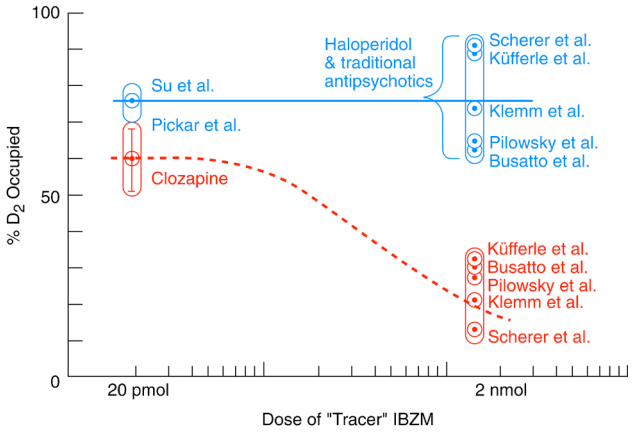
[a] The addition of 100 nM dopamine displaced [^{3}H]clozapine and [^{3}H]quetiapine from human cloned D_2 receptors about 100 times more quickly than [^{3}H]haloperidol, [^{3}H]olanzapine, and [^{3}H]chlorpromazine.

D_2 occupancy by PET is between 15 and 100 nmol, with an average of 36 nmol (A. Wilson and S. Kapur, personal communication, and references 55–59). This average dose, when rapidly distributed in 5 liters of blood, yields a peak molarity in plasma of the order of 7 nM, with a final free concentration in the plasma water of the order of 0.03–0.3 nM raclopride (see references 31, 53, and 54 for data on free molarities of antipsychotic drugs). This concentration range of raclopride may displace some of the clozapine bound to the D_2 receptors in a human subject's striatum within 10 minutes, at which time the brain imaging measurements are started. As shown in figures 1 and 2, the effect of raclopride was long-lasting and persisted beyond 1 hour. (Under clinical conditions, of course, the patient subsequently resumes his or her medication [clozapine] after brain imaging, and any clozapine-displacing action of the injected raclopride dissipates as the raclopride is removed from the body.)

In particular, for example, the peak [^{11}C]raclopride in striatum corresponds to about 0.005% of the injected radioactivity per gram of striatum (60). Taking the concentration of [^{11}C]raclopride in the cerebellum (F*, according to the terminology of reference 29) as 0.0015% of the injected radioactivity per gram of striatum (at the time of peak striatal activity) as a rough measure of the "nonspecifically bound" tracer in striatum, one arrives at 0.0035% of the injected radioactivity per gram of striatum for the specifically bound [^{11}C]raclopride (B*, according to the terminology of reference 29). Hence, as obtained by Volkow et al. (60), the ratio B*/F* is 2.3, in general agreement with the average value of 3.55 (SD=0.63) obtained by Farde et al. (29) for 15 drug-naive patients. Furthermore, as defined previously (29), receptor occupancy in a drug-treated patient is expressed as "the percent reduction of B*/F* from the value of 3.55."

Hence, if 36 nmol of [^{11}C]raclopride are injected, the concentration in the striatum will be about 1 pmol/

FIGURE 6. Dependence of the Clozapine Occupancy of Dopamine D_2 Receptors in Humans on the Amount of [^{123}I]Iodobenzamide Injected[a]



[a] Low occupancies of D_2 by clozapine were found by Küfferle et al. (51), Busatto et al. (20), Pilowsky et al. (21), Klemm et al. (22), and Scherer et al. (19), using [^{123}I]iodobenzamide ([123I]IBZM) admixed with nonradioactive iodobenzamide, such that between 1 and 2 nmol of [^{123}I]IBZM were injected into each volunteer. According to figure 4, these occupancies might be underestimated. Pickar et al. (70) and Su et al. (27), however, used [^{123}I]IBZM that was carrier-free, thus yielding D_2 occupancies by clozapine approaching 80%.

gram, in agreement with values reported earlier (61). If it is assumed that the concentration of the D_2 receptors in the human striatum is between 20 and 28 pmol/gram (62–64), the injected [^{11}C]raclopride will occupy between 3% and 5% of the total population of D_2 receptors. Thus, for example, an injection of 36 nmol of [^{11}C]raclopride might result in 1 pmol/gram, or 3%, of the total D_2 population being occupied in a drug-free subject. This control value of 1 pmol/gram, or 3% of total D_2, would be designated as "0% occupancy" (29). For an individual taking a tightly bound antipsychotic drug such as haloperidol, however, the injection of the 36 nmol of [^{11}C]raclopride would result in less specific binding of the [^{11}C]raclopride, with an observed B*/F* value of, say, 1.1 instead of 3.55 (56). This fall in B*/F* would correspond to a D_2 occupation by [^{11}C]raclopride of 0.3 pmol/gram, or 1% of total D_2. Hence, the drop from 3% to 1% of total D_2 would indicate that haloperidol occupied about 67% of the D_2 population.

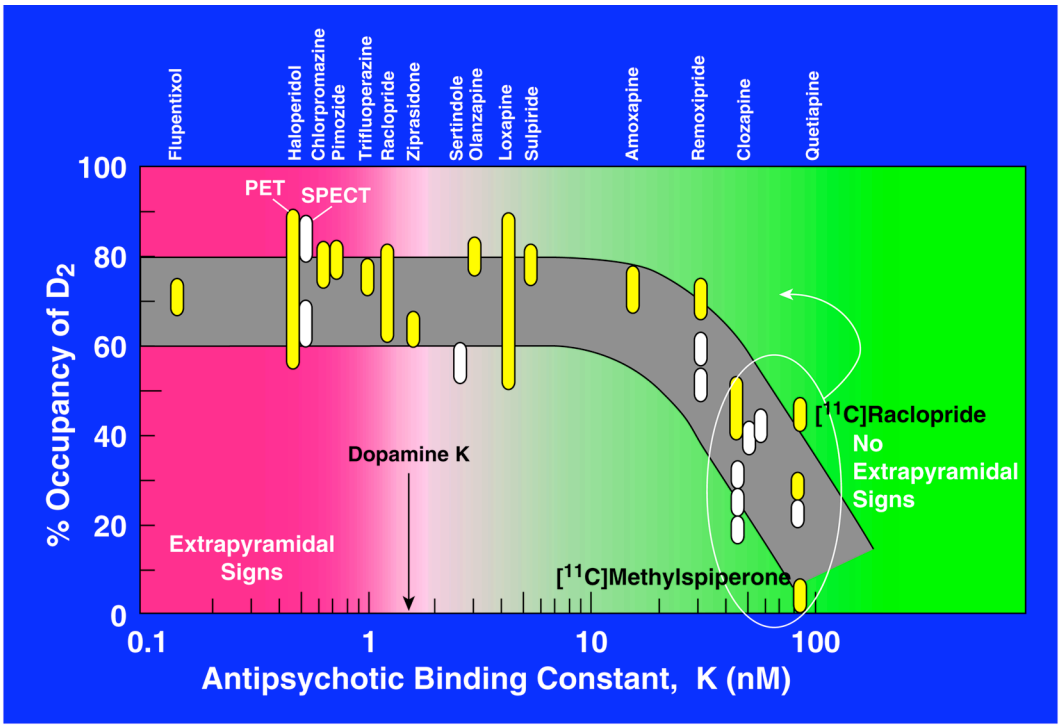
However, in the case of a patient taking clozapine (or quetiapine), the injection of 36 nmol of [^{11}C]raclopride (with a specific activity of 500–1,000 Ci/mmol) would displace some of the D_2-bound clozapine, resulting in a value for B*/F* that would be slightly higher than expected for the true occupancy by clozapine. For example, if clozapine tightly occupied 67% of the D_2 receptors, then one would observe the same data that were just mentioned for haloperidol, namely, an occupation of 1% of total D_2 by [^{11}C]raclopride. However, if some of the D_2-bound clozapine is displaced by [^{11}C]raclopride, the occupation by [^{11}C]raclopride will not be as low as 1% of the total density of D_2 receptors. For example, if such a displacement results in 2% of the total D_2 being occupied by [^{11}C]raclopride (compared to the control value of 3% of the total D_2 density), the apparent occupancy by clozapine would be seen to be only 33% instead of the expected 67%.

A similar situation obtains with [^{123}I]IBZM, used in SPECT, where between 1 and 2 nmol are injected. For example, European studies use [^{123}I]IBZM provided by Cygne B.V. (Eindhoven, The Netherlands) (20–22, 65), which prepares [^{123}I]IBZM by the method of Bobeldijk et al. (66). This method uses carrier-free Na^{123}I and *S*-benzamide, such that the final specific activity of *S*-[^{123}I]IBZM is the same as that of the carrier-free Na^{123}I, namely, >3000 Ci/mmol. However, as later noted by Verhoeff et al. (67), to enable the accurate measurement of the specific activity of [^{123}I]IBZM for both in vitro and in vivo studies, a small amount of carrier iodide is added to the labeling mixture, resulting in a specific activity of the prepared [^{123}I]IBZM in the range of 50–250 MBq/nmol. Thus, the Cygne B.V. specifications indicate that the [^{123}I]IBZM has 1 nmol/ml (0.4 μg/ml) of nonradioactive iodobenzamide. Usually, 185 MBq (or 5 mCi) are injected into the patient, and this dose corresponds to 1–2 nmol, depending on the time of injection. After being diluted in the bloodstream, and allowing for the fact that 4.4%–12.3% of the [^{123}I]IBZM is free in the plasma water (68, 69), the final molarity of free iodobenzamide in the plasma water of a human volunteer is of the order of 0.02 nM–0.05 nM. This concentration range would be expected to displace some of the D_2-bound clozapine or quetiapine from the human volunteer's striatum in a matter of minutes, as indicated in figure 4.

Thus, the data in figure 4 may account for the low occupancies of 12%–32% of D_2 by clozapine, as monitored by the [^{123}I]IBZM used in the European studies (19–22, 51). In other words, according to the data in figure 4, the D_2 occupancies by clozapine in these studies may be underestimated. In the United States, however, the [^{123}I]IBZM used by Pickar et al. (70) was carrier-free, yielding D_2 occupancies by clozapine approaching 80%. Hence, the clozapine occupancy of D_2 depends on the amount of [^{123}I]IBZM injected, as illustrated in the summary in figure 6.

Considering that iodobenzamide, as well as raclopride, may displace some of the D_2-bound clozapine or quetiapine in the human brain striatum, then clozapine or quetiapine may occupy more dopamine D_2 receptors in humans than are currently estimated. This is illustrated in figure 7, which summarizes the observations that all antipsychotic drugs occupy 60%–80% of D_2 receptors, except for clozapine and quetiapine. However, if allowance is made for some displacement of the D_2-bound clozapine or quetiapine by the injected radioligand, then the D_2 occupancies by clozapine and quetiapine may rise. This conclusion is the same as that derived previously by using the antipsychotic molarities in plasma water and using the ligand-independent dissociation constants of the antipsychotic drugs (54).

FIGURE 7. Summary of the Reported Ranges of D_2 Receptor Occupancy by Maintenance Doses of Antipsychotic Drugs in Schizophrenic Patients, as Measured by PET (Yellow Ovals) and by SPECT (White Ovals)[a]



[a] All antipsychotic drugs occupy 60%–80%, with the exception of clozapine and quetiapine, which occupy 20%–50% when [^{11}C]raclopride is used but less when [^{11}C]methylspiperone is used. When it is considered that the injected [^{11}C]raclopride or [^{123}I]iodobenzamide can displace some of the D_2-bound clozapine or quetiapine, then both clozapine and quetiapine may occupy higher levels of the D_2 receptors in humans under therapeutic conditions, as indicated by the arrow. The dissociation constant (K) for dopamine at the high-affinity state of the dopamine D_2 receptor is 1.6 nM. Antipsychotic drugs with a K value higher than 1.6 nM usually elicit fewer extrapyramidal signs. The horizontal axis gives the ligand-independent dissociation constants of the antipsychotic drugs (54). The references for the drug studies and the number of patients in each study are as follows: flupenthixol (15), N=2; haloperidol (15), N=2; (25), N=7; (36), N=12; (38), N=7; (45), N=5; (51), N=8; chlorpromazine (15), N=1; pimozide (15), N=1; trifluoperazine (15), N=1; raclopride (32), N=13; ziprasidone (39), N=7; (58), N=6; sertindole (23), N=6; (26), N=6; olanzapine (43), N=12; loxapine (40), N=7; (42), N=10; sulpiride (15), N=1; remoxipride (15), N=1; (20), N=4; (22), N=4; clozapine (15), N=5; (19), N=9; (20), N=10; (21), N=10; (22), N=6; (24), N=7; (25), N=5; (26), N=4; (27), N=4; (37), N=9; (51), N=6; quetiapine (25), N=4; (48), N=8; (49), N=10; (51), N=4.

A particularly surprising result of this study was the observation that preexposure of the cloned D_2 receptors to clozapine caused the receptors to be more readily displaced by raclopride. As shown in figure 3, when the radioactive clozapine and the nonradioactive raclopride were added simultaneously (as is done in the traditional competition-type method), the K value (dissociation constant) of raclopride was about 1 nM, in agreement with previous data in vitro (53, 54) as well as in vivo in human positron tomography. (Although the raclopride K value in the striatum of human volunteers is between 7.7 and 9.1 nmol/gram of striatum [64, 71], these values convert to 1.5–1.8 nM, given that the tissue/buffer partition coefficient for raclopride is 5 [nmol/gram]/[nmol/ml] [53, 72–74], and L. Farde, personal communication].) However, when the cloned D_2 receptors were preexposed to clozapine for 1 hour, raclopride displaced 50% of bound radioactive clozapine at 0.1 nM (figure 3). When the preexposure was shortened to 10 minutes, the results were the same as the results when the competition method was used (data not shown). In other words, the prolonged exposure of the cloned D_2 receptors to clozapine for 1 hour increased the D_2 affinity for raclopride. Although the molecular basis of this effect is not known, G protein-linked receptors can interact and cooperate to exist as dimers or multimers with multiple states of affinity for an antagonist, as found for muscarinic receptors (75, 76) and dopamine D_2 receptors (77). It is possible, therefore, that preexposure of D_2 receptors to clozapine may alter the affinity state of D_2 for raclopride, but such speculation must be directly tested in future experiments.

The present data (figures 1–5) are consistent with the time course of D_2 occupancy measured clinically. For example, whereas the D_2 occupancy by haloperidol in patients is long-lasting (78), the D_2 occupancy by clozapine or by quetiapine in patients continues only for a matter of hours (7, 50).

Although the data from this study show that clozapine and quetiapine are readily released from D_2, these data do not reveal the time course of release of these antipsychotic drugs from D_2 under ordinary clinical conditions (i.e., in the absence of brain imaging experiments). However, the data in figure 5, showing the

rapid displacement of clozapine from D_2 by 100 nM dopamine (which is the average level of dopamine in the synaptic space [79]) is consistent with the hypothesis that these antipsychotic drugs are displaced from the patient's D_2 receptors by endogenous dopamine (reviewed in reference 54), thereby contributing to the early reemergence of psychotic symptoms (1–11).

Additional data (not shown) revealed that an extremely high concentration of dopamine (30 μM) displaced 50% of [^{3}H]quetiapine in 4 seconds, 50% of [^{3}H]clozapine in 9 seconds, and 50% of [^{3}H]haloperidol in 7 minutes; the result with [^{3}H]haloperidol is in excellent agreement with the previously known value of 7.5 minutes for the time needed for 50% dissociation of haloperidol from the D_2 receptor (80). Lower concentrations of dopamine displace [^{3}H]clozapine more slowly, as just mentioned. The physicochemical factors determining the "loose binding" properties of quetiapine and clozapine are not known, although, presumably, the oil/water (or membrane/buffer) partition coefficients of these drugs are important.

Remoxipride has a K value of 30 nM, close to the values of 44 nM for clozapine and 78 nM for quetiapine (figure 7), yet it occupies about 70% of D_2 (56). As summarized in figure 7, however, it appears that the region between 30 and 40 nM for the antipsychotic dissociation constant is a transition region, above which displacement of the antipsychotic drug appears to occur, but below which displacement of the antipsychotic does not appear to occur. Melperone, however, has a dissociation constant of about 50 nM (54) yet occupies 70% of D_2 receptors (56). One of the problems in analyzing the action of melperone is that it has an active metabolite, FG5155 (81), which is also an antagonist at D_2 receptors (unpublished data), complicating the interpretation of the pharmacology involved.

The present data, indicating that [^{3}H]clozapine binds loosely to the D_2 receptor, are also consistent with the observation that clozapine occupies more D_2 receptors in the cerebral cortex of patients than in the striatum (82). That is, the higher output of endogenous dopamine in the striatum readily displaces more D_2-bound clozapine in the striatum, compared with the lower output of dopamine in the cerebral cortex (54).

The summary in figure 7 omits the important drug risperidone. The risperidone dose range of 2–6 mg/day occupies between 60% and 80% of D_2 receptors, with "mild" parkinsonism occurring in patients taking 6 mg/day and having D_2 occupancies higher than 75% (35, 37, 44, , 83, 84). Such extrapyramidal signs, however, are generally low or negligible in patients taking 2–4 mg/day of risperidone and having D_2 occupancies between 60% and 75% (35, 37, 44, , 83, 84).

The observations in the present study (figures 1–4) also illustrate the principle that D_2 occupancy by an antipsychotic drug depends on the radioligand used (53, 54). Other studies have also shown that a radioligand should have a lower affinity than the drug for which a site's occupancy is being probed (85–88). PET receptor occupancy studies are relevant to clinical realities, but technical aspects must be recognized before results can be usefully interpreted.

In conclusion, with respect to the apparently low D_2 occupancy of clozapine, it may not be that "less is better" for the patient (89), but rather that "loose is better."

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