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Dopamine D_2 and D_3 Receptor Occupancy in Normal Humans Treated with the Antipsychotic Drug Aripiprazole (OPC 14597): A Study Using Positron Emission Tomography and [^{11}C]Raclopride

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Aripiprazole (OPC 14597) is an antipsychotic drug that has high affinity for dopamine D_2 and D_3 receptors and the dopamine autoreceptor. It is being developed for treatment of patients with schizophrenia. The purpose of this study was to determine whether a dose response following graduated doses of aripiprazole could be quantified and correlated with its occupancy of the D_2 and D_3 dopamine receptors in the brain of living humans. Dopamine D_2 and D_3 receptor occupancy in fifteen normal male human brains was measured using positron emission tomography (PET) with [^{11}C]raclopride. PET studies were performed before and after two weeks of administration of aripiprazole. The dopamine D_2 receptor occupancy was quantified with two kinetic modeling methods without using a blood input function. Administration of aripiprazole for 14 days resulted in a dose-dependent receptor occupancy between 40 – 95% after the administration of 0.5mg, 1 mg, 2 mg, 10 mg, and 30 mg per day. These results suggest that an adequate occupancy can be obtained, and this may be useful to predict an appropriate therapeutic dose for an individual patient. Interestingly, even at striatal D_2 receptor occupancy values above 90%, which occurred with the higher doses, extrapyramidal side effects (EPS) were not observed. This underlines aripiprazole's unique mechanism of action as a partial dopamine receptor agonist, which might become a novel principle in the treatment of schizophrenia.

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Aripiprazole is a drug currently under development by Otsuka America Pharmaceutical, Inc., and Bristol-Myers Squibb Co. for the treatment of schizophrenia. It is a quinolinone derivative that appears to have a mechanism of action differing from currently marketed typical and atypical antipsychotics. Biochemically, aripipra-

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zole has been shown to be a partial agonist at the D_2 dopamine receptor (Lawler et al. 1999). In vivo, aripiprazole has been shown to exhibit antagonist properties in animal models of dopaminergic hyperactivity (e.g., blockade of apomorphine-induced stereotypy; Kikuchi et al. 1995) and agonist activity in an animal model of dopaminergic hypoactivity (blockade of increased dopamine synthesis in reserpine-treated rats; Kikuchi et al. 1995). These in vivo properties are consistent with the biochemical characterization of partial agonist activity. In addition, aripiprazole has been shown to exhibit partial agonist properties, ex vivo, via the inhibition of spontaneous prolactin release from isolated rat anterior pituitary slices (Inoue et al. 1996) and does not appear to upregulate D_2 receptors following repeated administration (21 days; Inoue et al. 1997). In cloned transfected cell lines, aripiprazole exhibits high affinity for D_2 (K_i = 0.8 nM) and D_3 dopamine receptors (K_i = 13 nM), a lower affinity for the D_4 receptor (K_i of approximately 200 nM), and negligible affinity for D_1-like dopamine receptors.

With regard to clinical effects, aripiprazole appears to be an efficacious and well-tolerated antipsychotic therapy. In the double-blind Phase 2 and 3 studies comparing aripiprazole against placebo and haloperidol, the 15-, 20-, and 30-mg doses of aripiprazole demonstrated significant superiority over placebo ($p < .05$), whereas, in general, aripiprazole was comparable to haloperidol and risperidone with regard to efficacy. The 2- and 10-mg doses were generally less efficacious on some measures. In three studies, aripiprazole demonstrated superior efficacy against negative symptoms compared with placebo. Aripiprazole appeared to be better tolerated than its active competitors, with extrapyramidal side effects (EPS) and prolactin plasma levels not differing from placebo (Carson et al. 2000; Daniel et al. 2000; Kane et al. 2000; Petrie et al. 1997).

Aripiprazole also has the most potent affinity to the presynaptic dopamine autoreceptor when compared with other neuroleptics available for common clinical use. The autoreceptor responds to transmitter molecules released from the same neuron. Aripiprazole acts as a partial agonist (Burris et al. 2000). Functionally, most autoreceptors appear to regulate transmitter release in such a way that the released transmitter, acting on autoreceptors, regulates additional release. The inhibitory action of the dopamine autoreceptor is mediated by exertion of negative feedback control on transmitter release. The dopamine autoreceptor occupancy by aripiprazole may affect the postsynaptic dopamine receptor regulation through a change in negative feedback of intrasynaptic dopamine release (Gründer 1993; Gründer et al. 1995).

There is a significant correlation between the binding of neuroleptics to D_2 receptors in animal brain and their antipsychotic effect in humans (Creese et al. 1996; Peroutka and Snyder 1980; Seeman et al. 1976). Potential effective therapeutic doses of a given drug may be determined based on its in vivo D_2 receptor occupancy (Smith et al. 1988; Farde et al. 1988; 1992; Bench et al. 1993; Kapur et al. 1996; 1999; Hirschowitz et al. 1997). In this, positron emission tomography (PET) study, we used [^{11}C]raclopride ([^{11}C]RAC) to measure the D_2 receptor occupancy of aripiprazole in the corpus striatum in fifteen normal male control subjects. PET studies were performed at baseline and after the administration of 5 different oral doses of aripiprazole for 14 days. The data was analyzed with two different quantitative procedures for occupancy estimation.

METHODS

Subjects

Fifteen normal male volunteers (mean +/− SD age: 32 ± 9 years old) participated in the study. All subjects gave written informed consent. The ethics committee of Johns Hopkins University approved the protocol. The Food and Drug Administration (FDA) has approved [^{11}C]RAC and aripiprazole for administration to humans.

Aripiprazole was administered orally once per day in the morning for 14 days. Four subjects received 30 mg, two subjects received 10 mg, three subjects received 2 mg, three received 1 mg, and three received 1/2 mg.

Assay of Aripiprazole in Plasma

The blood for aripiprazole plasma concentration assay was taken just before administration of the last dose (time zero), and at 3.5, 4.5, 5, 5.5, 24, 48, 72, 96, 120, and 180 h after day 14 administration. After blood centrifugation, the plasma samples were analyzed for OPC 14597, using a reverse phase HPLC system with ultraviolet detection.

Synthesis of [^{11}C]RAC

[^{11}C]RAC was synthesized in the laboratory of nuclear chemistry at Johns Hopkins Medical Institutions by O-alkylation of a phenolic (S)-precursor of raclopride with [^{11}C]methyl iodide. [^{11}C]methyl iodide was prepared from [^{11}C] carbon dioxide with $LiAlH_4$, tetrahydrofuran and hydriodic acid. [^{11}C]carbon dioxide was produced by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction. The radiotracer was purified using a reversed phase semipreparative column and the final specific activity was 1520–2990 mCi/μmol. This synthesis procedure was slightly modified from the original method of Ehrin (Ehrin et al. 1985).

PET Procedure

Dynamic PET scans were performed using a GE 4096 Plus PET system. On the 4096+ attenuation correction is

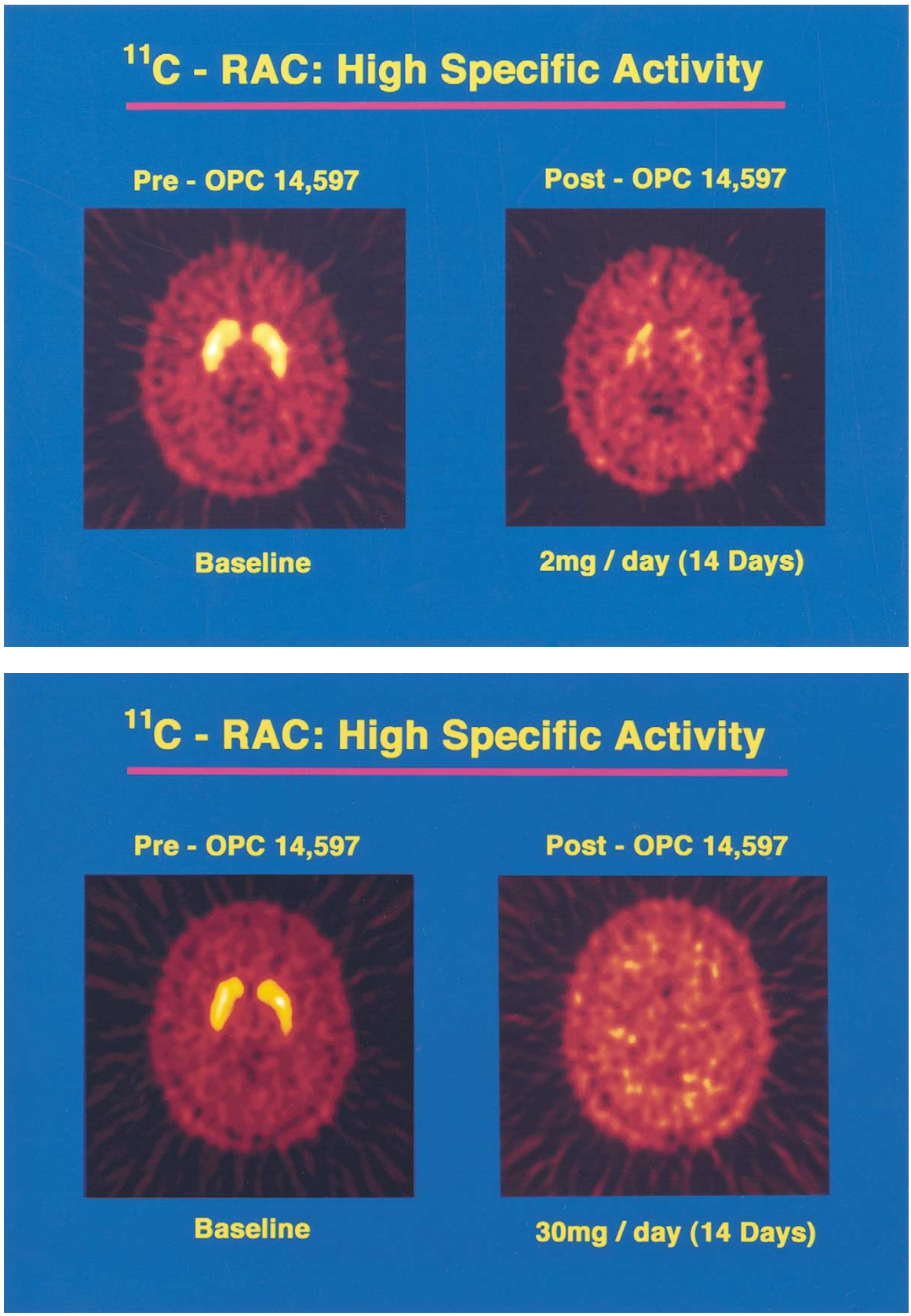

Figure 1. (Top) Typical [^{11}C]raclopride brain positron emission tomography (PET) images before and after 2 mg of aripiprazole (OPC 14597). (Bottom) Typical [^{11}C]raclopride brain PET images before and after 30 mg of aripiprazole (OPC 14597).

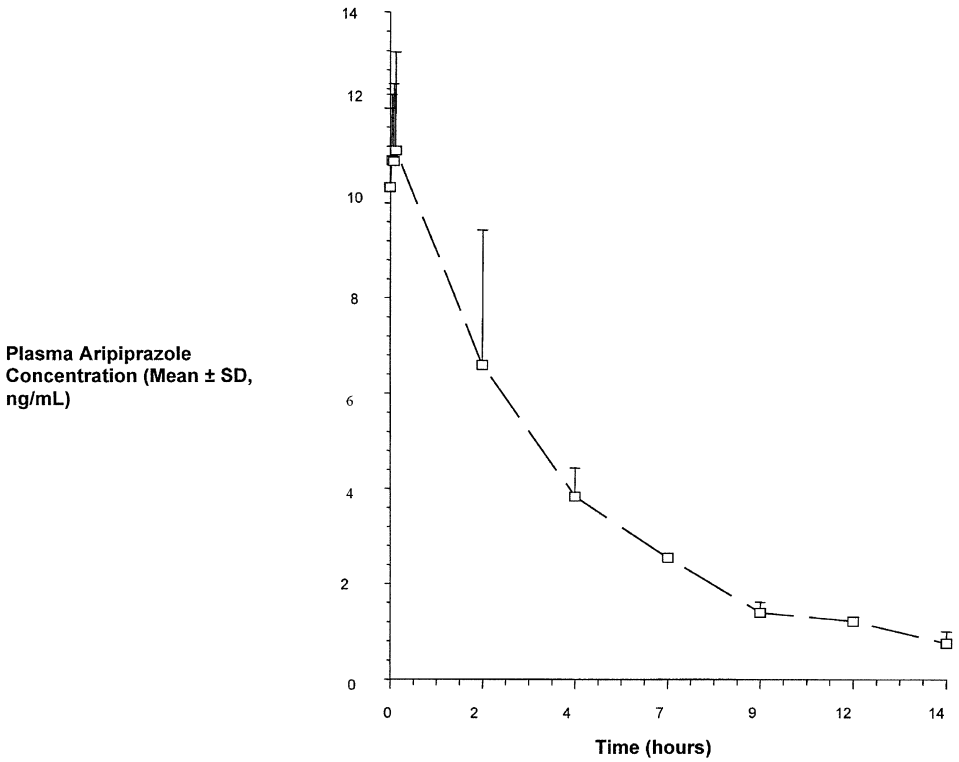

Figure 2. The mean ± SD plasma aripiprazole concentration-time profile for day 14 following 10 mg once daily administration.

carried out by measured transmission scan obtained in 2D. The 2D transmission data are directly used to correct the 2D emission data. Scatter correction is achieved by the convolution-subtraction method of Bergstrom (Bergstrom et al. 1983). Twenty mCi (740 MBq) of [^{11}C] RAC was injected intravenously over 30–40 s. The total acquisition time of the scan was 90 min. The duration of acquisition was 15 s in the first eight, 30 s in the following sixteen, 60 s in six, 120s in the following six, then 240 s in the next eleven, and 360 s in the last three PET frames.

Regions of interest (ROIs) were drawn on the first and last 10 summed PET images. ROIs were placed on the right and left caudate nuclei, putamen, and cerebellar cortices. Their dimensions were 4 × 4 pixels, 8 mm × 8 mm × 6 mm for the striatal structures, and 7 × 5 pixels, 14 mm × 10 mm × 6 mm for the cerebellar cortices. With regard to ROIs, small structures were drawn particularly on the cerebellum to ensure that regions were placed well within the gray matter. The precautions taken to ensure regions were placed in the same position were that both pre- and post-drug images were coregistered to one another, and the regions were adjusted only if there was significant patient movement. Because the thermal plastic mask with laser alignment was employed in most cases, the re-registration of the individual between PET scans (between control and blocked PET scan) was usually within a few millimeters. Time-activity curves (TACs) were obtained for the ROIs described above, and decay corrected for the time after tracer injection. These brain TACs were used for the kinetic modeling described below.

Calculation of D_2 Receptor Occupancy after Aripiprazole Administration

Two quantitative procedures were applied to calculate dopamine D_2 receptor occupancy, both using the cerebellum as a reference region to estimate the free [^{11}C] RAC concentration.

The first procedure was performed using the method of Farde (Farde et al. 1989; 1992).

Dopamine D_2 receptor occupancy (D_{2occ}) was expressed as percent change in the ratio of the *bound (B)* and the *free (F)* components of [^{11}C]RAC, obtained before and after the drug administration as expressed in the following equation:

$$D_2occ = \frac{(B/F)_{pre-drug} - (B/F)_{post-drug}}{(B/F)_{pre-drug}} \times 100\%$$

The ratio *(B)/(F)* of [^{11}C]RAC was obtained at the peak of the specific binding time-activity curve, which is assumed to represent transient equilibrium (Farde et al. 1989).

The specific binding in the corpus striatum was obtained by subtracting the cerebellar TAC from the fitted

TAC of the corpus striatum. This method is based on the assumption that the cerebellar compartment is equal to the free compartment, plus the nonspecifically bound compartment of the corpus striatum.

The second method used the procedure published by Lammertsma and Hume (1996). The D_{2occ} was expressed as the percentage reduction of the binding potential (BP) of [^{11}C]RAC as expressed by equation (2):

$$D2occ = \frac{(BP)_{pre-drug} - (BP)_{post-drug}}{(BP)_{pre-drug}} \times 100\%$$

A two-tissue compartment model was applied to estimate BP according to equation (3) (Lammertsma et al. 1996b):

$$C_T(t) = R_1 C_T(t) + \left\{ k_2 - \frac{R_1 k_2}{1 + BP} \right\} Cr(t) * \exp\left\{ \frac{-k_{2t}}{1 + BP} \right\}$$

where C_T is the total radioactivity concentration in brain tissue, C_r is the concentration of [^{11}C]RAC in the reference tissue (cerebellum), k_2 is the rate constant for transfer from the free compartment to plasma, BP is the binding potential, R_1 is the partition coefficient differences for target versus reference region, and t is time. The parameters R_1, k_2 and BP (k_3/k_4) were estimated with a nonlinear least square minimization procedure.

Statistical Analyses

Statistics employed in the methods section included Student's *t*-test for comparison of the occupancies between the two quantitative procedures described, a two-tailed assumption, and Pearson correlation coefficients for comparing relationships between plasma concentrations and occupancy values. Standard nonlinear regression techniques were employed as outlined in the Lammertsma method using the reference tissue calculation for binding potential (Lammertsma et al. 1996b).

RESULTS

The brain PET images before and after the administration of aripiprazole (2 mg and 30 mg) are shown in Figure 1. The mean plasma aripiprazole concentration-time profile for day 14 following 10 mg once daily is shown in Figure 2. A highly significant positive linear

Table 1. Dopamine Receptor Occupancy at Various Steady State Oral Doses

Aripiprazole Dose	Subjects	Region	D2+D3 Occ (%)	D2+D3 Occ (%) (mean ± SD)
30 mg	Subject 1	CN	92.8	
		Pu	95.2	Caudate Nucleus
	Subject 2	CN	91.7	92.3 ± 0.47
		Pu	88.5	
	Subject 3	CN	92.6	Putamen
		Pu	80.8	86.4 ± 6.9
	Subject 4	CN	92.3	
		Pu	81.2	
10 mg	Subject 5	CN	82.4	Caudate Nucleus
		Pu	80.1	85.5 ± 4.3
	Subject 6	CN	88.5	Putamen
		Pu	90.6	85.3 ± 7.4
2 mg	Subject 7	CN	71.9	
		Pu	61.0	Caudate Nucleus
	Subject 8	CN	76.9	74.3 ± 2.5
		Pu	80.2	Putamen
	Subject 9	CN	74.2	71.6 ± 9.8
		Pu	73.6	
1 mg	Subject 10	CN	39.2	
		Pu	54.2	Caudate Nucleus
	Subject 11	CN	56.5	49.1 ± 8.9
		Pu	53.7	Putamen
	Subject 12	CN	51.6	57.2 ± 5.6
		Pu	63.7	
0.5 mg	Subject 13	CN	26.8	
		Pu	28.0	Caudate Nucleus
	Subject 14	CN	41.5	30.4 ± 9.8
		Pu	45.8	Putamen
	Subject 15	CN	22.8	33.7 ± 10.5
		Pu	27.1	

The B/F ratio measured at the transient equilibrium state (Farde et al. 1989) before and after administration of aripiprazole (OPC 14597). CN (Caudate Nucleus); Pu (Putamen); Occ (Receptor Occupancy)

correlation between the oral dose and C_{max} values for day 14 could be observed ($r = 0.97$; $p < .01$).

Following the injection of [^{11}C]RAC, the radioactivity in the striatum increased rapidly and reached a peak at approximately 15 minutes after the injection. The striatal uptake of [^{11}C]RAC after aripiprazole administration was lower than striatal uptake found in the baseline PET study (Tables 1, 2). The uptake of aripiprazole (% injected dose of radiotracer) in the cerebellum was similar in the baseline study and after aripiprazole treatment with both 2 mg and 30 mg of doses (data not shown).

Individual and mean dopamine D_2 and D_3 receptor occupancy values, estimated with Farde's and Lammertsma's methods, are shown in Tables 1 and 2, respectively. The values of dopamine D_2 and D_3 occupancy, estimated with the two procedures, are similar and statistically significantly correlated (Tables 1 and 2, Figure 3; $r = 0.94$, $p < .05$). There was no significant difference in occupancy values determined with the two methods ($p = .46$). Both methods showed the well-known hyperbolic relationship between oral dose or plasma levels, respectively, of aripiprazole and striatal dopamine D_2 and D_3 receptor occupancy, with almost complete saturation of receptors occurring at peak plasma levels above 100–200 ng/ml (Figure 4).

DISCUSSION

The administration of increasing doses of aripiprazole correlated with a proportional increase in the plasma concentration of the drug and a decrease in the binding potential of [^{11}C]RAC. This indicates increasing brain dopamine D_2 and D_3 receptor blockade. Treatment with 0.5 mg of aripiprazole per day for 14 days resulted in receptor occupancy of about 40%. When the dose was increased to 30 mg per day for 14 days, the receptor occupancy increased to almost 95%. This type of pharmacokinetic data from chronic multiple doses is useful for the proper selection of a neuroleptic drug and establishing the appropriate drug dose for a specific patient. These results provide a direct correlation between the brain dopamine D_2 and D_3 receptor occupancy and

Table 2. Dopamine Receptor Occupancy at Various Steady State Oral Doses as Calculated using the Lammertsma Kinetic Model (Lammertsma and Hume 1996) before and after Administration of Aripiprazole (OPC 14597)

Aripiprazole Dose	Subjects	Region	D2+D3 Occ (%)	D2+D3 Occ (%) (mean ± SD)
30 mg	Subject 1	CN	89.5	Caudate Nucleus
		Pu	94.0	84.1 ± 11.1
	Subject 2	CN	95.5	Putamen
		Pu	92.3	93.7 ± 1.5
	Subject 3	CN	81.4	
		Pu	95.7	
	Subject 4	CN	69.9	
		Pu	92.7	
10 mg	Subject 5	CN	83.7	Caudate Nucleus
		Pu	76.5	86.3 ± 3.6
	Subject 6	CN	88.8	Putamen
		Pu	90.9	83.7 ± 10.2
2 mg	Subject 7	CN	81.8	Caudate Nucleus
		Pu	65.8	78.7 ± 3.1
	Subject 8	CN	78.7	Putamen
		Pu	78.0	71.3 ± 6.2
	Subject 9	CN	75.6	
		Pu	70.0	
1 mg	Subject 10	CN	43.7	Caudate Nucleus
		Pu	45.5	47.6 ± 4.9
	Subject 11	CN	53.0	Putamen
		Pu	56.2	53.3 ± 6.9
	Subject 12	CN	46.1	
		Pu	58.2	
0.5 mg	Subject 13	CN	33.6	Caudate Nucleus
		Pu	33.8	39.6 ± 5.7
	Subject 14	CN	44.8	Putamen
		Pu	42.7	39.0 ± 4.6
	Subject 15	CN	40.3	
		Pu	40.5	

CN (Caudate Nucleus); Pu (Putamen); Occ (Receptor Occupancy)

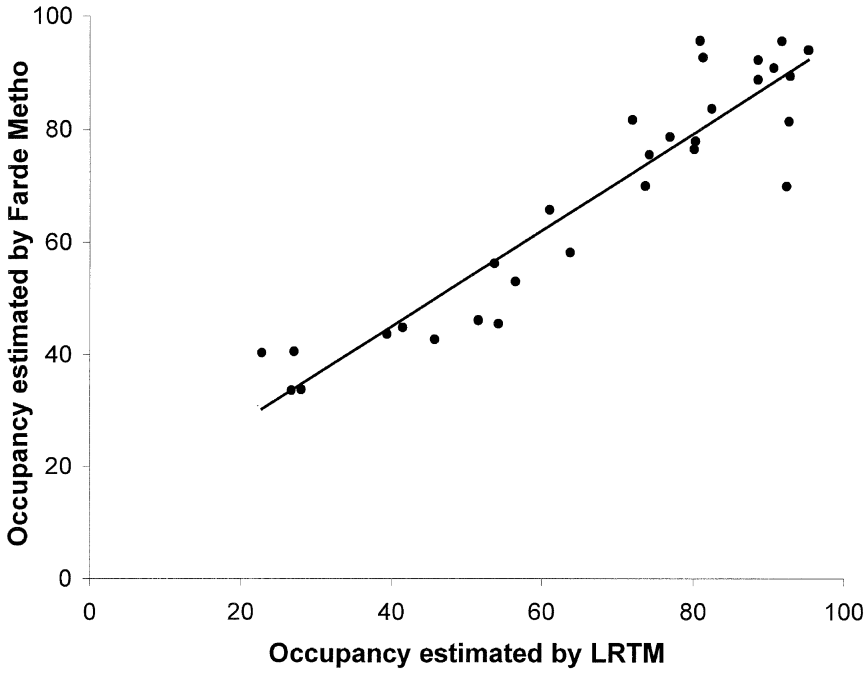

Figure 3. The highly significant linear correlation between striatal dopamine receptor occupancy values determined at various aripiprazole doses with Farde's method (Farde et al. 1989) and Lammertsma's method (Lammertsma and Hume 1996). r = 0.94, $p <$ .001.

plasma levels in living humans who are undergoing treatment with the neuroleptic drug, aripiprazole. This is clinically useful information that should help in decisions about drug dose and maintenance of the appropriate level of medication.

We applied two procedures to estimate dopamine D_2 and D_3 receptor occupancy. The first method was the classical method described by Farde et al. (1989). This Bound/Free (B/F) ratio at "transient equilibrium state" was assayed twice, both before and after the drug administration. The D_2 occupancy can be expressed as the percent change in the B/F ratio before and after drug administration. The theoretical problem with this method is that the equilibrium state is defined as the state when $dB/dt = 0$ ("transient equilibrium", Farde et al. 1989). The B/F ratio is calculated at the "transient equilibrium." The ratio is identical to the k_3/k_4 ratio if equilibrium is established simultaneously at this "transient equilib-

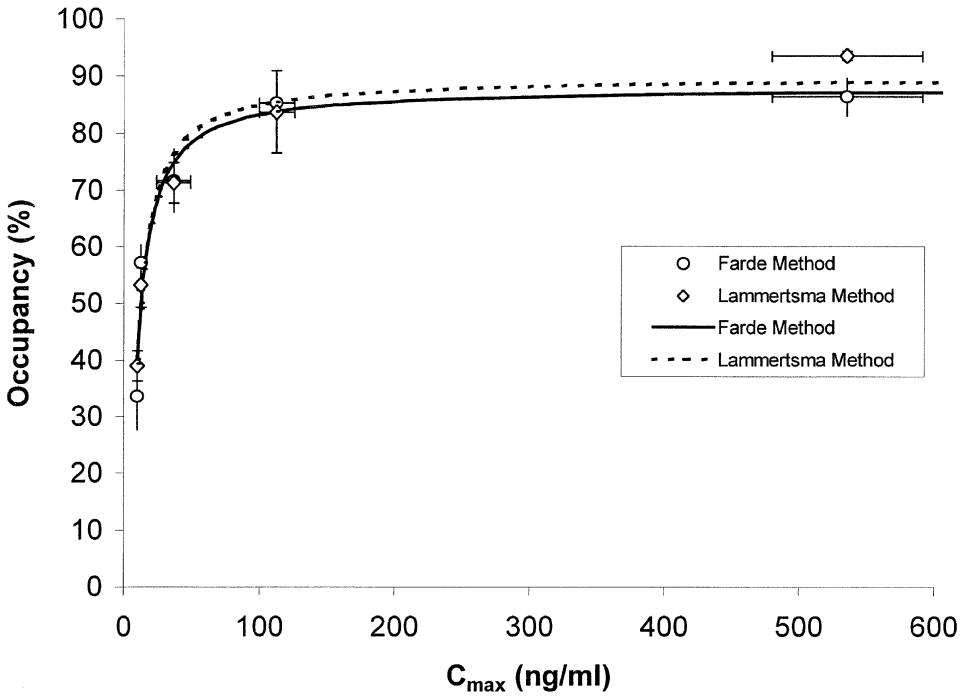

Figure 4. The typical hyperbolic relationship between peak plasma concentration of aripiprazole and dopamine D_2 and D_3 occupancy in the putamen. The dopamine receptor occupancy was estimated using two kinetic modeling procedures. The mean values (± SD) of dopamine occupancy and plasma concentration (± SD) at each drug dose are shown. The results in the putamen are similar to those obtained in the caudate nucleus.

rium" in each compartment, designated specifically as the bound compartment, the free compartment, or the plasma compartment. The free compartment, however, which is assumed to be represented by the cerebellar compartment, does not reach physiological equilibrium at "transient equilibrium." A single bolus injection technique may not achieve physiological equilibrium (Ichise et al. 1996). Although Farde's method contains these theoretical weak points, the values of D_2 occupancy are very similar to those obtained with the second method. A potential approach to minimize these uncertainties, using a plasma input function, might further improve these measurements (Wong et al. 1998).

The second model was described by Lammertsma and colleagues (Lammertsma et al. 1996; Lammertsma and Hume 1996). The four parameters were estimated without a plasma input function. The Lammertsma kinetic model can be applied if a reversible tracer like [^{11}C]RAC and a reference brain region like the cerebellum, in which the receptor density is negligible, is used. Both of these kinetic models require an assumption. In Farde's method, it is assumed that the free compartment, which includes the nonspecifically bound radiotracer, is the same in a receptor-rich brain region like the basal ganglia and in cerebellum. Lammertsma's model assumes that the partition coefficient (K_1/k_2 ratio) is the same in the basal ganglia and the cerebellum.

Most interestingly, however, our results seem to contradict the current opinion on the relationship between occupancy of striatal D_2-like dopamine receptors by neuroleptic drugs and clinical efficacy and EPS, respectively. Farde and collaborators in their pioneering work demonstrated that treatment of schizophrenic patients with neuroleptics in clinically used doses leads to a high occupancy of D_2-like DA receptors in the range between 65% and 90% (Farde et al. 1988; 1992). Patients with acute EPS were found to have higher DA receptor occupancies (average occupancy 82%) than those without such side effects (average occupancy 74%). Moreover, a recent study in schizophrenic patients, by the same group, demonstrated a significant relationship between the antipsychotic effect of raclopride and the degree of striatal DA D_2 receptor occupancy. The relationship between DA receptor occupancy and EPS could be confirmed in this study. It also suggests a "ceiling" of about 60% occupancy of striatal DA D_2 receptors for sufficient treatment response (Nordström et al. 1993), although such a high occupancy does not necessarily mean that every patient sufficiently improves (Wolkin et al. 1989). Studies with some of the newer "atypical" antipsychotics such as olanzapine or risperidone support Farde's suggestion (Kapur et al. 1999). The only exception from this rule seemed to be clozapine, which occupies 20–40% of striatal D_2-like dopamine receptors at clinically used doses (Farde et al. 1992), and probably also quetiapine (Kapur et al. 2000).

Our study clearly demonstrates that Farde's hypothesis may be true for most of the currently used antipsychotics, including some of the newer atypical agents, but not for some atypical compounds with particular pharmacological properties such as aripiprazole. Aripiprazole at doses shown to be the clinically most effective ones, namely 15–30 mg daily, leads to occupancy of striatal D_2-like dopamine receptors above 80%, in some subjects on 30 mg daily even above 90%. The incidence of EPS, however, even at those higher aripiprazole doses is not higher than under placebo (Carson et al. 2000; Daniel et al. 2000; Kane et al. 2000; Petrie et al. 1997). EPS could not be detected in our sample of normal volunteers, either, although Farde's hypothesis would predict severe EPS al least at the higher doses administered. The observation that high D_2 receptor occupancy may not necessarily be strictly related to EPS or even clinical efficacy was already made with the sigma ligand EMD 57445 (Gründer et al. 1999). In the case of aripiprazole, its partial dopamine agonism or dopamine autoreceptor agonism, respectively, may explain our unexpected findings.

First, it can be assumed that aripiprazole as a partial agonist exhibits a particular intrinsic efficacy at dopamine receptors (Lawler et al. 1999). Therefore, it is conceivable that even at a 90% receptor occupancy as defined by PET using aripiprazole, an antagonistic effect comparable to that expected with classic antipsychotics such as haloperidol does not occur. This is indeed the most likely explanation for the discrepancy between the high D_2 receptor occupancy determined in our study and the lack of EPS observed with the used aripiprazole doses.

Second, the induction of transmitter release by the investigational drug has to be carefully considered. It is now well established that the "total" striatal binding of an antipsychotic as measured with PET and SPECT represents not only binding of the drug to the receptor, but also that of endogenous dopamine (Laruelle et al. 1997). Factors increasing synaptic DA may lead to false overestimation of binding of the antipsychotic to the receptor. This would then be an explanation for the discrepancy between high striatal D_2 DA receptor occupancy and observed incidence of EPS. Stimulation of dopamine autoreceptors, however, theoretically leads to a decreased neuronal impulse rate and reduced release and synthesis of the neurotransmitter (Roth et al. 1995). Although it cannot be ruled out that a pharmacologically induced desensitization of dopamine autoreceptors subsequently leads to a dopamine release, the effects of this class of drugs are more likely explained by agonistic postsynaptic actions occurring at higher doses (Gründer et al. 1993; Wetzel et al. 1992). Thus, an aripiprazole-induced dopamine release is less likely to be the cause of the high observed D_2 receptor occupancy.

Third, it cannot be ruled out that aripiprazole as a partial dopamine agonist leads to internalization of

dopamine receptors, which might influence occupancy measurements (Laruelle 2000). Agonist-induced internalization of neuroreceptors that are coupled to G-proteins is a well-documented phenomenon (Maloteaux and Hermans 1994; Sternini et al. 2000). This concept has also been demonstrated to be true for D_2-like dopamine receptors (Barbier et al. 1997; Ito et al. 1999). Chugani et al. suggest that dopamine promotes the internalization of spiperone-receptor complexes (Chugani et al. 1988). This was more recently further investigated by Vickery et al. (1999). Consequently, Laruelle (2000) and Wong (2002) hypothesize that this could be the explanation for the paradoxical increase in spiperone binding following amphetamine administration, because the lipophilic spiperone is able to penetrate into the neuron, where it is trapped (Maloteaux et al. 1983). While preincubation of human retinoblastoma cells with dopamine leads to a profound increase in [^{3}H]NMSP binding, [^{125}I]iodosulpiride binding is decreased (Barton et al. 1991). This difference has been attributed to the different lipophilicity of the two tracers. While [^{3}H]NMSP with its higher lipophilicity is supposed to bind both to internalized and surface-exposed receptors, benzamides such as [^{125}I]iodosulpiride are proposed to bind only or, at least to a much lesser extent, to surface-exposed receptors (Barton et al. 1991). Young et al. (1991) suggested that agonist-mediated receptor internalization and the lower affinity often seen with benzamides might account for the different behavior of spiperone and benzamide radiotracers in binding studies following a dopamine stimulus. Laruelle (2000) proposed that the benzamides' low lipophilicity prevents their diffusion across plasma membranes and also contributed to this discrepancy. On the other hand, the same group demonstrated recently, that neither the affinity nor the lipophilicity of the used radiotracer is a reliable predictor of its vulnerability to competition with endogenous dopamine (Guo et al. 2000). With regard to a possible aripiprazole-induced receptor internalization, this should indeed lead to a reduction in [^{11}C]raclopride binding, because the internalized receptors would not be available for binding of the radiotracer. The high apparent striatal D_2 receptor occupancy under treatment with aripiprazole would accordingly not be just "true" occupancy by the drug, but could be attributed to the lower apparent receptor availability. But it is presently unknown, whether aripiprazole as a partial dopamine agonist induces receptor internalization.

Fourth, binding of the radiotracer itself to dopamine autoreceptors could explain that aripiprazole occupies a very high number of striatal dopamine receptors without inducing EPS. Most currently used radiotracers for D_2-like dopamine receptors such as [^{123}I]IBZM for SPECT and [^{11}C]raclopride or [^{18}F]fallypride for PET belong to the class of substituted benzamides, which are pharmacologically characterized by their preferential binding to dopamine autoreceptors. There are now several studies showing that neuroleptic drugs differ substantially in their relative potency at blocking dopamine autoreceptors (Kendler et al. 1982; Sanger et al. 1997). Antipsychotics with a relatively higher affinity for dopamine autoreceptors such as aripiprazole or the benzamide antipsychotics, sulpiride or amisulpride, should lead to a higher D_2 receptor occupancy, compared with classic neuroleptics such as haloperidol when measured with PET and a benzamide radiotracer, because a substantial amount of benzamide radiotracer binding is likely to represent binding to autoreceptors.

Finally, it is theoretically possible that receptor upregulation occurring with chronic administration of an antipsychotic confounds the occupancy measures. It has been reported that chronic neuroleptic administration results in the upregulation of dopamine D_2 receptors in animal models (Burt et al. 1977; Clow et al. 1979; 1980a,b; Murugaiah et al. 1985; Theodorou et al. 1981). Interestingly, repeated aripiprazole administration does not lead to significant receptor upregulation in the rat striatum (Inoue et al. 1997). This could suggest that receptor occupancy by conventional neuroleptics measured with PET might be underestimated in the chronically medicated state caused by upregulation of dopamine D_2 receptors in the brain, which may not occur with aripiprazole. The fact, however, that striatal receptor occupancies of more than 90%, which were observed under aripiprazole in our study, are hard to reach with single-dose haloperidol without inducing severe EPS (Nordström et al. 1992), speaks strongly against this view.

Aripiprazole represents a potential novel mechanism for the "atypicality" of antipsychotics that will further fuel the debate about the pharmacologic characteristics of those compounds. Recently, Kapur and Seeman (2001) proposed that the most successful atypical antipsychotics would have a fast dissociation from D_2-like receptors as determined by their in vitro k_{off}. They suggested that antagonism of 5-HT_2 receptors is irrelevant to atypicality. With aripiprazole, however, we also have a situation that may be independent of k_{off}, as aripiprazole's notable pharmacology as described above seems to lead to an atypical antipsychotic through a mechanism that may be independent of the dissociation from the D_2-like receptor. Indeed, a continued concern with the concept of an in vitro k_{off} as a predictor of atypicality is that in vitro k_{off} values as demonstrated by imaging studies are often discrepant because of synaptic rebinding and other factors associated with the obvious heterogeneity in in vitro tissue situations (Delforge 1996; Wong and Gjedde 1996). Careful studies of aripiprazole's dissociation rate in vivo and in vitro are needed to determine whether this fits the Kapur and Seeman hypothesis or, which is more likely, whether aripiprazole's mechanism of action represents yet another hypothesis for atypicality.

In conclusion, the occupancy of D_2 and D_3 dopamine receptors in 15 normal control subjects showed a good correlation with administered doses and plasma levels of aripiprazole. These results may be predictive of the appropriate therapeutic dose to administer to schizophrenic patients. Our study clearly illustrates, moreover, that a very high striatal D_2-like dopamine receptor occupancy measured with [^{11}C]raclopride PET does not necessarily lead to EPS, an observation that can be attributed to the distinguished pharmacology of the compound.

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