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Imaging D_2 Receptor Occupancy by Endogenous Dopamine in Humans

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The impact of endogenous dopamine on in vivo measurement of D_2 receptors in humans was evaluated with single photon emission computerized tomography (SPECT), by comparing the binding potential (BP) of the selective D_2 radiotracer [^{123}I]IBZM before and after acute dopamine depletion. Dopamine depletion was achieved by administration of the tyrosine hydroxylase inhibitor alpha-methyl-para-tyrosine (AMPT), given orally at a dose of 1 g every six hours for two days. AMPT increased [^{123}I]IBZM BP by 28 ± 16% (±SD, n = 9). Experiments in rodents suggested that this effect was due to removal of endogenous dopamine rather than D_2 receptor upregulation. Synaptic dopamine concentration was estimated as 45 ± 25 nM, in agreement with values reported in rodents. The amplitude and the variability of the AMPT effect suggested that competition by endogenous dopamine introduces a significant error in measurement of D_2 receptors in vivo with positron emission tomography (PET) or SPECT. However, these results also imply that D_2 receptor imaging coupled with acute dopamine depletion might provide estimates of synaptic dopamine concentration in the living human brain. ***[Neuropsychopharmacology 17**:162–174, **1997**]* *©1997 American College of Neuropsychopharmacology. Published by Elsevier Science Inc.*

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Over the last few years, several groups have documented that in vivo measurement of D_2 receptors is affected by changes in the concentration of endogenous dopamine. For example, increasing synaptic dopamine concentration with amphetamine or methylphenidate acutely reduced the striatal specific binding of several positron emission tomography (PET) and single photon emission computerized tomography (SPECT) radiotracers in baboons (Logan et al. 1991; Innis et al. 1992; Dewey et al. 1993; Kessler et al. 1993; Laruelle et al. 1997) and in humans (Farde et al. 1992; Volkow et al. 1994; Laruelle et al. 1995). These observations opened the possibility to use PET or SPECT neuroreceptor imaging to gain information about changes in neurotransmitter concentration in the "synaptic space," or more exactly, in the vicinity of the receptors, since not all D_2 receptors are located at synaptic junctions (Yung et al. 1995). Specifically, the amphetamine or methylphenidate challenges coupled with D_2 receptor imaging provide assessments of relative increase in dopamine release. Using this technique, we recently reported an increase in amphetamine-induced elevation of synaptic dopamine in drug-free patients with schizophrenia compared to controls (Laruelle et al. 1996a). However, these challenges do not allow measurement of dopamine con-

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centration at baseline (i.e., in the absence of pharmacological intervention).

In contrast, a pharmacological intervention that would induce a rapid and complete depletion of synaptic dopamine could theoretically provide a measure of D_2 receptor occupancy by dopamine at baseline and, providing knowledge of the in vivo affinity of dopamine for D_2 receptors, an absolute quantification of synaptic dopamine concentration. Preclinical studies indicated that in vivo D_2 receptor occupancy by dopamine is far from negligible. Acute dopamine depletion induced a 10 to 60% increase in the in vivo binding of D_2 receptor radioligands in rodents (Van der Werf et al. 1986; Ross and Jackson 1989b; Ross and Jackson 1989a; Seeman et al. 1990; Ross 1991; Young et al. 1991). In baboons, we observed a 30% increase in the specific binding of the D_2 receptor radiotracer [^{123}I](*S*)-(-)-3-iodo-2-hydroxy-6-methoxy-N-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide ([^{123}I]IBZM) following acute exposure to the tyrosine hydroxylase inhibitor alpha-methyl-para-tyrosine (AMPT) (Laruelle et al. 1997).

The purpose of the present investigation was to estimate D_2 receptor occupancy by dopamine in the resting state in young healthy volunteers. For each subject, we compared [^{123}I]IBZM binding potential (BP) at baseline and during dopamine depletion as achieved by oral administration of AMPT (1 g every six hours for two days). AMPT is a competitive and reversible inhibitor of tyrosine hydroxylase, the rate limiting enzyme for dopamine and norepinephrine synthesis (Spector et al 1965; Udenfriend et al. 1965). We selected AMPT as the depleting agent because this drug is approved for human use and because, in contrast to reserpine, AMPT effects are rapidly reversible. The dose and frequency of AMPT administration was selected to provide and maintain significant inhibition of tyrosine hydroxylase activity (Engelman et al. 1968). AMPT was given for two days, based on the expectation that this duration of treatment would be adequate to produce marked dopamine depletion but too short to induce significant D_2 receptor upregulation. To verify that this regimen does not affect D_2 receptor density, rats were treated with either saline or AMPT for two days and in vitro D_2 receptor density was compared between the two groups.

To minimize delay between scan 1 (baseline state) and scan 2 (depleted state), AMPT treatment was initiated at the end of scan 1, and scan 2 was performed two days after scan 1. Plasma levels of AMPT were measured on each subject on the day of the second scan. Plasma levels of the dopamine metabolite homovanillic acid (HVA) and norepinephrine metabolite 3-methoxy-4- hydroxyphenylethyleneglycol (MHPG) were measured at the time of each scan, to assess the effect of AMPT on these metabolites. Clinical ratings were obtained each day of the study to document subjective and motor side effects of AMPT administration.

MATERIALS AND METHODS

Human Subjects

The study was approved by Yale Institutional Review Board for human investigation. Eleven healthy volunteers participated in this study (males, age 25 ± 4 years, with these and subsequent values given as mean ± SD). All subjects gave written informed consent after complete explanation of the risks and possible consequences of the study. The absence of medical, neurological and psychiatric history (including alcohol and drug abuse) was assessed by history, review of systems, physical examination, routine blood tests, urine toxicology and EKG.

Depletion Regimen and Clinical Monitoring

The study lasted four days. Each subject was scanned twice, at 48-hour interval, in the baseline state (scan 1, day 1) and after dopamine depletion (scan 2, day 3). Dopamine depletion was induced by oral administration of AMPT, 1 g every six hours for 48 hours. The first AMPT dose was given on day 1, at the end of scan 1 (8 PM). On day 2, 1 g AMPT was administered at 6 AM, noon, 6 PM, and midnight. On day 3, 1 g AMPT was given at 6 AM, noon, and just prior the beginning of the second scanning session (5–6 PM). To prevent the formation of AMPT crystals in the urine, subjects were instructed to drink at least 3 liters of fluids per day, starting the day before the study (Engelman et al. 1968; Lang and Marsden 1982).

Clinical status of the subjects was evaluated daily at 10 AM from day 1 to day 4. Five subjective feelings, known to be affected by monoamine depletion, were rated by the subjects on a visual analog scale ranging from 1 ("not at all") to 10 ("most ever"): sleepiness, happiness, anxiety, energy and restlessness. Subjects were also evaluated for the presence of extrapyramidal side effects with the Simpson Angus scale (Simpson and Angus 1970). Urine samples were collected daily and examined for the presence of AMPT crystals.

Radioiodination

[^{123}I]IBZM was prepared by direct electrophilic radioiodination of the phenolic precursor BZM [(*S*)(-)-*N*-[1-ethyl-2-pyrrolidinyl)methyl]-2-hydroxy-6-methoxybenzamide] with high-purity sodium [^{123}I]iodide in 0.1 M NaOH (no-carrier-added, radionuclidic purity > 99.8%) as previously described (Kung and Kung 1990). [^{123}I]IBZM was obtained in average radiochemical purity of 95 ± 2%. The specific activity was estimated to be at least 5,000 Ci/mmol. Sterility was confirmed by incubation in two media (USP XXIII 1995), and apyrogenicity was confirmed by the LAL test (Endosafe, Charleston, NC). [^{125}I]IBZM was labeled with the same technique, and the specific activity was measured by UV detection at 2,200 Ci/mmol.

Scan Protocol

The previously described radiotracer constant infusion technique was used to perform the experiments under sustained equilibrium binding conditions (Laruelle et al. 1994, 1995). To decrease radiation exposure to the thyroid gland, subjects received 0.6 g potassium iodide 60 minutes prior to [^{123}I]IBZM injection. Four fiducial markers filled with 10 μCi of [^{99m}Tc]$NaTcO_4$ were glued on each side of the subject's head at the level of the cantho-meatal line. An intravenous catheter was inserted in each arm of the subjects, for drug administration and blood sampling, respectively. A total [^{123}I]IBZM dose of 5.79 ± 0.76 mCi was given as a bolus (2.57 ± 0.33 mCi) followed by a continuous infusion at a rate of 0.66 ± 0.08 mCi/h (bolus to hourly infusion rate of 3.90 ± 0.02 h) for the duration of the experiment (294 ± 13 min, with these and all subsequent times given with reference to the beginning of the radiotracer administration). This bolus to infusion ratio was originally calculated from kinetic experiments performed in baboons. This protocol of administration was shown, in previous experiments, to induce a state of sustained [^{123}I]IBZM binding equilibrium: both the striatal and nonspecific activities remained at a constant level from 150 minutes to the end of the experiment (Laruelle et al. 1995). The relatively long time interval needed to achieve equilibrium in all subjects (>150 minutes) is due to limitation in tracer delivery to the brain and diffusional barriers inside the brain (see full discussion in Laruelle et al. 1994). Each subject received the same dose of [^{123}I]IBZM for scans 1 and 2.

During the first 210 minutes of the infusion, subjects were allowed to relax in a comfortable setting, in a room adjacent to the camera room. SPECT data were acquired with the three headed PRISM 3000 camera (Picker, Cleveland, Ohio) equipped with high resolution fan beam collimators (resolution at full width half-maximum, 11 mm; ^{123}I point source sensitivity, 16.5 counts/sec/μCi). Continuous scanning was performed during 80 min from 214 ± 13 min to 294 ± 13 min. Ten acquisitions of 8 minutes each were obtained. As opposed to one long acquisition, repeated consecutive acquisitions allowed: 1) correction for shift or drift in head's position; 2) measurement of changes over time in regional activities; 3) measurement of within-experiment standard deviation (noise). Venous plasma samples were obtained every 20 minutes during the imaging session (n = 4) to measure plasma [^{123}I]IBZM, HVA and MHPG. Plasma [^{123}I]IBZM determination was not repeated during scan 2.

[^{123}I]IBZM Plasma Analysis

The plasma was separated by centrifugation for 15 minutes at 3500 rpm and 200 μL aliquots were assayed in a calibrated gamma counter to measure concentration of total plasma radioactivity. Plasma (2.5 mL) was extracted three times with 5 mL of ethyl acetate. The combined organic extracts were evaporated to dryness under vacuum, the residue was taken up in 150 μL CH_3OH, diluted with 300 μL of water, and analyzed by reversed phase HPLC (C_{18}, $CH_3OH/H_2O/Et_3N$, 80/20/0.1, 1 mL/min). Determination of the parent compound fraction was calculated as previously described (Zoghbi et al. 1992). Measurement of plasma [^{123}I]IBZM free fraction (f_1) was performed in triplicate by ultrafiltration (Centrifree, Amicon, Danvers, MA) as previously described (Gandelman et al. 1994). To control for between assay variability in f_1 measurement, a standard sample (aliquoted from a pool of plasma obtained from 10 healthy volunteers) was run in parallel in triplicate, and the subject sample f_1 value was corrected using the standard sample. Change over time of the free parent compound was evaluated by linear regression, with the slope expressed as percentage of the average value. Plasma clearance of the parent compound (L/h) was calculated as the ratio of the rate of infusion (μCi/h) to the total (i.e., free plus plasma protein bound) parent compound plasma concentration at steady state (C_{ss}, μCi/L).

HVA Plasma Analysis

Plasma HVA and MHPG were assayed as previously described by gas chromatography and mass spectrometry, using deuterated internal standards (Maas et al. 1976; Bacopoulos et al. 1978). The intra-assay and inter-assay coefficients of variation were 2% and 5%, respectively, for both HVA and MHPG.

AMPT Plasma Analysis

On Day 3, one 5 mL venous blood sample was collected 60 minutes before the last administration of AMPT. AMPT was measured by reverse phase HPLC with fluorescence detection. The procedure used was adapted from that of Anderson et al. (1981) for tyrosine and tryptophan. Sample preparation was similar to that originally used by Engelman et al. (1968) to measure plasma AMPT, except that following protein precipitation we used HPLC to enable separation of AMPT from tyrosine. Following addition of alpha-methyl-metatyrosine as internal standard, equal volumes of serum and 0.7 M $HClO_4$ were mixed and centrifuged. An aliquot of the protein free filtrate was submitted to chromatography as follows: column, Spherisorb ODS-1; mobile phase, 4% ethanol and 1.5% acetic acid, pH 5.0; flow rate, 2 mL/min; fluorescence detector, excitation 285 nm, emission 317 nm. Standards and controls prepared in serum were carried through the entire procedure and linear squares regression of relative peak

height versus concentration was used to fit the standard curve and calculate unknowns. Within run coefficients of variation at 5 and 10 μg/mL were 4.5% and 2.4%, respectively. All samples were processed in the same run.

Image Analysis

Count projections were prefiltered using a Wiener 0.5 filter and backprojected using a ramp filter. SPECT images were reoriented to the cantho-meatal line as visualized by the four external fiducial markers. The four slices with highest striatal uptake were summed and attenuation corrected assuming uniform attenuation within an ellipse drawn around the subject's head. For each subject, the same attenuation ellipse was used for scans 1 and 2. Striatal and occipital regions of interest were positioned on the summed images. Standard regions of interest of constant size (striatum 556 mm^2, occipital 2204 mm^2) were used to analyze all studies. Right and left striatal regions were averaged. Specific binding was calculated as striatal minus occipital activity. The occipital region was selected as the background region because 1) the density of dopamine D_2 receptors is negligible in this region compared to the striatum (Lidow et al. 1989); 2) this region can be identified with greater reliability than the cerebellum; 3) in humans, [^{123}I]IBZM activity in the occipital region is equal to the nonspecific activity in the striatum (Seibyl et al. 1992). The quality of the equilibrium state was assessed by measuring the change over time of regional activity, expressed relative to the average regional activity.

Outcome Measures

At equilibrium, the [^{123}I]IBZM specifically bound concentration (B) is related to the [^{123}I]IBZM free concentration (F), the maximal number of D_2 receptors (B_{max}) and the equilibrium dissociation rate constant of [^{123}I]IBZM for D_2 receptors (K_D) by the Michaelis-Menten equation:

$$B = \frac{B_{max}F}{K_D + F}. \quad (1)$$

Because [^{123}I]IBZM was given at tracer doses, F was negligible relative to K_D and this equation simplified to:

$$B = \frac{B_{max}F}{K_D}. \quad (2)$$

The [^{123}I]IBZM binding potential (BP, mL g^{-1}), corresponding to the product of the receptor density (B_{max}, nM or pmol per g of brain tissue) and affinity ($1/K_D$, nM^{-1}, or mL of plasma per pmol), was thus equal to the B over F ratio. B (μCi per g of brain tissue) was measured as the difference between striatal total activity (STR) and occipital activity (OCC):

$$BP = \frac{B_{max}}{K_D} = \frac{B}{F} = \frac{(STR - OCC)}{F}. \quad (3)$$

Since [^{123}I]IBZM crosses the blood brain barrier by passive diffusion, the free [^{123}I]IBZM concentration equilibrates on both sides of the blood brain barrier under sustained equilibrium conditions (Kawai et al. 1991; Laruelle et al. 1994). Therefore, the intracerebral free [^{123}I]IBZM concentration was estimated by the steady-state free unmetabolized plasma [^{123}I]IBZM concentration (f_1C_{SS}, μCi per mL of plasma). Assuming that AMPT does not modify [^{123}I]IBZM nonspecific binding, the free [^{123}I]IBZM during scan 2 (F_2) could be derived from:

$$F_2 = F_1(OCC_2/OCC_1), \quad (4)$$

where F_1 is [^{123}I]IBZM f_1C_{SS} during scan 1; and OCC_1 and OCC_2 are the occipital activities measured during scans 1 and 2, respectively. This calculation avoided the need to repeat [^{123}I]IBZM f_1C_{SS} measurement during the second experiment.

In the presence of a competitive inhibitor such as endogenous dopamine, equation 1 becomes:

$$B = \frac{B_{max}F}{K_D(1 + F_{DA}/K_I) + F}, \quad (5)$$

where F_{DA} (nM) is the temporal average concentration of free dopamine in the vicinity of receptors, and K_I is the inhibition constant of dopamine for [^{123}I]IBZM binding to D_2 receptors. Assuming complete dopamine depletion during scan 2, BP_1 [^{123}I]IBZM BP measured during scan 1, i.e., in the presence of endogenous dopamine) and BP_2 ([^{123}I]IBZM BP measured during scan 2, i.e., in the absence of dopamine) are given by

$$BP_1 = \frac{B_{max}}{K_D(1 + F_{DA}/K_I)} \text{ and } BP_2 = \frac{B_{max}}{K_D}. \quad (6\text{–}7)$$

The percentage occupancy of D_2 receptors by dopamine during scan 1 was calculated as $100*((BP_2 - BP_1)/BP_2)$. The free synaptic dopamine concentration (F_{DA}) was obtained by combining and rearranging equations 6 and 7 and defining "a" as the BP_2/BP_1 ratio,

$$F_{DA} = K_i(a - 1). \quad (8)$$

Thus F_{DA} can be expressed as a fraction of K_I. To calculate the absolute value of F_{DA}, the value of K_I must be known. For this calculation, we used the K_I value of dopamine for [^{125}I]IBZM binding measured in vitro at room temperature in the presence of sodium ions and antioxidants (160 nM, Brücke et al. 1988). This model assumes competitive inhibition at one receptor site. In vitro, D_2 receptors present high and low affinity-states for dopamine (Sibley et al. 1982; Hamblin et al. 1984) and since D_2 antagonists have similar affinity for both sites, the interaction between dopamine and D_2 antagonists appears non-competitive under certain conditions

(Sibley and Creese 1980). However, the proportion of high- versus low-affinity agonist-binding states of D_2 receptors might be much higher in vivo (90% versus 10%, for the high- and low-affinity states, respectively) than in vitro (50% for each subtype) (Richfield et al. 1986). The uncertainty about the exact proportion of high- versus low-affinity states of D_2 receptors in vivo precluded incorporating these factors in the model.

Experiment in Rodents

Male Sprague-Dawley rats, 200–250 g, were treated with either AMPT (200 mg/kg) or saline subcutaneous injections twice a day for two days (n = 7 per group). This dose (400 mg/kg per day) was about 7 times the dose used in the human study (4 g per day, or 57 mg/kg per day in a 70 kg subject). Three hours after the last injection, rats were killed by decapitation. Striatum was immediately dissected on ice and frozen at −80°C. On the day of the assay, samples were homogenized using a Polytron (Kinematica, Kriens–Lu, Switzerland, setting 6 for 10 sec) in 1/40 g of tissue/mL of buffer (50 mM Tris, pH 7.4, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$). Homogenates were incubated for 10 minutes at 37°C and washed three times by centrifugation (20,000g, 4°C, 10 min). Incubation was initiated by the successive addition of 100 μL [^{125}I]IBZM (final concentration of 5 pM), 100 μl buffer or nonradioactive compound (unlabeled IBZM at 0.3 nM or unlabeled raclopride at 1 μM, final concentrations) and 800 μL of tissue solution (1/1250 g of tissue/mL of buffer, final tissue dilution). Each incubation was performed in quadruplicate (twelve tubes per tissue). Incubation was carried for 45 minutes at 22°C and terminated by rapid filtration through GF/B filters on a Cell Harvester (Brandel, Gaithersburg, MD, USA). Filters were rapidly washed three times with 5 mL ice-cold buffer and counted in a COBRA 5010 gamma counter (Packard, Meriden, CT, USA). The nonspecific binding was defined as the binding remaining in the presence of 1 μM raclopride. Protein concentration was measured with the method of Lowry et al. (1951). For each animal, B_{max} and K_D were derived by weighted nonlinear regression analysis using the program LIGAND (Munson and Rodbard 1980). Unlabeled IBZM and raclopride were obtained from Research Biochemicals International, Natick, MA.

Statistical Analyses

All values are provided as mean ± SD. AMPT effects on clinical ratings, plasma catecholamine metabolite, and scan measurements were assessed by repeated measures ANOVA. Correlations were analyzed with simple or multiple linear regression analyses. Significant differences from zero were evaluated by two tailed, one sample *t*-tests. In vitro B_{max} and K_D values were compared between saline and AMPT treated rats with unpaired, two tailed, *t*-tests. Probability value of 0.05 was used as the significance level.

RESULTS

Clinical Effects of AMPT

One subject could not be scanned in the depleted state (scan 2) because of delayed radioisotope ([^{123}I]NaI) delivery. Another subject was withdrawn from the study because of a side effect of AMPT (diarrhea). Thus, nine out of eleven subjects completed the protocol. AMPT crystals were detected in one subject (day 3 sample). This subject acknowledged poor compliance with fluid intake instructions. AMPT crystals were not present in an additional urine sample collected six hours later, nor in the day 4 sample. Plasma urea and creatinine were measured in this subject on days 3, 4, and 7, and were found unchanged compared to baseline. Thus, no impact of AMPT crystalluria on renal function was observed. AMPT crystalluria was not detected in the other ten subjects.

AMPT effects on clinical ratings were analyzed with repeated measures ANOVA in the nine subjects who completed the protocol. Sleepiness, happiness, restlessness, and Parkinsonism ratings were significantly affected by AMPT treatment (Table 1, Figure 1). No significant effects of AMPT were noted on anxiety or energy ratings. Sleepiness increased steadily from baseline, peaked on day 3, and resolved on day 4. Happiness significantly decreased from day 1 to day 3, and regained

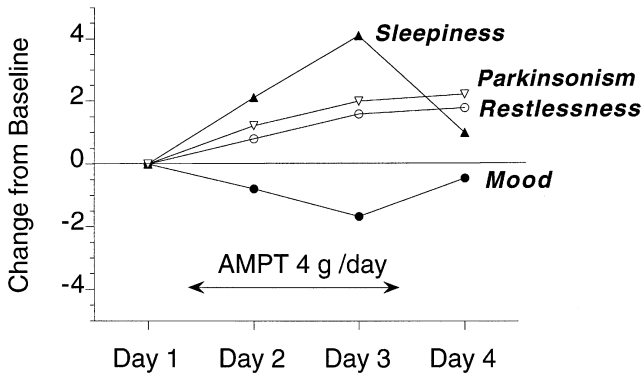

Figure 1. Effect of AMPT on clinical ratings obtained from day 1 (prior to AMPT administration) to day 4 (16 h after last AMPT dose). Data are presented in change from baseline (day 1) ratings. Sleepiness, restlessness, and mood were self-rated on a 10 points scale from 0 ("not at all") to 10 ("most ever"). Parkinsonism was rated using the Simpson and Angus scale. Error bars were omitted for clarity (see values of SD in Table 1). Symptoms presented on the graph showed a significant time effect (Repeated Measure ANOVA, $p < 0.05$).

Table 1. Effect of AMPT Administration on Clinical Ratings

Symptoms	Day 1	Day 2	Day 3	Day 4	*p*
Sleepiness	3.67 ± 2.50	5.78 ± 2.22	7.78 ± 1.99	4.67 ± 1.94	0.002
Happiness	7.56 ± 1.33	6.78 ± 1.48	5.89 ± 1.69	7.11 ± 1.45	0.027
Anxiety	5.22 ± 2.17	3.11 ± 2.42	3.33 ± 1.58	3.67 ± 1.66	0.070
Energy	5.56 ± 1.24	4.67 ± 1.94	4.67 ± 2.40	5.89 ± 2.15	0.346
Restlessness	2.11 ± 1.27	3.11 ± 1.96	3.89 ± 1.54	4.11 ± 2.67	0.041
Parkinsonism	0.00 ± 0.00	1.22 ± 0.97	2.00 ± 1.37	2.22 ± 1.79	0.003

Ratings were obtained at 10 AM before (Day 1), during (Day 2 and 3), and after AMPT administration. Subjective ratings (sleepiness, mood, anxiety, energy, and restlessness) were provided by the subjects on a 1 to 10 visual analog scale (1 being "not at all," 10 being "most ever"). Parkinsonism was evaluated by the clinical rater using the Simpson Angus scale.

p is the probability value of repeated measure ANOVA.

baseline level on day 4. Restlessness increased, and this effect persisted 16 hours after the last AMPT dose (day 4).

Clinical examination revealed mild signs of Parkinsonism in all subjects. These signs were still noted on day 4. Face akinesia, hypo/bradykinesia and resting tremors were the extrapyramidal signs most often noted. Two subjects experienced an acute dystonic reaction (spasms of muscles of the neck and jaw) during the evening of day 3. In one case, this reaction necessitated injection of diphenhydramine 50 mg i.m., with complete and immediate response.

Plasma AMPT

AMPT levels were obtained in 7 out of 9 subjects. The average AMPT plasma concentration was 21 ± 7 μg/mL (range 13 to 31 μg/mL).

Plasma HVA

AMPT significantly decreased plasma HVA by 70 ± 12% (repeated measures ANOVA, $p < 0.0001$, range from 54% to 84%, Table 2) and plasma MHPG by 66 ± 6% ($p < 0.0001$, range from 57% to 73%). Plasma HVA and MHPG were correlated at baseline ($r^2 = 0.65$, $p = 0.008$), but not after AMPT ($r^2 < 0.01$, $p = 0.98$).

SPECT Results

Venous samples for plasma [^{123}I]IBZM concentration analysis were obtained during scan 1 in all subjects. From 214 to 294 minutes, unmetabolized [^{123}I]IBZM accounted for 17 ± 4% of plasma activity. Total plasma activity significantly increased during this interval (+7.0 ± 2.6%/h, one-sample *t*-test, $p < 0.001$). In contrast, plasma level of unmetabolized [^{123}I]IBZM was stable (change of (−1.01 ± 8%/h, one-sample *t*-test, $p = 0.72$). Plasma [^{123}I]IBZM clearance was 14 ± 3 L/h and [^{123}I]IBZM plasma free fraction (f_1) was 0.034 ± 0.006.

The quality of the equilibrium state in the brain was assessed by measuring the change over time of occipital and striatal activities. During scan 1, striatum and occipital activities changed by −1.7 ± 6.4%/h and +1.1 ± 2.3%/h, respectively. During scan 2, striatal and occipi-

Table 2. Effect of AMPT Administration on Plasma Homovanillic Acid

Subject #	Age (years)	AMPT (μg/mL)	HVA_1 (ng/mL)	HVA_2 (ng/mL)	Change HVA (%)
1	29	—	7.1 ± 0.4	1.1 ± 0.1	−84%
2	22	31	11.9 ± 1.2	4.4 ± 0.4	−63%
3	27	18	4.3 ± 0.4	1.3 ± 0.2	−69%
4	21	13	7.9 ± 0.3	1.1 ± 0.1	−86%
5	22	—	7.7 ± 0.3	1.8 ± 0.1	−77%
6	22	21	6.7 ± 0.4	2.7 ± 0.1	−60%
7	22	28	4.2 ± 0.1	1.9 ± 0.1	−54%
8	25	17	7.7 ± 0.2	1.7 ± 0.1	−78%
9	33	19	10 ± 0.1	4.1 ± 0.4	−59%
Mean ± SD	25 ± 4	21 ± 7	7.5 ± 2.4	2.2 ± 1.3	−70 ± 12%

AMPT was measured on day 3, 60 minutes before the last administration of AMPT. HVA_1 and HVA_2 are plasma HVA concentrations during the scan obtained before and at the end of AMPT administration, respectively. Values for HVA and MHPG are the mean ± SD of 4 independent determinations on 4 plasma samples obtained at 20-minute intervals. Change in HVA was calculated as 100 * ($HVA_1 - HVA_2$)/HVA_1.

Table 3. Effect of AMPT Administration on [^{123}I]IBZM Binding Potential

Subject #	BP_1 (mL/g)	BP_2 (mL/g)	p	Change BP (%)
1	175 ± 16	270 ± 19	<0.001	+54%
2	207 ± 26	227 ± 27	0.117	+10%
3	216 ± 46	301 ± 34	<0.001	+39%
4	257 ± 16	343 ± 28	<0.001	+34%
5	191 ± 48	263 ± 35	0.008	+38%
6	355 ± 91	368 ± 33	0.864	+4%
7	211 ± 33	277 ± 51	0.002	+31%
8	286 ± 50	368 ± 59	0.004	+29%
9	245 ± 40	285 ± 36	0.029	+16%
Mean ± SD	238 ± 56	300 ± 49		+28 ± 16%

BP_1 and BP_2 are [^{123}I]IBZM binding potential to D_2 receptors before and at the end of AMPT administration, respectively. Values are the mean ± SD of 10 consecutive SPECT acquisitions.

p is the probability value of a significant difference between BP_1 and BP_2 for each subject (two tailed t-test). Change BP is the increase of BP due to AMPT, calculated as $100 * (BP_2 - BP_1)/BP_1$.

tal activities changed by +1.4 ± 4.2%/h and +0.7 ± 2.8%/h, respectively. None of these values were significantly different from zero (one-sample t-tests, $p > 0.05$). No effect of AMPT was observed on the slopes of the regional activities (repeated measures ANOVA, striatum: $p = 0.28$; occipital: $p = 0.74$). Thus, both scan 1 and 2 were performed under appropriate equilibrium conditions.

Table 3 presents the individual values of [^{123}I]IBZM BP at baseline (scan 1) and at the end of AMPT administration (scan 2). We observed a significant increase in [^{123}I]IBZM BP following AMPT administration (repeated measures ANOVA, $p = 0.0003$, Figure 2 and 3, Table 3). The average increase was 28 ± 16% (range from 4% to 54%). Comparing the 10 pre-AMPT with the 10 post-AMPT acquisitions, we observed a significant increase in [^{123}I]IBZM BP in 7 out of 9 subjects (Table 2). Assuming a complete depletion of dopamine following AMPT, these results indicate a baseline D_2 receptor occupancy by dopamine of 21 ± 10%, which would correspond to a synaptic dopamine concentration equal to 0.28 ± 0.16 K_I (or 45 ± 25 nM, using a K_I value of 160 nM).

AMPT plasma levels on day 3 were not correlated with AMPT-induced change in [^{123}I]IBZM BP ($r^2 = 0.18$, $p = 0.33$). An almost significant relationship was observed between the decrease in plasma HVA and the AMPT-induced increase in [^{123}I]IBZM BP ($r^2 = 0.43$, $p = 0.054$, Figure 4A). This correlation suggested that a relative decrease in plasma HVA could be an indicator of

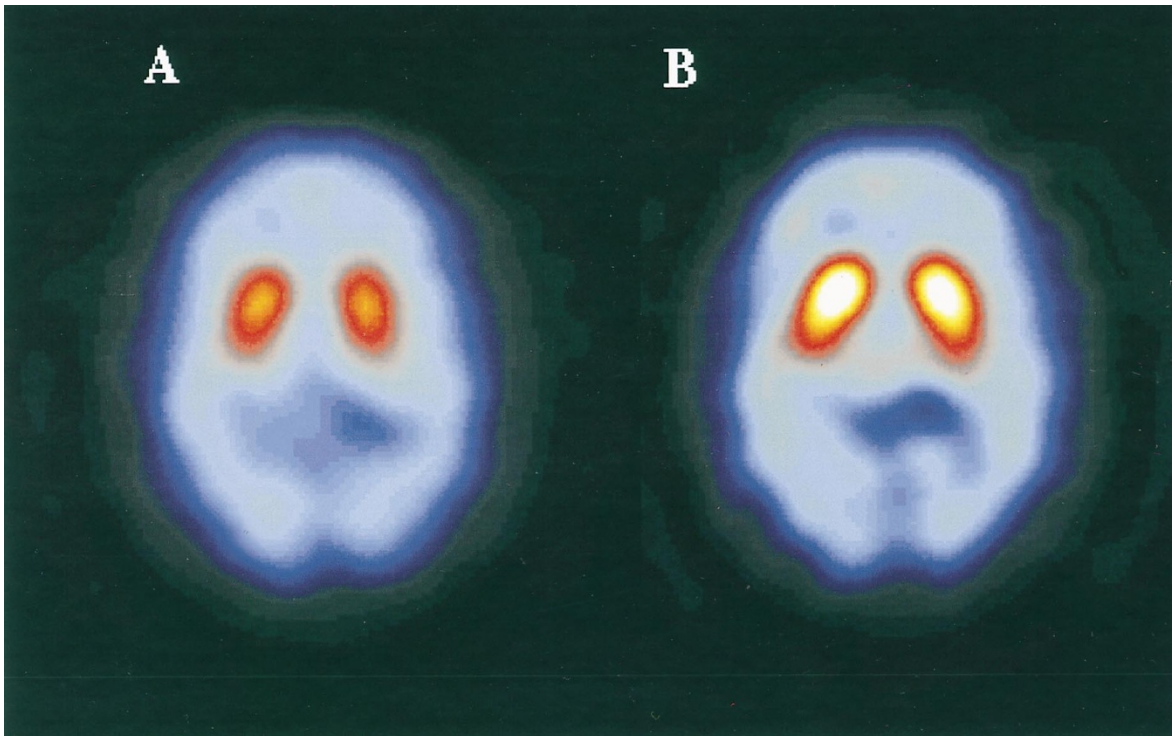

Figure 2. [^{123}I]IBZM activity distribution during scan 1 (A, baseline scan) and scan 2 (B, scan obtained at the end of AMPT administration, 1 g four times a day for 2 days) in a 29 year-old male healthy volunteer. Both images were normalized to the [^{123}I]IBZM infusion rate, decay corrected for the beginning of the infusion and color coded. Striatal to occipital activity ratio was increased in the post-AMPT scan compared to the baseline scan. The depletion in endogenous dopamine induced by AMPT resulted in a 54% increase in the striatal [^{123}I]IBZM binding potential.

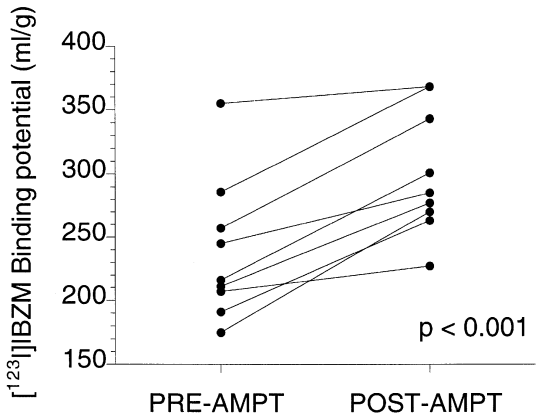

Figure 3. [123I]IBZM BP measured at baseline (pre-AMPT) and at the end of AMPT administration (1 g four times a day for two days). AMPT-induced depletion in endogenous dopamine resulted in a significant increase in [123I]IBZM BP (Repeated Measures ANOVA, $p = 0.0003$). The average increase was 28 ± 16%.

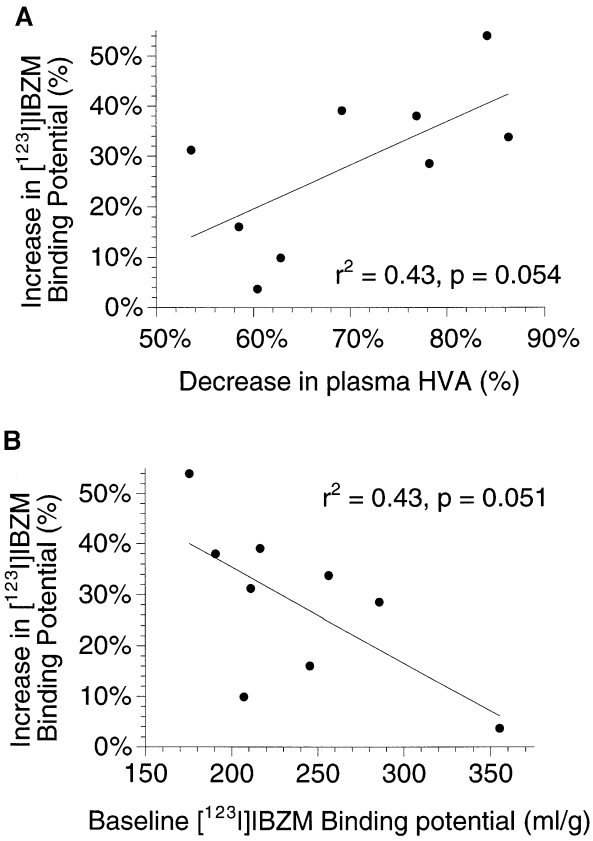

Figure 4. Factors associated with the AMPT-induced increase in [123I]IBZM BP. (A) Relationship between the AMPT effect on plasma HVA and [123I]IBZM BP. (B) Relationship between baseline [123I]IBZM BP and AMPT-induced increase in [123I]IBZM BP.

the relative decrease in striatal synaptic dopamine. In contrast, changes in plasm MHPG were not correlated with the increase in [123I]IBZM BP ($r^2 = 0.17$, $p = 0.26$). Baseline [123I]IBZM BP was inversely correlated with AMPT-induced increase in [123I]IBZM BP $r^2 = 0.43$, $p = 0.051$, Figure 4B). This correlation suggested that a relatively high receptor occupancy by dopamine might contribute to a relatively low [123I]IBZM BP at baseline. The increase in [123I]IBZM BP was correlated with the mood depressing action of AMPT ($r^2 = 0.63$, $p = 0.01$, Figure 5), but not with the level of sleepiness, restlessness or Parkinsonism.

Experiment in Rodents

No significant difference was observed in [125I]IBZM K_D between saline (0.13 ± 0.04 nM) and AMPT treated rats (0.12 ± 0.02 nM, unpaired *t*-test, $p = 0.40$), which suggested that the washing procedure effectively removed dopamine from the preparation in the saline treated rats. No significant difference was observed in D_2 receptor B_{max} between saline (228 ± 63 fmol/mg of protein) and AMPT treated rats (256 ± 28 fmol/mg of protein, unpaired *t*-test, $p = 0.31$), which indicated the failure to demonstrate significant D_2 receptor upregulation in the AMPT treated rats.

DISCUSSION

This study showed that AMPT administration (1 g every six h for 2 days) induced a significant increase (+28 ± 16%) in measured [123I]IBZM BP in nine young healthy volunteers. The magnitude of this effect by far exceeds our test-retest variability for repeated [123I]IBZM BP measurements in humans. In a previous study (Laruelle et al. 1995), seven subjects were scanned twice at two-

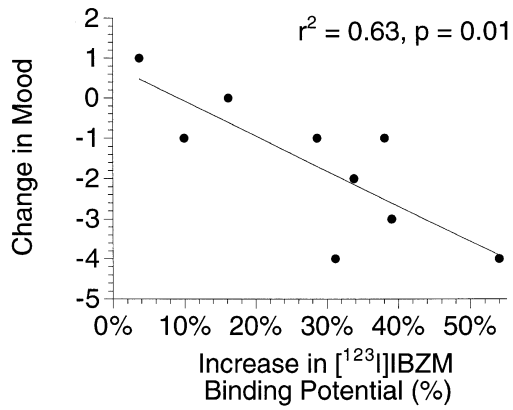

Figure 5. Relationship between AMPT-induced decrease in synaptic dopamine (as measured by the increase in [123I] IBZM BP) and AMPT-induced decrease in mood (as self–rated by the subjects on a 1 to 10 analog scale).

week intervals under identical conditions [^{123}I]IBZM constant infusion). The average difference in [^{123}I]IBZM BP between test and retest was −0.1 ± 6.4% (range from −6.6% to +9.4%). The average absolute difference was 5.6 ± 2%. This reproducibility corresponded to an intraclass coefficient of correlation close to unity (0.96).

The AMPT effect on [^{123}I]IBZM BP suggested that endogenous dopamine occupied at least 20 ± 10% of D_2 receptors in these subjects. Before adopting this conclusion, potential sources of artifactual results must be considered: 1) The increase in [^{123}I]IBZM BP measured after AMPT might have reflected receptor upregulation rather than removal of endogenous dopamine. Yet, in vitro measurement of D_2 receptor density in rodents following 2 days of AMPT administration failed to reveal significant receptor upregulation. This experiment confirmed an earlier study that could not demonstrate significant D_2 receptor upregulation after one week of dopamine depletion induced by 6-hydroxy-dopamine (Narang and Wamsley 1995). Similarly, acute pretreatment of mice with reserpine (5 mg/kg) resulted in a significant increase in the in vivo specific binding of [^{3}H]raclopride whereas no changes in D_2 receptor density could be detected in vitro (Ross and Jackson, 1989a). Thus, to the extent that these results can be extrapolated to humans, receptor upregulation does not seem to play a major role in the observed effect. 2) AMPT administration might have increased cerebral blood flow and accelerated the radiotracer delivery to the brain. Yet, [^{123}I]IBZM BP measurements were obtained under sustained equilibrium conditions, i.e., when no net radiotracer transfer occurs across the blood brain barrier. Therefore, [^{123}I]IBZM BP measurements could not have been affected by potential effects of AMPT on cerebral blood flow. 3) AMPT and fluids administration, by increasing [^{123}I]IBZM peripheral clearance, might have affected the quality of equilibrium achieved during scan 2, and such a factor might bias the results of the equilibrium analysis (Carson et al. 1992). However, changes over time in striatal and occipital [^{123}I]IBZM activities were minimal (around 1%/h) during both scans 1 and 2, and were not affected by the AMPT administration. This observation indicated that an appropriate state of equilibrium was reached and maintained during the scanning session for both pre- and post-AMPT treatment. Together, these considerations clearly implicate the reduction in endogenous dopamine as the main, if not the only, mechanism responsible for the increase in [^{123}I]IBZM BP.

Plasma AMPT levels measured in this study were in good agreement with pharmacokinetic data previously published. Engelman et al. (1968) showed that peak AMPT plasma concentration of 12–14 μg/mL were achieved about 2 hours after oral ingestion of a single dose of 1 g AMPT, and that plasma AMPT declined with a half-life of about 4 hours. Therefore, the administration of AMPT every six hours was expected to yield plasma concentrations of about 19-22 μg/mL at steady-state. Values observed in this study (21 ± 7 μg/mL) were in accordance with this expectation. In this AMPT concentration range, the large variation observed between subjects (10–30 μg/mL) would not be expected to have significant consequences for the pharmacodynamic effect of AMPT. Given an average plasma tyrosine level of 10 μg/mL, a tyrosine affinity constant for tyrosine hydroxylase of 62.5 μM, and an AMPT affinity constant for tyrosine hydroxylase of 17 μM (Udenfriend et al. 1965), AMPT concentrations of 13 μg/mL (smallest value observed in this study), 21 μg/mL (mean value) and 31 μg/mL (highest value) are expected to produce about the same degree of enzymatic inhibition (68%, 78% and 83%, respectively, Figure 6). The lack of correlation between AMPT plasma levels and the AMPT-induced increase in [^{123}I]IBZM BP is in agreement with this prediction. As pointed out by Engelman et al. (1968), higher doses of AMPT would not induce significantly more blockade of enzyme, because of the nonlinearity between inhibitor concentration and enzymatic inhibition (Figure 6). However, these considerations rest on the assumption that AMPT plasma levels reflect cellular levels, an assumption that has not been validated yet.

To calculate D_2 receptor occupancy by dopamine (21 ± 10%), we assumed that this AMPT regimen induced a complete depletion of synaptic dopamine. This assump-

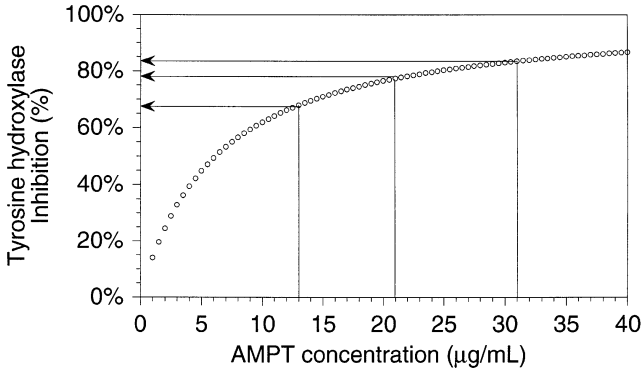

Figure 6. Relationship between AMPT concentration (μg/mL, x axis) and tyrosine hydroxylase inhibition (%, y axis). This simulation was obtained using a tyrosine concentration of 10 μg/mL, and an affinity constant for tyrosine hydroxylase of 17 μM for AMPT and 62.5 μM for tyrosine (Udenfriend et al. 1965). Arrows indicate the percentage inhibition that would correspond to AMPT concentrations of 13, 21 and 31 μg/mL, i.e., the lowest, mean, and highest steady state AMPT plasma concentrations in this study. Because of the nonlinearity between percentage inhibition and inhibitor concentration, large differences in AMPT levels observed in this study results in similar inhibition of tyrosine hydroxylase (68%, 78%, and 83% for the lowest, mean, and highest observed AMPT values, respectively).

tion might not be valid, since AMPT does not completely block tyrosine hydroxylase. Since dopamine synthesis was not completely blocked, the magnitude of striatal synaptic dopamine depletion achieved by this regimen is uncertain, and the D_2 receptor occupancy by dopamine might be more than 21 ± 10%.

The decrease in plasma HVA might provide an estimate of the decrease in striatal synaptic dopamine. At 4 g per day, AMPT induced cerebrospinal fluid HVA depletion (68 to 77%, Brodie et al. 1971) similar to the one measured in the plasma in this study (70 ± 12%). Following AMPT, cerebrospinal fluid HVA depletion reflects striatal dopamine depletion (Mignot and Laude 1985). These considerations would locate the dopamine depletion achieved in this study in the 70% range. Furthermore, it is often stated that 80% dopamine depletion or 80% D_2 receptor blockade is needed to produce extrapyramidal symptoms (Hornykiewicz and Kish 1987; Farde et al. 1989). While our subjects presented extrapyramidal symptoms, the moderate level of these symptoms likewise suggested depletion in the 70 to 80% range.

If we assume for each subject a decrease in striatal dopamine equal to the decrease in plasma HVA (F_{DA2}/F_{DA1} = plasma HVA_2/HVA_1), and defining "b" as HVA_2/HVA_1, equation 8 becomes:

$$F_{DA} = K_i(1 - a)/(ab - 1). \quad (9)$$

Under this assumption, dopamine occupancy of D_2 receptors would be 29 ± 13% (rather than 21 ± 10%) and baseline dopamine concentration would be 0.45 ± 0.25 K_I (rather than 0.28 ± 0.16 K_I). Thus, dopamine occupies at least 20% and possibly as much as 30% of D_2 receptors *in vivo* in young healthy subjects. These data indicate that endogenous dopamine competition significantly affects *in vivo* measurement of D_2 receptors with PET or SPECT.

To derive absolute synaptic dopamine concentration, we used the published K_I value of dopamine for [^{123}I]IBZM binding (160 nM), measured at room temperature (60 min incubation) in the presence of sodium ions and antioxidants (Brücke et al. 1988). This seemed a logical choice, given the large range of values of dopamine affinity at D_2 receptors that are reported in the literature (Seeman 1993). The in vitro measurement of dopamine K_D for D_2 receptors is very sensitive to the conditions of the assay and is also affected by the choice of the radiotracer. For example, dopamine has lower affinity for D_2 receptors in the presence than in the absence of sodium ions (Hamblin et al. 1984; Watanabe et al. 1985) and is more potent at competing with [^{3}H]raclopride than [^{3}H]spiperone binding to the D_2 receptors (Hall et al. 1990). This situation is further complicated by the existence of high and low affinity states of D_2 receptors for dopamine and dopamine agonists in vitro (Sibley et al. 1982) and by the absence of information on the proportion of high versus low affinity states in vivo (Richfield et al. 1986).

Despite these limitations, our estimates of synaptic dopamine concentration (from 45 ± 25 nM, assuming complete depletion, to 72 ± 40 nM, assuming partial depletion) were very close to values previously measured in rodents using a radiolabelled agonist. Comparing the in vivo binding of the D_2 receptor agonist [^{3}H]*N-n*-propylnorapomorphine in mouse striatum at baseline and following dopamine depletion induced by γ–butyrolactone or reserpine, synaptic dopamine concentration in mice striatum was estimated to be in the 40 to 60 nM range (Ross and Jackson 1989b; Ross 1991), in good agreement with values derived from this study (45 to 72 nM).

When considering these results, the extrasynaptic location of a significant number of D_2 receptors should be kept in mind. As much as 40% of D_2 receptors might be located at nonsynaptic sites (Yung et al. 1995), where the concentration of dopamine is lower than in the synapse. As a result, synaptic values estimated by in vivo competition studies are likely to underestimate true synaptic dopamine concentration. Using fast-scan voltametry measurement of dopamine release at the synaptic interface, Kawagoe et al. (1992) estimated that the synaptic concentration of dopamine rapidly varies from as high as 200 nM to as low as 6 nM, with a temporal average of about 100 nM. Given the underestimation of synaptic dopamine concentration introduced by the presence of nonsynaptic D_2 receptors, this value is in reasonable agreement with the values derived from receptor competition studies.

Despite the homogeneity of the subjects' demographics (all young males), we observed a large between-subject variability in the AMPT effect (range from 4 to 54%). This variability could relate to differences in the magnitude of AMPT-induced dopamine depletion or to differences in baseline synaptic dopamine concentration. Analysis of variance suggested a significant contribution of both factors. The relationship between plasma HVA depletion and the increase in [^{123}I]IBZM BP suggested that between-subject differences in AMPT-induced dopamine depletion (due to differences in the pharmacodynamic effect of AMPT) might have contributed to the variability of the dependent effect. The inverse relationship between baseline [^{123}I]IBZM BP and AMPT-induced increase in [^{123}I]IBZM BP might indicate that a relatively high synaptic dopamine concentration at baseline is associated with both a relatively low [^{123}I]IBZM BP and a relatively large increase in [^{123}I]IBZM BP following dopamine depletion. Studies that would include measurements of AMPT plasma and cerebrospinal fluid levels would be useful to further characterize the sources of the between-subject variability.

In addition to documenting the impact of endogenous dopamine on in vivo D_2 receptors measurement,

this study suggests the possibility to use such a paradigm to estimate endogenous dopamine at baseline in the living human brain. Such an application would require the AMPT administration to result in a similar level of tyrosine hydroxylase inhibition across subjects. Alternatively, the relative decrease in plasma HVA could be used to correct for between-subjects variation in AMPT pharmacodynamic effects equation 9. While absolute levels of plasma HVA cannot be readily used as an index of striatal dopamine concentration (for review see Amin et al. 1995), the relative decrease in plasma HVA induced by AMPT might reflect the degree of tyrosine hydroxylase blockade in both brain and periphery, and might potentially be used to estimate the relative central dopamine depletion. The relationship between AMPT-induced decrease in plasma and cerebrospinal HVA will be further investigated to validate such a method.

In conclusion, this study demonstrates that competition by endogenous dopamine results in a 20 to 30% underestimation of D_2 receptor density in vivo and suggests the possibility to take advantage of this phenomenon to measure striatal synaptic dopamine concentration in humans. With the recently developed ability to measure extrastriatal D_2 receptors (Halldin et al. 1995), this technique might also allow measurement of dopamine activity in extrastriatal regions, such as the cerebral cortex. Such a method would be very valuable to better understand regional alterations of dopamine activity in schizophrenia and other neuropsychiatric illnesses, and to relate these alterations to the clinical symptomatology.

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REFERENCES

Amin F, Davidson M, Kahn RS, Schmeidler J, Stern R, Knott PJ, Apter S (1995): Assessment of the central dopaminergic index of plasma HVA in schizophrenia. Schiz Bull 21:53– 66

Anderson GM, Young GJ, Cohen DJ, Schlicht KR, Patel N (1981): Liquid-chromatographic determination of serotonin and tryptophan in whole blood and plasma. Clin Chem 27:775–776

Bacopoulos N, Henninger G, Roth R (1978): Effects of haloperidol and probenecid on plasma and CSF dopamine metabolites in the rhesus monkey (Macacca mulatta). Life Sci 23:1805–1812

Brodie HK, Murphy DL, Goodwin FK, Bunney W Jr (1971): Catecholamines and mania: the effect of alpha-methyl-para-tyrosine on manic behavior and catecholamine metabolism. Clin Pharmacol Ther 12:218–224

Brücke T, Tsai Y, McLellan C, Singhanyom W, Kung H, Cohen R, Chiueh C (1988): In vitro binding properties and autoradiographic imaging of 3-iodobenzamide ([^{125}I] IBZM): a potential imaging ligand for D-2 dopamine receptors in SPECT. Life Sci 42:2097–2104

Carson RE, Channing MA, Blasberg RG, Dunn BB, Cohen RM, Rice KC, Herscovitch P (1992): Comparison of bolus and infusion methods for receptor quantification: application to [^{18}F]cyclofoxy and positron emission tomography. J Cereb Blood Flow Metab 13:24–42

Dewey SL, Smith GS, Logan J, Brodie JD, Fowler JS, Wolf AP (1993): Striatal binding of the PET ligand ^{11}C-raclopride is altered by drugs that modify synaptic dopamine levels. Synapse 13:350–356

Engelman K, Jequier E, Udenfriend S, Sjoerdsman A (1968): Metabolism of alpha-methyltyrosine in man: relationship to its potency as an inhibitor of catecholamine biosynthesis. J Clin Invest 47:568–576

Farde L, Nordström AL, Wiesel FA, Pauli S, Halldin C, Sedvall G (1992): Positron emission tomography analysis of central D_1 and D_2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. Arch Gen Psychiatry 49:538–544

Farde L, Wiesel FA, Nordström AL, Sedvall G (1989): D1– and D2–dopamine receptor occupancy during treatment with conventional and atypical neuroleptic. Psychopharmacology 99:S28–S31

Gandelman MS, Baldwin RM, Zoghbi SS, Zea-Ponce Y, Innis RB (1994): Evaluation of ultrafiltration for the free fraction determination of single photon emission computerized tomography (SPECT) radiotracers: β–CIT, IBF and iomazenil. J Pharmaceutical Sci 83:1014–1019

Hall H, Wedel I, Halldin C, J Kopp, Farde L (1990): Comparison of the in vitro binding properties of N-[^{3}H]methylspiperone and [^{3}H]raclopride to rat and human brain membranes. J Neurochem 55:2048–2057

Halldin C, Farde L, Hogberg T, Mohell N, Hall H, Suhara T, Karlsson P, Nakashima Y, Swahn CG (1995): Carbon-11-FLB 457: a radioligand for extrastriatal D2 dopamine receptors. J Nucl Med 36:1275–1281

Hamblin MW, Lef SF, Creese I (1984): Interactions of agonists with D-2 dopamine receptors: evidence for a single receptor population existing in multiple agonist affinity-states in rat striatal membranes. Bioch Pharmacol 33:877–887

Hornykiewicz O, Kish SJ (1987): Biochemical pathophysiology of Parkinson's disease. Adv Neurol 45:19–34

Innis RB, Malison RT, Al-Tikriti M, Hoffer PB, Sybirska EH,

Seibyl JP, Zoghbi SS, Baldwin RM, Laruelle MA, Smith E, Charney DS, Heninger G, Elsworth JD, Roth RH (1992): Amphetamine-stimulated dopamine release competes in vivo for [^{123}I]IBZM binding to the D_2 receptor in non-human primates. Synapse 10:177–184

Kawagoe KT, Garris PA, Wiedemann DJ, Wightman RM (1992): Regulation of transient dopamine concentration gradients in the microenvironment surrounding nerve terminals in the rat striatum. Neuroscience 51:55–64

Kawai R, Carson RE, Dunn B, Newman AH, Rice KC, Blasberg RG (1991): Regional brain measurement of B_{max} and K_D with the opiate antagonist cyclofoxy: equilibrium studies in the conscious rat. J Cereb Blood Flow Metab 11:529–544

Kessler RM, Votaw JR, de Paulis T, Bingham DR, Ansari SM, Mason NS, Holburn G, Schmidt DE, Votaw DB, Manning RG, Ebert MH (1993): Evaluation of 5-[^{18}F]fluoropropylepidepride as a potential PET radioligand for imaging dopamine D_2 receptors. Synapse 15:169–176

Kung M-P, Kung H (1990): Peracetic acid as a superior oxidant for preparation of [^{123}I]IBZM, a potential dopamine D-2 receptor imaging agent. J Lab Comp Radiopharm 27:691–700

Lang AE, Marsden CD (1982): Alpha methylparatyrosine and tetrabenazine in movement disorders. Clin Neuropharmacol 5:375–387

Laruelle M, Abi-Dargham A, Al-Tikriti MS, Baldwin RM, Zea-Ponce Y, Zoghbi SS, Charney DS, Hoffer PB, Innis RB (1994): SPECT quantification of [^{123}I]iomazenil binding to benzodiazepine receptors in nonhuman primates. II. Equilibrium analysis of constant infusion experiments and correlation with in vitro parameters. J Cereb Blood Flow Metab 14:453–465

Laruelle M, Abi-Dargham A, van Dyck CH, Gil R, De Souza CD, Erdos J, McCance E, Rosenblatt W, Fingado C, Zoghbi SS, Baldwin RM, Seibyl JP, Krystal JH, Charney DS, Innis RB (1996a): Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug free schizophrenic subjects. Proc Natl Acad Sci 93:9235–9240

Laruelle M, Abi-Dargham A, van Dyck CH, Rosenblatt W, Zea-Ponce Y, Zoghbi SS, Baldwin RM, Charney DS, Hoffer PB, Kung HF, Innis RB (1995): SPECT imaging of striatal dopamine release after amphetamine challenge. J Nucl Med 36:1182–1190

Laruelle M, Iyer RN, Al-Tikriti MS, Zea-Ponce Y, Malison R, Zoghbi SS, Baldwin RM, Kung HF, Charney DS, Hoffer PB, Innis RB, Bradberry CW (1997): Microdialysis and SPECT measurements of amphetamine-induced dopamine release in nonhuman primates. Synapse 25:1–14

Lidow MS, Goldman-Rakic PS, Rakic P, Innis RB (1989): Dopamine D_2 receptors in the cerebral cortex: distribution and pharmacological characterization with [^{3}H]raclopride. Proc Natl Acad Sci 86:6412–6416

Logan J, Dewey SL, Wolf AP, Fowler JS, Brodie JD, Angrist B, Volkow ND, Gatley SJ (1991): Effects of endogenous dopamine on measures of [^{18}F]N-methylspiroperidol binding in the basal ganglia: comparison of simulations and experimental results from PET studies in baboons. Synapse 9:195–207

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951): Protein measurement with folin phenol reagent. J Biol Chem 193:263–274

Maas JW, Hattox SE, Landis DH (1976): The determination of a brain arteriovenous difference for 3-methoxy-4-hydroxyphenethyleneglycol (MHPG). Brain Res 118: 167–173

Mignot E, Laude D (1985): Study of dopamine turnover by monitoring the decline of dopamine metabolites in rat CSF after alpha-methyl-p-tyrosine. J Neurochem 45: 1527–1533

Munson P, Rodbard D (1980): LIGAND: A versatile computerized approach for characterization of ligand binding systems. Ann Biochem 107:220–239

Narang N, Wamsley JK (1995): Time dependent changes in DA uptake sites, D_1 and D_2 receptor binding and mRNA after 6-OHDA lesions of the medial forebrain bundle in the rat brain. J Chem Neuroanatomy 9:41–53

Richfield EK, Young AB, Penney JB (1986): Properties of D2 dopamine receptor autoradiography: high percentage of high-affinity agonist sites and increased nucleotide sensitivity in tissue sections. Brain Res 383:121–128

Ross SB (1991): Synaptic concentration of dopamine in the mouse striatum in relationship to the kinetic properties of the dopamine receptors and uptake mechanism. J Neurochem 56:22–29

Ross SB, Jackson DM (1989a): Kinetic properties of the in vivo accumulation of the dopamine D-2 receptor antagonist raclopride in the mouse brain in vivo. Naunyn Schmiedeberg Arch Pharmacol 340:6–12

Ross SB, Jackson DM (1989b): Kinetic properties of the in vivo accumulation of [^{3}H](-)-N-n-propylnorapomorphine in the mouse brain. Naunyn Schmiedeberg Arch Pharmacol 340:13–20

Seeman P (1993): Receptor Tables. Vol. 2: Drug Dissociation Constants for Neuroreceptors and Transporters. Toronto, Canada

Seeman P, Niznik HB, Guan H-C (1990): Elevation of dopamine D2 receptors in schizophrenia is underestimated by radioactive raclopride. Arch Gen Psychiatry 47:1170–1172

Seibyl J, Woods S, Zoghbi S, Baldwin R, Dey H, Goddard A, Zea-Ponce Y, Zubal G, Germinne M, Smith E, Heninger GR, Charney DS, Kung H, Alavi A, Hoffer P, Innis R (1992): Dynamic SPECT imaging of D_2 receptors in human subjects with iodine-123-IBZM. J Nucl Med 33:1964–1971

Sibley DR, Creese I (1980): Pseudo non-competitive interactions with dopamine receptors. Eur J Pharmacol 65:131–133

Sibley DR, De Lean A, Creese I (1982): Anterior pituitary receptors: Demonstration of interconvertible high and low affinity states of the D_2 dopamine receptor. J Biol Chem 257:6351– 6361

Simpson GM, Angus JWS (1970): A rating scale for extrapyramidal side effects. Acta Psychiatr Scand 212:1–58

Spector S, Sjoerdsma A, Udenfriend S (1965): Blockade of endogenous norepinephrine synthesis by alpha-methyltyrosine, an inhibitor of tyrosine hydroxylase. J Pharmacol Exp Ther 147:86–95

Udenfriend S, Nagatsu T, Zaltzman-Nirenberg P (1965):

Inhibitors of purified beef adrenal tyrosine hydroxylase. Biochem Pharmacol 14:837–847

USP XXIII (1995): Sterility test. In: (eds), The United States Pharmacopeia. Rockville, MD, United States Pharmacopeial Convention, Inc., pp 1686–1690

Van der Werf JF, Sebens JB, Vaalburg W, Korf J (1986): In vivo binding of N-n-propylnorapomorphine in the rat brain: regional localization, quantification in striatum and lack of correlation with dopamine metabolism. Eur J Pharmacol 87:259–270

Volkow ND, Wang G-J, Fowler JS, Logan J, Schlyer D, Hitzemann R, Lieberman J, Angrist B, Pappas N, MacGregor R, Burr G, Cooper T, Wolf AP (1994): Imaging endogenous dopamine competition with [^{11}C]raclopride in the human brain. Synapse 16:255–262

Watanabe M, George SR, Seeman P (1985): Dependence of dopamine receptor conversion from agonist high to low-affinity state on temperature and sodium ions. Biochem Pharmacol 34:2459–2463

Young LT, Wong DF, Goldman S, Minkin E, Chen C, Matsumura K, Scheffel U, Wagner HN (1991): Effects of endogenous dopamine on kinetics of [^{3}H]methylspiperone and [^{3}H]raclopride binding in the rat brain. Synapse 9:188–194

Yung KK, Bolam JP, Smith AD, Hersch SM, Ciliax BJ, Levey AI (1995): Immunocytochemical localization of D1 and D2 dopamine receptors in the basal ganglia of the rat: light and electron microscopy. Neuroscience 65:709–730

Zoghbi SS, Baldwin RM, Seibyl JP, Al-Tikriti MS, Zea-Ponce Y, Laruelle M, Sybirska EH, Woods SW, Goddard AW, Charney DS, Smith EO, Hoffer PB, Innis RB (1992): Pharmacokinetics of the SPECT benzodiazepine receptor radioligand [^{123}I]iomazenil in human and nonhuman primates. Nucl Med Biol 19:5