SPECT Imaging of Striatal Dopamine Release after Amphetamine Challenge

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This study assesses the feasibility of using SPECT to image intrasynaptic dopamine release in human striatum following dextroamphetamine sulfate (d-amphetamine) challenge testing. **Methods:** A bolus plus constant infusion administration schedule of the D2 receptor radiotracer [^{123}I]iodobenzamide ([^{123}I]IBZM) was used to obtain a stable baseline for reliable quantitation of the d-amphetamine effect. Eight healthy subjects first underwent a controlled experiment to demonstrate that stable levels of striatal and occipital activities could be maintained from 150 to 420 min during programmed infusion of the tracer. Next, seven subjects underwent the experiment with d-amphetamine. The experimental conditions were identical except that 0.3 mg/kg amphetamine was injected intravenously at 240 min. The behavioral effects of d-amphetamine were measured by self-rating on the following analog scales: euphoria, alertness, restlessness and anxiety. **Results:** The d-amphetamine injection induced a 15% ± 4% (mean ± s.d.) decrease in D2 receptor availability, measured as the specific-to-nonspecific equilibrium partition coefficient (V_3''). The d-amphetamine injection induced marked increase in euphoria, alertness and restlessness scores. The intensity of these behavioral responses correlated with the decrease in D2 availability measured with SPECT. In contrast, the anxiety response was milder and not correlated with the decrease in D2 availability. **Conclusion:** These studies demonstrate the feasibility of using [^{123}I]IBZM programmed infusion and SPECT imaging to measure endogenous dopamine release after d-amphetamine challenge and to study brain neurochemical correlates of emotions.

Key Words: single-photon emission computed tomography; dopamine D2 receptors; iodine-123-IBZM; equilibrium analysis

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Alterations of dopamine function are associated with multiple neuropsychiatric disorders, including Parkinson's disease (*1*), schizophrenia (*2*) and cocaine abuse (*3*). PET and SPECT neuroimaging are now well-established techniques to study pre- and postsynaptic dopaminergic receptors in the living human brain (*4*). In addition, both PET and SPECT imaging techniques have recently emerged as potential noninvasive tools to study endogenous dopamine release (*5–8*). The in vivo competition between endogenous dopamine and dopaminergic radiotracers for binding to dopamine receptors is the fundamental mechanism allowing us to study this functional aspect of synaptic transmission.

The in vivo competition between endogenous transmitters and radiotracers for receptor binding was initially described in rodents. Amphetamine, which releases dopamine and thereby increases endogenous dopamine concentration (*9,10*), reduced the in vivo binding of the dopamine D2 receptor agonist [^{3}H]N-propylnorapomorphine (*11,12*) and the dopamine D2 receptor antagonist ^{3}H-raclopride (*13,14*). Reduced in vivo accumulation of D2 receptor tracers was also reported in rodents following other agents that increase dopamine intrasynaptic concentration, such as the dopamine uptake inhibitors amfonelic acid and methylphenidate and the dopamine precursor L-DOPA (*12,15*). The opposite effect (i.e., increased tracer accumulation) was induced by drugs that decrease dopamine endogenous concentration, such as reserpine and gamma-butyrolactone (*12,14,16–18*).

These results suggested that PET or SPECT could be used to measure in vivo competition between radiotracers and endogenous transmitters. Logan et al. (*5*) reported that the rate of uptake of the PET D2 tracer [^{18}F]N-methylspiroperidol was decreased in baboons pretreated with d-amphetamine (1 mg/kg). Innis et al. (*7*) showed that the striatal washout rate of the SPECT D2 tracer ^{123}I-iodobenzamide ([^{123}I]IBZM) was increased by dextroamphetamine sulfate (d-amphetamine; 0.3 to 1.5 mg/kg) in baboons and that this effect was attenuated by reserpine pretreatment (1 mg/kg). Dewey et al. (*6*) reported decreased distribution volume of [^{11}C]raclopride in baboons pretreated with d-amphetamine (1 mg/kg) or with the dopamine uptake inhibitor GBR 12909 (1.5 to 3 mg/kg).

The first experiments in humans were reported by Farde et al. (*19*) who showed that the in vivo binding of ^{11}C-raclopride decreased by 6% to 16% after oral administra-

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TABLE 1
Control Experiments: Iodine-123-IBZM Distribution Volumes

Subject no.	Sex	Age (yr)	f_1 —	C_L ($L\ hr^{-1}$)	V_2 ($ml\ g^{-1}$)	D2 BP ($ml\ g^{-1}$)
1	F	20	0.045	1226	143	121
2	F	26	—	—	—	—
3	F	28	0.033	1542	176	142
4	M	29	0.042	1711	235	141
5	M	30	0.046	1632	204	96
6	M	22	0.034	1224	152	82
7	M	26	0.046	1687	214	169
8	M	25	0.042	1344	127	70
Mean ± s.d.		26 ± 3	0.041 ± 0.005	1481 ± 213	179 ± 40	117 ± 36

Equilibrium analysis was applied to data of control experiments in which free parent compound, steady-state plasma concentration (C_{SS}) was measured. f_1 = free fraction (i.e., unbound to plasma proteins) of parent compound in plasma; C_L = plasma clearance of the free parent compound, calculated as the ratio of rate of infusion to C_{SS}; V_2 = nonspecific equilibrium volume of distribution, calculated as the ratio of occipital activity during Session 2 (150 to 240 min) to C_{ss}; BP = binding potential, calculated as the ratio of striatal specific binding (calculated as striatal minus occipital equilibrium activities during Session 2) to C_{SS}. No blood samples could be obtained in Subject 2.

tion of d-amphetamine (30 mg, n = 3). More recently, Volkow et al. (*8*) reported a 23% ± 15% decrease in ^{11}C-raclopride distribution volume in humans after intravenous administration of the uptake blocker methylphenidate (0.5 mg/kg, n = 6). These results suggested the feasibility of measuring changes in endogenous dopamine concentration in the living human brain following pharmacological challenge tests.

In this study, we applied a tracer programmed infusion technique (*20,21*) with the SPECT D2 receptor probe [^{123}I]IBZM (*22*) to establish a stable baseline for evaluation of the d-amphetamine induced dopamine release in humans. Iodine-123-IBZM is a dopamine D2 and D3 antagonist characterized by high selectivity and relatively moderate affinity (K_D at 37°C is 0.43 n*M*) for both D2 and D3 (Hank Kung, personal communication). Iodine-123-IBZM has negligible affinity for the D4 receptors (in this article, we use the term D2 receptor to denote D2 and D3 receptors but not D4). We selected [^{123}I]IBZM because of its moderate affinity and high lipophilicity, properties which both enhance tracer vulnerability to competition by the endogenous transmitter (*18,23,24*).

Eight healthy subjects never exposed to d-amphetamine were studied twice under [^{123}I]IBZM bolus plus constant infusion conditions. The first experiment (control study) was performed to document the stability of the specific to nonspecific equilibrium partition coefficient (V_3'', a measure of dopamine D2 receptor availability) under tracer constant infusion conditions. The second experiment (d-amphetamine study) was performed to assess the effect of d-amphetamine (0.3 mg/kg, intravenously) on V_3''. Self rating of the subjective effects of the d-amphetamine injection were obtained by analog scales and compared to the SPECT results.

METHODS

Subjects

Eight healthy subjects participated in these studies (5 men and 3 women; aged 26 ± 3 yr) (Table 1). Inclusion criteria were: age between 18 and 35 yr; absence of any prior exposure to d-amphetamine; absence of past or present medical, neurological and psychiatric conditions (including alcohol and drug abuse) as assessed by complete medical and psychiatric history, physical examination, routine blood tests, urine toxicology and EKG; and absence of pregnancy as controlled by a plasma pregnancy test. All subjects gave written informed consent. Studies were approved by the respective human investigation committees and were performed under an investigational new drug (IND) protocol from the Federal Drug Administration. Subjects received 0.6 g potassium iodide (SSKI solution) 24 hr prior to the scan.

Radiolabeling

Iodine-123-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 (*25*). Iodine-123-IBZM was obtained in average radiochemical purity of 95% ± 2% (with this and subsequent values expressed as mean ± s.d.). The specific activity was too great to measure with the sensitivity of the UV detector on the HPLC and was estimated to be at least 5000 Ci/mmole. Sterility was confirmed by incubation in two media (*26*) and apyrogenicity was confirmed by the LAL test (Endosafe, Charleston, NC).

SPECT Protocol

Seven subjects underwent two SPECT experiments (control and d-amphetamine studies, respectively) separated by approximately 2 wk. One subject underwent the control study but was not available for the d-amphetamine study. Both experiments were performed using the identical protocol except that in the first experiment, venous blood samples were obtained to measure the free, metabolite-corrected, plasma [^{123}I]IBZM concentration,

and, in the second experiment, d-amphetamine was injected intravenously (0.3 mg/kg) at 240 min.

Four fiducial markers filled with 10 μCi [^{99m}Tc]pertechnetate were glued on each side of the subject's head at the level of the cantho-meatal line. These fiducial markers were used to control adequate positioning of the subject's head in the gantry before tracer injection and to identify the cantho-meatal plane during image analysis. An indwelling catheter was inserted in a forearm vein for tracer infusion. In the control experiment, a second intravenous catheter was inserted in the controlateral forearm for blood collection.

Iodine-123-IBZM was injected as a bolus followed by a constant infusion according to the following protocol: bolus activity, 4.73 ± 0.60 mCi; rate of infusion, 1.15 ± 0.17 mCi/hr; bolus to hourly infusion ratio 4.11 ± 0.26 hr; duration of infusion, 7 hr; total injected dose decay corrected to the beginning of the experiment, 12.8 ± 1.7 mCi (dosimetric calculation has established that a dose of 15 mCi [^{123}I]IBZM provides less than 5 rads exposure to each organ).

Patients were scanned on the PRISM 3000 (Picker, Bedford Heights, OH) equipped with low-energy ultra high-resolution fan-beam collimators (system resolution, 6.8 mm; ^{123}I point source sensitivity, 8.56 cps/μCi). Each experiment included three scanning sessions. Subjects were scanned during the following intervals: 0–90 min (Session 1), 150–240 min (Session 2) and 300–420 min (Session 3). Each session consisted of 8 to 14 consecutive acquisitions of 8 min each. Acquisition parameters were: photopeak window, 159 ± 16 keV; acquisition mode, continuous; matrix, 64 × 64 × 32; angular range, 120; angular steps, 3; seconds per step, 12; acquisition time, 8 min; radius of rotation, 14.1 cm; magnification factor, 1. Subjects relaxed between the scanning sessions, outside of the camera.

Plasma Analysis

Nine venous samples (10 ml) were collected every 30 min from 180 min to 420 min in heparin-treated evacuated tubes and stored at 0–4°C until analyzed (typically 15–18 hr after the scan) as previously described (*27*). Briefly, the plasma was separated by centrifugation for 10 min at 3500 rpm and 50-μl aliquots were assayed in a calibrated gamma counter to measure concentration of total plasma radioactivity. Remaining plasma was extracted three times with an equal volume 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 an equal volume of water and analyzed by reversed-phase HPLC (C_{18}, $CH_3OH/H_2O/Et_3N$, 75/25/0.2, 1 ml/min). Determination of the plasma-free fraction (f_1) was performed by ultrafiltration as previously described (*28*). Change over time of the free parent compound was evaluated by linear regression with the slope expressed as the percentage of the average value. Plasma clearance of the free parent compound (liter/hr) was calculated as the ratio of the rate of infusion (μCi/hr) to the free parent compound plasma concentration at steady state (C_{SS}, μCi/liter).

D-amphetamine Challenge Test

During the second experiment, d-amphetamine (0.3 mg/kg) was intravenously injected over 30 sec at 240 min (a few minutes after the end of session 2). EKG and vital signs were monitored continuously after the d-amphetamine injection. Behavioral response was measured by self rating with a simplified version of the Amphetamine Interview Rating Scale (*29*). Four items were investigated: euphoria ("feel good"), alertness ("feel energetic"), restlessness ("feel like moving") and anxiety ("feel anxious"). Self ratings were obtained by analog scales at the following times (min) relative to the d-amphetamine injection: −5, −2.5, 0, 2.5, 5, 7.5, 10, 15, 20, 25, 30, 40, 50 and 60 min. At 60 min post d-amphetamine injection, subjects were positioned in the gantry for Session 3 acquisition. Response to each of these items was calculated as the peak minus baseline scores.

Data Analysis

SPECT data were analyzed blind to the presence or absence of d-amphetamine injection. Projections from photopeak window were prefiltered using a Wiener 0.5 filter. Images were reconstructed with filtered backprojections using a ramp filter on a 64 × 64 × 32 matrix (corresponding to a voxel volume of 4.1 × 4.1 × 7.1 mm, = 122 mm^3). Images were then exported to the CERASPECT image analysis software (Digital Scintigraphics, Cambridge, MA). A series of 32 images were reoriented so that the cantho-meatal line, as identified by the fiducial markers, corresponded to the transaxial plane of the data set. The three slices with highest striatal uptake were summed and attenuation corrected with a Chang algorithm (*30*), assuming uniform attenuation within an ellipse drawn around the skull. The attenuation coefficient (μ) value was derived from phantom studies performed with an ^{123}I distributed source. A flat profile of the phantom was recovered using a micron value of 0.12 cm^{-1}. This value is lower than the theoretical attenuation coefficient of 150 keV photons in water (0.15 cm^{-1}) because of the higher density of scattered events in the middle compared to the periphery of the object.

Striatal and occipital regions of interest (ROIs) were positioned on the summed images. A standard ROI profile of constant size and shape was used to analyze the studies. The occipital region was selected as the background region because:

1. The density of dopamine D2 receptors is negligible in this region compared to the striatum (*31*).
2. The assumption of attenuation uniformity might not be valid for the cerebellum because of air-filled cavities such as the mouth and the sinuses.
3. Among the neocortical regions, the occipital pole is the most distant from the striatum, which reduces the contamination by scattered events of striatal origin. The assumption that occipital activity is equal to striatal nonspecific activity in humans was validated by two experiments in which an intravenous injection of 20 μg/kg haloperidol rapidly reduced the striatal activity to the level observed in the occipital area (*27*).

Regional averages of counts per minute per milliliter were decay-corrected to the beginning of the experiment and were transformed in μCi/ml using a sensitivity of 10.22 ± 0.18 cps/μCi. This sensitivity was measured with a 20-cm diameter cylindrical phantom filled with ^{123}I-NaI in water (≈0.5 μCi/ml, n = 5). The distributed source sensitivity (10.22 ± 0.18 cps/μCi) was thus 19% higher than the point source sensitivity (8.56 cps/μCi), which was expected because of the detection of scattered events in the distributed source. No attempts were made to correct for the scatter fraction in the photopeak window.

The stability of plasma and regional brain activity was assessed for the control experiments by linear regression of the regional activity from the beginning of Session 2 (150 min) to the end of Session 3 (420 min). The slope of these regressions was expressed as the percentage of the average value in this time interval (150–420 min). Average change over time was calculated as the average

value of the slopes. The absolute change was also obtained as the average of the absolute value of the slopes.

A three-compartment model was used to analyze the data. This model included the plasma compartment (C_1), the nondisplaceable compartment (C_2, including the free tracer in the brain and the nonspecific binding) and the receptor compartment (C_3, including the specific binding). Specific binding at equilibrium was calculated as striatum (S) minus occipital (O) equilibrium activity. The binding potential (BP, ml g^{-1}), corresponding to the product of the receptor density (B_{max}, nM or pmole per g of brain tissue) and affinity ($1/K_D$, nM^{-1} or ml plasma per pmole), was calculated as the ratio of the striatal specific activity to the free unmetabolized plasma tracer activity (F) at equilibrium (*20*).

$$BP = \frac{S - O}{F}. \qquad \text{Eq. 1}$$

The equilibrium distribution volume of the nondisplaceable compartment (V_2, ml g^{-1}) was calculated as the ratio of occipital activity to the free unmetabolized plasma tracer activity (F) at equilibrium.

$$V_2 = \frac{O}{F}. \qquad \text{Eq. 2}$$

The derivation of BP and V_2 requires plasma measurements. BP and V_2 were thus calculated for the control experiments only.

The specific to nonspecific equilibrium partition coefficient (V_3'', unitless), corresponding to the ratio of BP to V_2 (*32*) was calculated as:

$$V_3'' = \frac{BP}{V_2} = \frac{(S - O)}{O}. \qquad \text{Eq. 3}$$

Thus, in contrast to BP and V_2, the measurement of V_3'' does not require plasma measurement. For each session, V_3'' was calculated as the average of 8 to 14 acquisitions of 8 min each.

For the d-amphetamine experiments, the effect of d-amphetamine was measured by comparing V_3'' after d-amphetamine, i.e. during Session 3, to the baseline V_3'', i.e. during Session 2. The difference in V_3'' between Sessions 2 and 3 was expressed as a percentage of V_3'' in Session 2. Assuming that the nonspecific distribution volume (V_2) is not affected by d-amphetamine, the relative reduction in V_3'' following d-amphetamine is equal to the relative reduction in D2 BP (Eq. 3). For both control and d-amphetamine experiments, the significance of the difference in V_3'' between Sessions 2 and 3 was assessed by a two-tailed Student's t-test.

Factor analysis was performed on the four dimensions of the subjective response to d-amphetamine to reduce the number of statistical tests and because these dimensions appeared highly correlated. Factor analysis was performed using orthogonal varimax solution (*33*). Correlations were evaluated between subjective factors and decrease in D2 availability. For each correlation, the coefficient of determination (r^2) as well as the significance level of the correlation coefficient for a two-tailed test are provided.

RESULTS

Control Experiments

Plasma Analysis. Venous samples were obtained in seven of eight control experiments. In Subject 2, no blood samples were obtained because of difficulties in inserting a second catheter. The parent compound accounted for 16% ± 7% of the plasma activity in the samples collected from 180 to 420 min, whereas the polar and lipophilic fractions contributed 68% ± 8% and 15% ± 3% of the plasma activity, respectively. The free fraction (f_1) was 0.041 ± 0.006 (Table 1). The free parent compound content was stable during this interval, changing by 1.7% h^{-1} ± 3.2% h^{-1}. Therefore, the steady-state free-parent compound concentration was calculated as the average concentration from 180 to 420 min. The clearance was calculated as 1481 h^{-1} ± 213 liter/h^{-1} (Table 1).

Brain Analysis. Striatal and occipital activities were stable from 150 min (beginning of Session 2) to 420 min (end of Session 3; Fig. 1A). Changes of striatal activity over time ranged from −3.1% h^{-1} to +3.6% h^{-1}, with an average of −0.1% h^{-1} ± 2.6% h^{-1} and an average absolute change of 2.1% h^{-1} ± 1.7% h^{-1}. Occipital activity change over time ranged from −3.6% h^{-1} to +5.2% h^{-1}, with an average of +0.7% h^{-1} ± 3.1% h^{-1} and an average absolute change of 2.6% h^{-1} ± 1.6% h^{-1}. The stability of plasma and brain regional activities allowed equilibrium analysis (Eqs. 1, 2) for derivation of equilibrium distribution volumes (V_2, BP, and V_3''). V_2 corresponded to 179 ml g^{-1} ± 40 ml g^{-1}, BP corresponded to 117 ml g^{-1} ± 36 ml g^{-1} (Table 1), and V_3'' corresponded to 0.650 ± 0.134 (Table 2). Individual BP values were only moderately correlated with individual V_3'' values ($r^2 = 0.50$, $p = 0.07$) because of the between-subject variation in occipital V_2.

The stability of V_3'' between 150 and 420 min was further assessed by comparing V_3'' during Sessions 2 and 3 for each subject with a two-tailed t-test (Table 2). No significant differences in V_3'' between Sessions 2 and 3 were observed ($p > 0.10$ in each case, Table 2). The difference in V_3'' between Sessions 2 and 3, expressed as percentage of Session 2, ranged from +5% to −5%, with an average value of −2% ± 3%. The noise in the measurement of V_3'' was expressed as the percentage coefficient of variation (%CV, calculated as 100 × s.d./mean) for each session. V_3'' %CV was 9.8% ± 4.8% in Session 2 and 11% ± 4.7% in Session 3.

D-amphetamine Experiments

Brain Analysis. In all subjects, the injection of d-amphetamine at 240 min resulted in significantly lower V_3'' in Session 3 compared to Session 2 ($p < 0.05$ for all subjects, Table 3, Fig. 1B). The average V_3'' in Session 3 was reduced by 15% ± 4% of the V_3'' value measured in Session 2 (ranged from 8% to 21%). The stability over time of V_3'' values during Session 3, following d-amphetamine injection (average change, −1.0% ± 2.1%/hr; average absolute change, 4.7% ± 1.5%/hr) was identical to the stability of V_3'' during Session 3 in control conditions (average change, −0.2%/hr ± 4.1%/hr; average absolute change, 3.5% ± 1.4%/hr, ns), indicating that a new state of equilibrium was reached within 60 min of the d-amphetamine injection and maintained over the duration of Session 3. V_3'' %CV in

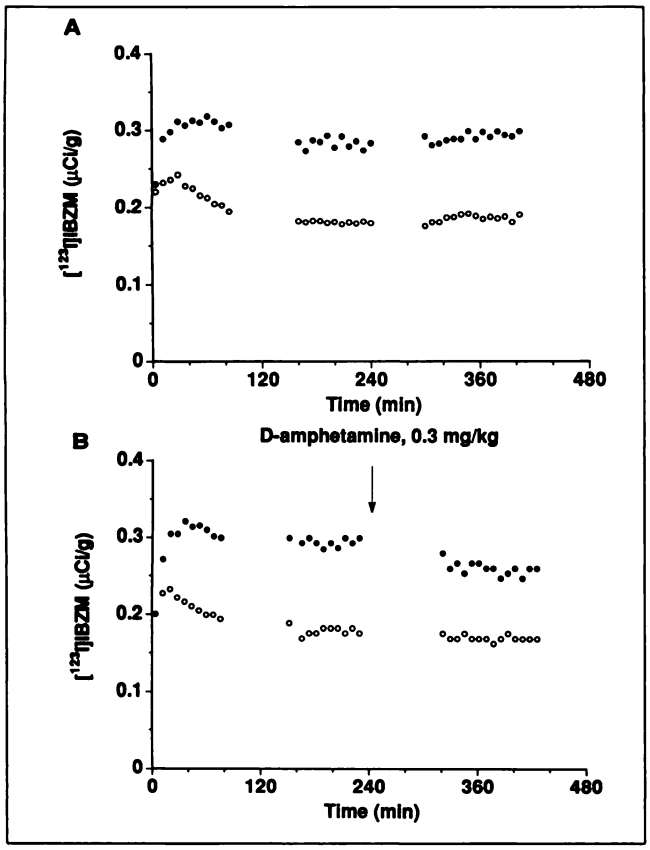

FIGURE 1. Striatal (closed circles) and occipital (open circles) time-activity curves during bolus plus constant infusion of [^{123}I]IBZM in a 22-yr-old man (Subject 4 of Tables 1, 2, 3). (A) Control experiment. The administration protocol (bolus 4.44 mCi, rate of infusion: 1.05 mCi/hr, bolus to hourly infusion ratio 4.23 hr) allowed achievement of stable levels of striatal and occipital activity from beginning of Session 2 to end of Session 3. (B) D-amphetamine experiment. The subject was retested under similar experimental conditions (bolus to hourly infusion ratio of 4.22 hr), with the exception that d-amphetamine (0.3 mg/kg, i.v.) was injected at the end of the second session (240 min, arrow). As a result, V_3'' in Session 3 (0.461 ± 0.068) was significantly lower than V_3'' in Session 2 (0.531 ± 0.056, t-test, p = 0.002).

Sessions 2 and 3 was 8.7% ± 4.1% and 9.2% ± 1.8%, respectively.

Somatic Side Effects. Systolic blood pressure increased from 123 ± 10 mmHg at baseline to 165 ± 12 mmHg at 5 ± 3 min post d-amphetamine (= average time of peak of systolic blood pressure), then slowly decreased to 141 ± 13 mmHg at 60 min. At 120 min, systolic blood pressure remained above baseline (131 ± 9 mmHg). Diastolic blood pressure changes paralleled systolic blood pressure changes: 64 ± 7 mmHg at baseline, 85 ± 12 mmHg at 5 min, 71 ± 8 mmHg at 60 min and 67 ± 9 mmHg at 120 min. The increase in heart rate occurred later than the increase in blood pressure, rising from 64 ± 9 bpm at baseline to 88 ± 15 bpm at 17 ± 10 min (= average peak time of pulse), and remained slightly elevated thereafter (69 ± 14 bpm at 60 min, and 71 ± 9 bpm at 120 min). All subjects remained in sinus rhythm at all times. One subject exhibited eight premature ventricular contractions during the 60 min following d-amphetamine injection. Side effects included palpitations (n = 5), chest tightness (n = 4), lightheadedness (n = 3), shortness of breath (n = 2), cold or warm feeling (n = 4), leg tingling (n = 2) and blurred vision (n = 2). These side effects were transient and resolved within 30 min without medical intervention.

Behavioral Response. Euphoria, alertness and restlessness markedly increased after the d-amphetamine injection. These responses were maximal between 10 and 20 min post-d-amphetamine injection, then gradually decreased (Fig. 2). In contrast, anxiety scores showed an initial moderate increase, followed by a return to baseline at 10 min, then decreased below baseline for the remainder of the experiment. The correlation matrix showed that euphoria, alertness and restlessness responses were correlated within subjects. Factor analysis revealed that behavioral responses could be reduced to two factors. Euphoria, alertness and restlessness had high loading scores on factor 1 (0.89, 0.72 and 0.80, respectively) and low negative scores on factor 2 (−0.03, −0.17 and −0.37, respectively). Factor 1 was referred to as the subjective activation factor. Anxiety had a high loading score on factor 2 (0.98) and a low negative score on factor 1 (−0.17). Factor 2 was referred to as the anxiety factor.

A significant correlation was observed between the subjective activation scores (calculated as the sum of euphoria, alertness and restlessness) and the decrease in V_3'' following d-amphetamine (r^2 = 0.84, p = 0.003). A similar correlation was observed between the factor 1 scores (calculated as the sum of the product of each three items by their loading score on factor 1) and the decrease in V_3'' (r^2 = 0.77, p = 0.008). Weaker correlations were observed with individual components of the activation score: euphoria (r^2 = 0.57, p = 0.04), alertness (r^2 = 0.62, p = 0.003), and restlessness (r^2 = 0.54, p = 0.05). No correlations were observed between d-amphetamine induced decrease in V_3'' and anxiety factor (r^2 = 0.14), peak systolic blood pressure (r^2 = 0.06), peak diastolic blood pressure (r^2 = 0.01), peak pulse (r^2 = 0.05) or age (r^2 = 0.27). No correlation was observed between the d-amphetamine-induced decrease in V_3'' and baseline V_3'' (r^2 = 0.09). No correlation between the decrease in V_3'' and baseline subjective states of the subjects was observed.

DISCUSSION

These experiments in humans demonstrate the feasibility of: (a) obtaining a stable brain regional activity of [^{123}I]IBZM using a bolus plus constant infusion protocol, (b) using this infusion schedule to measure dopamine D2 BP by equilibrium analysis and (c) using this stable baseline to quantify the reduction in D2 availability following d-amphetamine challenge testing.

The advantages of the programmed infusion/equilibrium analysis for derivation of BP have been extensively discussed elsewhere (*20,21,34*). The programmed infusion/equilibrium analysis method is easier to implement than the

TABLE 2
Control Experiments: Comparison of V_3'' between Sessions 2 and 3

Subject no.	Session 2 V_3'' ± s.d.	Session 3 V_3'' ± s.d.	Difference V_3''	p Value
1	0.850 ± 0.126	0.895 ± 0.042	5%	0.24
2	0.683 ± 0.072	0.660 ± 0.091	−3%	0.59
3	0.716 ± 0.051	0.689 ± 0.050	−4%	0.16
4	0.600 ± 0.079	0.584 ± 0.063	−3%	0.70
5	0.483 ± 0.092	0.489 ± 0.095	1%	0.94
6	0.545 ± 0.064	0.526 ± 0.037	−3%	0.39
7	0.800 ± 0.038	0.785 ± 0.054	−2%	0.38
8	0.524 ± 0.036	0.499 ± 0.040	−5%	0.46
Mean ± s.d.	0.650 ± 0.134	0.641 ± 0.145	−2% ± 3%	

V_3'', the equilibrium specific to nonspecific partition coefficient, was calculated as the specific (striatal minus occipital activity) to occipital activity ratio during scanning Session 2 (150 to 240 min) and Session 3 (300 to 420 min). Each session included 8 to 14 consecutive acquisitions (8 min each). No statistically significant differences in V_3'' were observed between Sessions 2 and 3.

single bolus/kinetic analysis method and more accurate than semiquantitative ratio methods. Most of the clinical studies performed with [^{123}I]IBZM use the striatal-to-occipital or the striatal-to-frontal ratio after single-bolus injection as the outcome measure (*35–37*). Two major problems are associated with this semiquantitative method: (a) the magnitude of the specific binding measured during the plateau phase is affected by the rate of washout of the tracer from the plasma (*21*) and (b) the use of this outcome measure implies the assumption that the nonspecific binding does not vary significantly between subjects.

In this study, we observed significant between-subject variation in V_2, i.e., significant between-subject variation in nonspecific binding. As a result V_3'', which is the ratio of BP over V_2, was only moderately correlated with BP. Thus, the use of V_3'', or any ratio method, is not optimal for quantification of D2 receptor density when between-subject comparison is required. V_3'' is an adequate outcome measure for within-subject comparison, such as before and after a challenge test, if one assumes that the challenge test does not modify the nonspecific binding. In contrast, the derivation of BP by programmed infusion/equilibrium analysis is not affected by the nonspecific binding and thus provides an easy and accurate method for in vivo quantitation of D2 receptors for between subjects comparison. In addition, the programmed infusion method creates optimal conditions for within-experiment quantification of the effects of pharmacological challenge tests. This property was utilized in this study to measure the reduction in D2 receptor availability after d-amphetamine challenge.

We initially observed that d-amphetamine (0.3 mg/kg to 1.5 mg/kg) injection increased [^{123}I]IBZM striatal washout rate after [^{123}I]IBZM single bolus injection in baboons (*7*). Since d-amphetamine has negligible affinity for D2 receptors (K_i of d-amphetamine is > 100 μM for [^{125}I]IBZM, unpublished data), the increase in the washout rate pre-

TABLE 3
D-amphetamine Experiments: Comparison of V_3'' between Sessions 2 and 3

Subject no.	Session 2 V_3'' ± s.d.	Session 3 V_3'' ± s.d.	Difference V_3''	p Value
1	0.892 ± 0.067	0.701 ± 0.055	−21%	<0.001
2	0.639 ± 0.059	0.586 ± 0.028	−8%	<0.050
3	0.695 ± 0.065	0.588 ± 0.040	−15%	<0.001
4	0.567 ± 0.038	0.473 ± 0.040	−16%	<0.001
5	0.531 ± 0.056	0.461 ± 0.068	−13%	<0.005
6	0.574 ± 0.069	0.475 ± 0.066	−17%	<0.005
7	0.761 ± 0.073	0.671 ± 0.031	−12%	<0.005
Mean ± s.d.	0.650 ± 0.132	0.547 ± 0.095	−15% ± 4%	

Subjects 1 to 7 are the same subjects as in Table 2. Experimental conditions were identical, except that d-amphetamine was injected i.v. (0.3 mg/kg) at 240 min (end of Session 2). V_3'', the equilibrium specific to nonspecific partition coefficient, was calculated as the specific (striatal minus occipital activity) to occipital activity ratio during scanning Sessions 2 (150 to 240 min) and 3 (300 to 420 min). Each session included 8 to 14 consecutive acquisitions (8 min each). In each case, V_3'' in Session 3 was significantly lower than in Session 2.

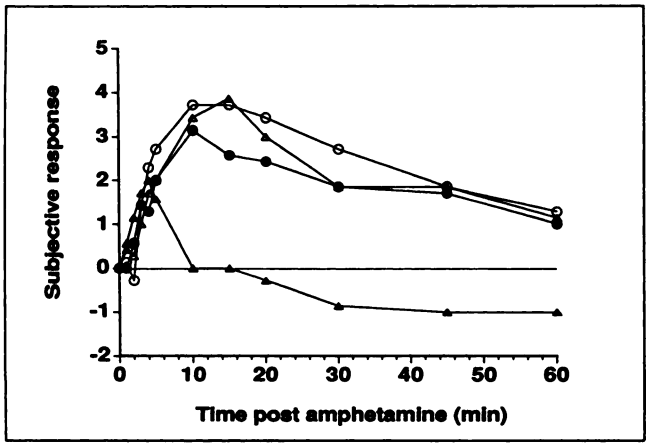

FIGURE 2. Subjective response to d-amphetamine i.v. administration (0.3 mg/kg) in six healthy volunteers. Each point represents the mean change relative to baseline. The response was assessed by self-rating analog scales at frequent times after d-amphetamine injection on the following items: euphoria (open circles), restlessness (open triangles), alertness (closed circles) and anxiety (closed triangles). Euphoria, restlessness and alertness showed a similar profile (marked increase, peak effect at 10 to 20 min, slow decrease) and correlated well within subjects. In contrast, anxiety showed an early and moderate peak, returned to baseline at 10 min and then decreased below baseline levels.

sumably reflected increased competition by endogenous dopamine. The use of this outcome measure (washout rate after the tracer single-bolus injection) to quantify the d-amphetamine effect was, however, limited by two factors: (1) the large between-animal variability in the spontaneous washout rate, inducing the need to perform two separate experiments to appreciate the d-amphetamine effect; and (2) the bias introduced in this outcome measured by potential d-amphetamine effects on the blood flow or the peripheral clearance.

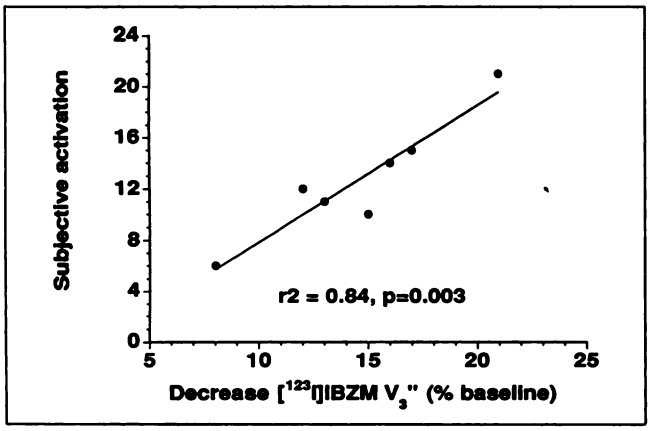

FIGURE 3. Correlation between the subjective activation score, calculated as the sum of euphoria, restlessness and happiness peak increase following d-amphetamine (0.3 mg/kg) and the reduction in D2 receptor availability, measured by the decrease in [^{123}I]IBZM V_3'' after d-amphetamine in six healthy subjects ($r^2 = 0.84$, $p = 0.003$). These data suggest that the intensity of the subjective response may be correlated with the magnitude of the dopamine release in the striatum following d-amphetamine challenge.

These two limitations were addressed by developing the bolus plus constant infusion method. Under equilibrium conditions, both baseline and post d-amphetamine levels can be measured in the same experiment, which protects the quantification of the d-amphetamine effect from between-scan variability. Furthermore, under equilibrium conditions, these measurements are not affected by potential effects of d-amphetamine on blood flow or on peripheral clearance. Experiments in baboons showed that, under equilibrium conditions achieved by tracer programmed infusion, d-amphetamine injection decreased [^{123}I]IBZM V_3'' (unpublished data). This decrease was dose dependent in the range of 0.3 mg/kg to 1 mg/kg d-amphetamine, an observation consistent with the dose dependency of endogenous dopamine release measured in primates with microdialysis (Bradberry C, *personal communication*, 1995). Furthermore, this decrease was prevented by pretreatment with the tyrosine hydroxylase inhibitor, alpha-methyl-para-tyrosine (unpublished data). Since tyrosine hydroxylase is the rate-limiting enzyme for the synthesis of dopamine, this observation added further evidence that the decrease in [^{123}I]IBZM V_3'' following d-amphetamine challenge was mediated by dopamine release.

Despite its low specific to nonspecific ratio, we selected [^{123}I]IBZM for these humans studies because experiments in primates showed that in vivo binding of [^{123}I]IBZM was more vulnerable than [^{123}I]iodobenzofuran [^{123}I]IBF) to competition by endogenous dopamine (*32,38,39*). As compared to [^{123}I]IBF, [^{123}I]IBZM has a lower affinity and higher lipophilicity, both properties that enhance vulnerability to endogenous dopamine (*24*).

The present study was designed to extend these observations to humans. The bolus-to-hourly infusion ratio used in this study (4.1 hr), derived from previous experiments in baboons, was adequate to obtain a state of equilibrium at 150 min in these eight subjects. A relatively homogenous group of young and healthy subjects was recruited. Iodine-123-IBZM clearance may be different in older subjects or in subjects with abnormal liver or renal function. In this case, the bolus-to-hourly infusion ratio might be too high or too low to establish equilibrium by 150 min, leading to over- or under-estimation of the d-amphetamine effect. Therefore, a larger number of subjects of different age groups should be studied before implementing this method. A better knowledge of the variability of the clearance would allow a better definition of the optimal bolus to infusion ratio and of the optimal imaging times (*34*).

Preliminary experiments in humans established that it takes about 60 min for the tracer to come to a new equilibrium level after the d-amphetamine injection. Because the outcome measure was the difference between pre- and post-d-amphetamine equilibrium levels, it seemed reasonable to optimize the definition of this difference by performing multiple scans before the injection (but after establishment of equilibrium) and 60 min after d-amphetamine injection. To limit the total scanning time, we thus decided not to scan immediately after d-amphet-

amine administration. This protocol also allowed the patient to be out of the camera during the 60 min following d-amphetamine, which facilitated the evaluation of the d-amphetamine behavioral effects.

A disadvantage of the method is the length of the experiment (7 hr). Both the pre- and post-d-amphetamine measurements, however, can be performed within the same experiment. If a single bolus/kinetic analysis method was used, two experiments would be needed. Because of the relatively slow isotope decay, these experiments should be performed on different days. Such an approach would require two separate isotope preparations, the placement of two arterial lines and multiple blood sample analysis. Thus, the programmed infusion technique is a more practical approach.

We observed a significant decrease in V_3'' after d-amphetamine injection in each subject. The magnitude of this decrease was small (15% ± 4%) and comparable to the decrease in [^{123}I]IBZM V_3'' observed in baboons after the same dose of d-amphetamine (20% ± 4%). Similar results were reported with PET in humans using ^{11}C-raclopride: Farde et al. (*19*) observed a 10% ± 5% decrease in ^{11}C-raclopride specific binding after 30 mg d-amphetamine p.o. (n = 3) and Volkow et al. (*18*) observed a 23% ± 15% decrease in [^{11}C]raclopride distribution volume after 0.25 mg/kg methylphenidate intravenously (n = 6). In baboons, higher doses of d-amphetamine resulted in greater reduction in D2 availability (28% ± 8% after d-amphetamine 0.5 mg/kg, 38% ± 11% after d-amphetamine 1 mg/kg, n = 3 for each dose, unpublished results). Microdialysis experiments in monkeys suggested that the magnitude of the increase in dopamine release does not change with doses higher than 1 mg/kg. This suggests that the highest response observed with SPECT would be a reduction of 30% to 40%. Thus, the dose used in these studies (0.3 mg/kg) is located in the middle of the dose response curve, which is optimal to detect increased responsiveness of dopamine neurons to d-amphetamine exposure.

The knowledge of the between-scan variability in V_3'' measurement can be used in a power analysis to define the minimal number of 8-min acquisitions needed in each session. The average within-session s.d. of V_3'', expressed as a percentage of mean V_3'', was 9.8% ± 4.0%. The average d-amphetamine effect (15%) is thus equal to 1.5 the average within-session s.d. of V_3''. Thus, four scans per session are needed to detect a 15% between session difference with a significance level (α) of 0.05 and a power ($1 - \beta$) of 0.8 (*41*). With eight scans per session, equivalent to 1 hr of scanning, a difference of 9% can be detected with the same power. As the between-acquisition variability in V_3'' is probably related to head movement, coregistration of each image to a common template such as an MRI image might improve the between-acquisition V_3'' reproducibility.

In this study, we observed significant between-subject variability in the d-amphetamine induced reductions in V_3''. Since we did not measure d-amphetamine plasma levels, we cannot rule out that these different responses are due to different rates of metabolism of d-amphetamine. Against this hypothesis was the fact that reductions in V_3'' were not correlated to changes in vital signs following d-amphetamine. Thus, these data are consistent with the existence of between-subject differences in neuronal sensitivity to d-amphetamine.

The small number of subjects and the absence of a placebo injection are two important limitations when considering the behavioral data. It was interesting to observe, however, that euphoria, alertness and restlessness were highly correlated, suggesting that these various subjective effects may be induced by a common mechanism of action of d-amphetamine. The good correlation between these effects and the reduction in D2 availability, if replicated in a larger sample, suggests that they are mediated by dopamine release. In contrast, anxiety was not correlated with dopamine release and may be related to a different action of d-amphetamine, such as norepinephrine release.

CONCLUSION

This study suggests the feasibility of measuring, in the same experiment, the baseline dopamine D2 BP and the reduction in D2 availability due to increased dopamine release elicited by d-amphetamine challenge. Additional experiments are needed to further validate this method in a larger group of subjects. These preliminary data also suggest that this method could be used to study the biochemical mechanism associated with the subjective effects of the drug. In addition, this method can be useful to study the biochemical correlates of increased sensitivity to the psychotogenic effects of d-amphetamine in schizophrenic patients (*42*).

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