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Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects

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ABSTRACT **The dopamine hypothesis of schizophrenia proposes that hyperactivity of dopaminergic transmission is associated with this illness, but direct observation of abnormalities of dopamine function in schizophrenia has remained elusive. We used a newly developed single photon emission computerized tomography method to measure amphetamine-induced dopamine release in the striatum of fifteen patients with schizophrenia and fifteen healthy controls. Amphetamine-induced dopamine release was estimated by the amphetamine-induced reduction in dopamine D_2 receptor availability, measured as the binding potential of the specific D_2 receptor radiotracer [^{123}I](*S*)-(−)-3-iodo-2-hydroxy-6-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide ([^{123}I]IBZM). The amphetamine-induced decrease in [^{123}I]IBZM binding potential was significantly greater in the schizophrenic group (−19.5 ± 4.1%) compared with the control group (−7.6 ± 2.1%). In the schizophrenic group, elevated amphetamine effect on [^{123}I]IBZM binding potential was associated with emergence or worsening of positive psychotic symptoms. This result suggests that psychotic symptoms elicited in this experimental setting in schizophrenic patients are associated with exaggerated stimulation of dopaminergic transmission. Such an observation would be compatible with an abnormal responsiveness of dopaminergic neurons in schizophrenia.**

The dopamine hypothesis of schizophrenia, formulated over 30 years ago, proposes that hyperactivity of dopaminergic transmission is associated with this illness (1). This hypothesis is based on the observation that dopamine D_2 receptor antagonists alleviate symptoms of the illness (mostly positive symptoms), while dopamine agonists can induce psychotic states characterized by some salient features of schizophrenia (2). These pharmacological effects suggest, but do not establish, a dysregulation of dopamine systems in schizophrenia. Despite decades of effort to validate this hypothesis, documentation of abnormalities of dopamine function in schizophrenia has remained elusive. Postmortem studies measuring dopamine and its metabolites in the brain of schizophrenic patients have yielded inconsistent results (for review, see ref. 3). Increased density of striatal dopamine D_2 and D_2-like receptors has been reported in postmortem studies, but this observation is difficult to interpret, given that neuroleptic drugs upregulate these receptors (4, 5). Positron emission tomography and single photon emission computerized tomography (SPECT) studies of striatal D_2 and D_2-like receptors density in neuroleptic-naive schizophrenic patients have been inconclusive. While one group reported increased striatal D_2-like receptors density in schizophrenia (6, 7), other groups reported negative results (8–12). The lack of clear evidence for increased dopaminergic indices in schizophrenia might indicate that dopaminergic transmission is enhanced only relative to other systems, such as serotonergic or glutamatergic systems (13, 14). On the other hand, the absence of data supporting the dopamine hypothesis of schizophrenia might be due to the difficulty of obtaining direct measurement of dopamine transmission in the living human brain.

Over the past few years, several groups have provided evidence that competition between neurotransmitters and radioligands for neuroreceptor binding allows measuring changes in synaptic neurotransmitter levels with *in vivo* binding techniques. In rodents, decreased uptake of D_2 radioligands has been measured following amphetamine and other dopamine enhancing drugs, whereas the opposite effect (i.e., increased tracer accumulation) has been induced by drugs that decrease dopamine concentration (15–17). In baboons, decreased specific uptake of positron emission tomography or SPECT D_2 radiotracers has been reported following amphetamine challenge (18–20). In humans, decreased accumulation of the D_2 antagonist [^{11}C]raclopride has been observed following challenges with amphetamine (21) or methylphenidate (22).

We recently developed and validated a protocol to measure amphetamine-induced dopamine release with SPECT and [^{123}I](*S*)-(−)-3-iodo-2-hydroxy-6-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide ([^{123}I]IBZM). This radiotracer, an iodinated analog of raclopride, is a selective antagonist at the D_2 and D_3 receptors (23). We initially observed that amphetamine challenge reduced the [^{123}I]IBZM binding potential in baboons (the binding potential is the product of the density and affinity of free receptors; ref. 24). Since amphetamine is devoid of significant affinity for D_2 receptors (amphetamine IC_{50} for [^{125}I]IBZM *in vitro* is >100 μM; unpublished results), we postulated that this effect was mediated by increased dopamine release and displacement of [^{123}I]IBZM specific binding by dopamine. This mechanism was confirmed by the observation that pretreatment with the dopamine depleter α-methyl-*p*-tyrosine prevented the effect of amphetamine on [^{123}I]IBZM binding potential (24). In addition, we established the existence of a good correlation between amphetamine-induced dopamine release measured with microdialysis and amphetamine-induced decrease in [^{123}I]IBZM binding potential measured with SPECT (24).

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Abbreviations: SPECT, single photon emission computerized tomography; [^{123}I]IBZM, [^{123}I](*S*)-(−)-3-iodo-2-hydroxy-6-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide.

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Therefore, measuring the reduction in [^{123}I]IBZM binding potential following amphetamine provides a noninvasive method to estimate the magnitude of amphetamine-induced dopamine release in the vicinity of the receptors (which includes, but is not restricted to, the synaptic space). Preliminary experiments in healthy volunteers demonstrated the feasibility of this method in humans (25).

Acute exposure to amphetamine induces emergence or worsening of positive symptoms in schizophrenic patients at doses that do not produce psychotic symptoms in healthy subjects (for review, see ref. 26). The neuronal mechanisms underlying this sensitivity of schizophrenic patients to the psychotogenic effect of amphetamine are not known. Preclinical data suggest that this exaggerated response might be associated with enhanced dopamine release (27). To test this hypothesis, we measured the amphetamine-induced reduction in [^{123}I]IBZM binding potential in fifteen drug free patients with schizophrenia and fifteen healthy controls matched for age, gender, race, and parental socioeconomic status.

METHODS

The study was performed according to protocols approved by Yale School of Medicine and West Haven Veterans Affairs Internal Review Boards. Inclusion criteria for patients were as follows: (*i*) diagnosis of schizophrenia according to Diagnostic and Statistical Manual (DSM-IV); (*ii*) no other DSM-IV axis I diagnosis; (*iii*) no history of alcohol or substance abuse or dependence; (*iv*) absence of any psychotropic medication for at least 21 days before the study (with the exception of lorazepam, which was allowed at a maximal dose of 3 mg per day up to 24 h before the study); (*v*) no concomitant or past severe medical conditions; (*vi*) no pregnancy; (*vii*) no current suicidal or homicidal ideation; and (*viii*) ability to provide informed consent. After explanation of the nature and risks of the study, the ability of the patient to provide informed consent was formally evaluated by asking the patient to complete a multiple-choice questionnaire (available on request). According to the recommendation of the National Alliance for the Mentally Ill (Arlington, VA), consent from involved family members was also obtained. All patients were admitted to a research ward for the duration of the study (including the washout period).

Inclusion criteria for healthy controls were as follows: (*i*) absence of past or present neurological or psychiatric illnesses; (*ii*) no concomitant or past severe medical conditions; (*iii*) no pregnancy; and (*iv*) informed consent. Healthy controls were individually matched to patients for age (±5 years), gender, race, and parental socioeconomic level. Socioeconomic level was assessed by education and employment using the Hollingstead scale (A. Hollingstead, Four-Factor Index of Social Status; work published by the author, 1975).

SPECT experiments were carried out as described (25). [^{123}I]IBZM with specific activity >5000 Ci/mmol and radiochemical purity >95% was prepared by direct electrophilic radioiodination of the desiodoprecursor BZM. An i.v. catheter was inserted in each arm of the subject, for drug administration and blood sampling, respectively. A total [^{123}I]IBZM dose of 10.5 ± 0.5 mCi (with this and subsequent values expressed as mean ± SEM) was given as a bolus (4.0 ± 0.2 mCi) followed by a continuous infusion at a rate of 1.1 ± 0.1 mCi/h for the duration of the experiment (375 min, with this and all subsequent times given in reference to the beginning of the radiotracer administration). This protocol of administration (bolus plus constant infusion) was shown, in preliminary experiments, to induce a state of sustained binding equilibrium: in the absence of amphetamine injection, both the specific and nonspecific activity remained at a constant level from 150 min to the end of the experiment (25).

SPECT data were acquired on the PRISM 3000 (Picker, Cleveland, OH) with high-resolution fan beam collimators (resolution at full-width half-maximum, 11 mm; ^{123}I point source sensitivity, 16.5 counts/s per μCi). Two scanning sessions were obtained for each subject (before and after amphetamine injection). Each scanning session consisted of eight consecutive acquisitions of 8 min each. The first scanning session was obtained from 180 min to 244 min. After completion of the first scanning session, amphetamine (dextroamphetamine sulfate) was injected i.v. at a dose of 0.3 mg/kg over 30 s. Experiments in baboons established that it takes ≈60 min for [^{123}I]IBZM displacement to be achieved after amphetamine challenge. Therefore, subjects were not scanned during the 60 min following the amphetamine injection and were available for evaluation of the psychiatric response to amphetamine. The second scanning session (post-amphetamine session) was obtained from 310 min to 374 min.

Plasma metabolite-corrected [^{123}I]IBZM steady-state concentration (C_{SS}) was measured by extraction followed by high-pressure liquid chromatography on nine venous samples collected at 20-min intervals from 180 to 300 min (25). Determination of the plasma [^{123}I]IBZM free fraction (f_1) was performed by ultrafiltration (Centrifree; Amicon) (28). Plasma [^{123}I]IBZM clearance was calculated as the ratio of C_{SS} to infusion rate. Amphetamine plasma concentration was measured by gas chromatography (National Medical Services, Willow Grove, PA) on three venous samples obtained at 10, 20, and 40 min post-amphetamine injection. Because no statistically significant differences were observed between these three amphetamine measurements (repeated measures ANOVA, $P = 0.17$), the average values were used in subsequent analyses.

The clinical response to the amphetamine challenge was evaluated with the Positive and Negative Symptom Scales (29). Baseline ratings were obtained 60 min before the first scanning session. Post-amphetamine ratings were obtained 30 min after the injection of amphetamine (i.e., during the interval between the first and second scanning session). For positive and negative subscales, a change of at least four points relative to baseline was considered clinically significant. Behavioral response was also rated by the subjects using a simplified version of the Amphetamine Interview Rating Scale (30). Self ratings for euphoria, restlessness, alertness, and anxiety were obtained at various intervals, using a ten-point analog scale (25). Responses were calculated as peak minus baseline scores.

SPECT data were analyzed blind to the diagnosis. 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 four external fiducial markers glued to the subject's head. The four slices with highest striatal uptake were summed and attenuation corrected assuming uniform attenuation. Standard region of interest profiles (striatum 556 mm^2; occipital 2204 mm^2) were positioned on the summed images. The camera resolution did not allow differentiating counts originating from the dorsal (sensorimotor) or ventral (limbic) striatum. Thus, the striatal region included both components. Right and left striatal regions were averaged. Striatal specific binding was calculated as striatal minus occipital activity. The occipital region was selected as the background region because (*i*) the density of dopamine D_2 receptors is negligible in this region compared with the striatum (31); (*ii*) this region can be identified with greater reliability than the cerebellum; and (*iii*) in humans, [^{123}I]IBZM activity in the occipital region is equal to the nonspecific activity in the striatum (32).

The baseline [^{123}I]IBZM binding potential (ml·g^{-1}), corresponding to the product of the free 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 calculated as the ratio of striatal specific binding (μCi per g of brain tissue) to the steady-state free unmetabolized plasma tracer concentration (f_1C_{SS}, μCi

per ml of plasma) measured during scanning session 1 (25). For each scanning session, the specific to nonspecific equilibrium partition coefficient was calculated as the ratio of striatal minus occipital to occipital activity. Under steady-state conditions, the decrease in specific to nonspecific partition coefficient is equivalent to the decrease in binding potential (see equations in ref. 25). Amphetamine-induced decrease in [^{123}I]IBZM binding potential was expressed in percentage of pre-amphetamine value.

Unless otherwise specified, between-groups comparisons were performed with two-tailed unpaired *t* tests. Relationships between continuous variables were analyzed with the Pearson product moment correlation coefficient. A probability value of 0.05 was selected as significance level. Because of lack or loss of the second i.v. line, plasma samples for [^{123}I]IBZM measurement could not be obtained in one patient and one control. In these subjects, the relative decrease in [^{123}I]IBZM binding potential could be calculated, but not the absolute value of the baseline binding potential. For similar reasons, plasma samples for amphetamine measurement could not be obtained in three patients and one control.

RESULTS

Eighteen patients with schizophrenia were recruited for this study. One patient was neuroleptic-naive, eight patients were neuroleptic-free at the time of recruitment for reasons unrelated to the study (such as noncompliance or intolerance), and nine patients were taking neuroleptics and/or other psychotropic drugs at the time of recruitment. Because of clinical deterioration, medication was initiated or resumed before the end of the washout period in three patients. Thus, a total of 15 patients completed the protocol with a mean time off neuroleptic medication of 192 ± 141 days (range 21 days to 5 years). Five patients received lorazepam during the washout period. Mean duration of illness was 14 ± 2 years. Brief Psychiatric Rating Scale (33) scores were 37 ± 3 points, and Positive and Negative Symptom Scales scores at baseline were 16.6 ± 1.7 points for positive symptoms subscale and 14.9 ± 1.5 points for negative symptoms subscale. One patient presented mild tardive dyskinesia at baseline. Other patients were free of motor symptoms. Controls were matched with patients for age, gender, race, and parental socioeconomic status (Table 1).

The two groups did not differ in experimental parameters such as [^{123}I]IBZM total injected dose (schizophrenics, 11.0 ± 0.6 mCi; controls, 10.4 + 0.7 mCi; $P = 0.51$), effective bolus to hourly infusion ratio (schizophrenics, 3.88 ± 0.02 h; controls 3.87 ± 0.02 h; $P = 0.59$), or scanning time (start of session 1: schizophrenics, 179 ± 7 min; controls, 180 ± 8 min; $P = 0.74$; start of session 2: schizophrenics, 319 ± 7 min; controls, 316 ± 8 min; $P = 0.75$). No between-group differences were observed in [^{123}I]IBZM plasma clearance (schizophrenics, 70.3 ± 6.1 liter/h; controls, 71.6 ± 5.2 liter/h; $P = 0.87$) or in the [^{123}I]IBZM plasma free fraction (schizophrenics, 3.1 ± 0.3%; controls, 3.5 ± 0.1%; $P = 0.87$). The steady-state quality of the plasma input function was evaluated by the slope of the metabolite-corrected plasma [^{123}I]IBZM concentration from 180 to 300 min. This slope was small and not different between groups (schizophrenics, +2.1 ± 2.5% per h; controls, +2.1 ± 1.7% per h; $P = 0.98$). Similarly, the slope of the occipital activity from the beginning of scanning session 1 to the end of scanning session 2 was negligible and did not differ between the groups (schizophrenics, +1.0 ± 0.7% per h, controls, +1.1 ± 1.2% per h; $P = 0.94$). None of these slope distributions had a mean value significantly different from zero (one-sample *t* test, plasma [^{123}I]IBZM, $P = 0.18$; occipital [^{123}I]IBZM, $P = 0.13$), indicating that an adequate steady-state input function was achieved in both groups.

Table 1. Demographic characteristics

Measure	Control subjects	Patients with schizophrenia
n	15	15
Age	41 ± 2	42 ± 2
Sex	14 M, 1 F	14 M, 1 F
Race	9 C, 5 AA, 1 H	9 C, 5 AA, 1 H
Parental SES	31 ± 3	37 ± 4
Subject SES	37 ± 4	24 ± 3*

Values are mean ± SEM. M, male; F, female; C, Caucasians; AA, African-Americans; H, Hispanics; and SES, socioeconomic status.
*Unpaired two-tailed *t* test; $P = 0.011$.

In agreement with previous data obtained with [^{123}I]IBZM and [^{11}C]raclopride in neuroleptic-naive schizophrenic patients (8, 10, 11), the density of D_2 receptors at baseline was not statistically different between schizophrenics and controls ($t = 1.35$, df = 26, $P = 0.18$; Table 2). Thus, potential D_2 receptor upregulation induced by previous neuroleptic treatment was not observed at the time of the study. The variance of baseline [^{123}I]IBZM binding potential was not statistically different between the groups (*F*-test for variance ratio: $F = 1.61$, $P = 0.40$).

The amphetamine-induced decrease in [^{123}I]IBZM binding potential was significantly larger in schizophrenic patients (−19.5 ± 4.1%) than in controls (−7.6 ± 2.1%; $t = 2.62$, df = 28, $P = 0.014$, Fig. 1 and Fig. 2 and Table 2). Schizophrenic patients exhibited larger displacement than control subjects in 12 of 15 pairs (paired *t* test: $t = 2.73$, df = 14, $P = 0.016$). The variance of the amphetamine effect on [^{123}I]IBZM binding potential was larger in the schizophrenic than in the control groups (variance ratio 3.85, $F = 3.85$, $P = 0.0167$). Therefore,

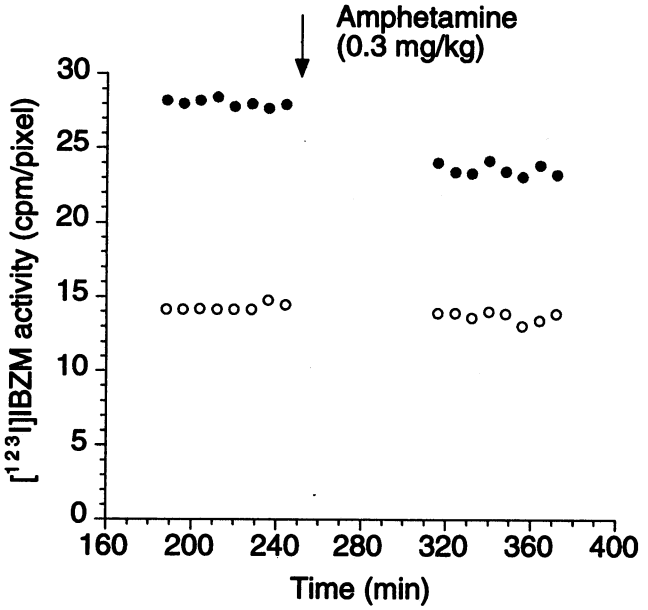

FIG. 1. [^{123}I]IBZM activity (in μCi/ml) in striatum (●) and occipital (○) before and after amphetamine challenge in a patient with schizophrenia. [^{123}I]IBZM was given as a bolus (4.6 mCi), followed by infusion at a constant rate of 1.2 mCi/h for the duration of the experiment (375 min). After establishment of steady state, a first scanning session was obtained from 180 to 244 min. Amphetamine (0.3 mg/kg i.v. bolus) was injected just after completion of the first scanning session (250 min, arrow). A second scanning session was obtained from 310 min to 374 min. Occipital activity was used to estimate the nonspecific binding in the striatum. Striatal specific binding to D_2 receptors was calculated by the difference between striatal and occipital activities. The reduction in [^{123}I]IBZM binding potential induced by amphetamine was calculated as the difference between the specific to nonspecific ratio measured during the first (0.99 ± 0.02) and second (0.77 ± 0.02) session and expressed in percentage of the baseline value (−22%).

Table 2. Measurement of D_2 receptor availability at baseline and after amphetamine

Measure	Control subjects	Patients with schizophrenia
Baseline [^{123}I]IBZM binding potential, ml/g	178 ± 12 (14)	204 ± 15 (14)
Amphetamine effect on [^{123}I]IBZM binding potential, % decrease	−7.6 ± 2.1% (15)	−19.5 ± 4.1% (15)*
Amphetamine plasma level, ng/ml	32 ± 3 (14)	35 ± 9 (12)

Values are mean ± SEM. Number of subjects (n) are in parentheses.
*Unpaired two-tailed t test; $P = 0.014$.

we also performed nonparametric analyses on this variable with similar results (unpaired analysis: Mann-Whitney U, $P = 0.034$; paired analysis: Wilcoxon Signed Rank, $P = 0.014$).

No significant between-groups difference was observed in amphetamine plasma levels ($P = 0.41$; Table 2). No correlation was observed between amphetamine plasma levels and amphetamine-induced decreases in [^{123}I]IBZM binding potential, either in the entire sample ($r = 0.08$, $P = 0.68$) or in each group considered separately (schizophrenics, $r = 0.24$, $P = 0.45$; controls, $r = 0.14$, $P = 0.63$). Consequently, the group difference in amphetamine-induced [^{123}I]IBZM binding potential decrease could not be attributed to differences in amphetamine disposition. Amphetamine induced a transient (60–90 min) increase in systolic and diastolic blood pressure. The blood pressure response did not differ between the groups (peak systolic blood pressure above baseline: schizophrenics, 43 ± 5 mm Hg; controls, 49 ± 4 mm Hg, $P = 0.31$; peak diastolic above baseline: schizophrenics, 18 ± 2 mm Hg; controls, 22 ± 2 mm Hg, $P = 0.11$).

No psychotic symptoms were observed after the amphetamine injection in controls. In patients, the clinical response was heterogeneous. Amphetamine induced clinically significant worsening in positive psychotic symptoms in six patients, improvement in three patients, and no significant change in six patients. This distribution was consistent with the previously reported prevalence of psychotic reactions to acute challenge with dopamine agonists in schizophrenia (26). Negative symptoms improved significantly in four patients and did not change in 11 patients. All observable clinical changes were transient and, by the end of the experiment, patients had recovered their pre-amphetamine clinical status.

Schizophrenic patients who experienced worsening in positive symptoms showed larger reductions in [^{123}I]IBZM binding potential (−27.6 ± 6.4%, $n = 6$) than schizophrenic patients whose positive symptoms did not worsen (−14.1 ± 4.6%, $n = 9$) and healthy controls (−7.6 ± 2.1%, $n = 15$, ANOVA: $F = 6.31$, $P = 0.0056$, Kruskal–Wallis: $P = 0.031$). In the schizophrenic group, the magnitude of the amphetamine effect on [^{123}I]IBZM binding potential was positively correlated with changes in positive symptoms ($r = 0.53$, $P = 0.038$; Fig. 3). Such a correlation was not observed with changes in negative symptoms ($r = 0.40$, $P = 0.14$). Schizophrenic and controls did not differ in general behavioral activation scores measured with the Amphetamine Interview Rating Scale: euphoria (schizophrenics, +2.0 ± 0.5; controls, +2.7 ± 0.5, $P = 0.35$), restlessness (schizophrenics, +2.6 ± 0.6; controls, +1.8 ± 0.5, $P = 0.34$), alertness (schizophrenics, +2.5 ± 0.4; controls, +2.5 ± 0.7, $P = 1$), and anxiety (schizophrenics, +2.7 ± 0.5; controls, +2.6 ± 0.6, $P = 0.87$).

In the schizophrenic group, the amphetamine effect on [^{123}I]IBZM binding potential was not correlated with severity of positive symptoms at baseline ($r = 0.03$, $P = 0.92$), duration of illness ($r = 0.09$, $P = 0.75$), duration of neuroleptic-free interval before the scan ($r = 0.30$, $P = 0.30$), or lifetime neuroleptic exposure ($r = 0.33$, $P = 0.22$). No difference was observed in the amphetamine effect on [^{123}I]IBZM binding potential between the schizophrenic patients who received lorazepam during the 21 days before the study (−16.6 ± 6.5%, $n = 5$) and the ones who did not (−21.0 + 5.3%, $n = 10$, $P = 0.63$). The effect of age on amphetamine-induced decrease in [^{123}I]IBZM binding potential could not be studied in these samples, because of the narrow age range of the subjects.

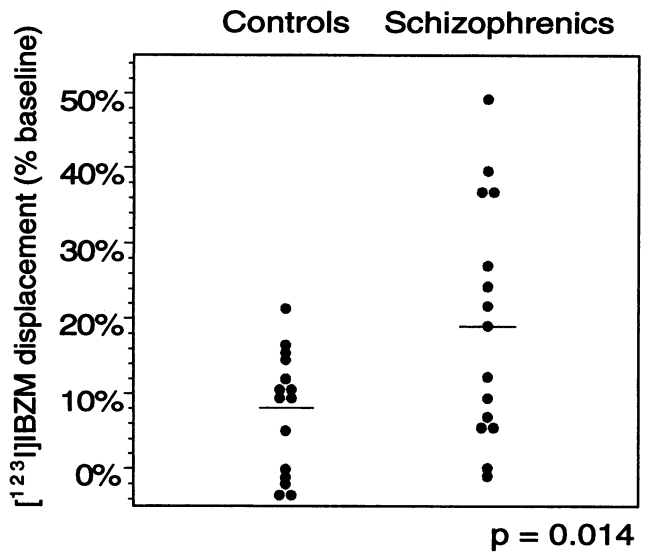

FIG. 2. Amphetamine-induced relative decrease in [^{123}I]IBZM binding potential in 15 healthy controls and 15 patients with schizophrenia, matched for age, sex, race, and parental socioeconomic level.

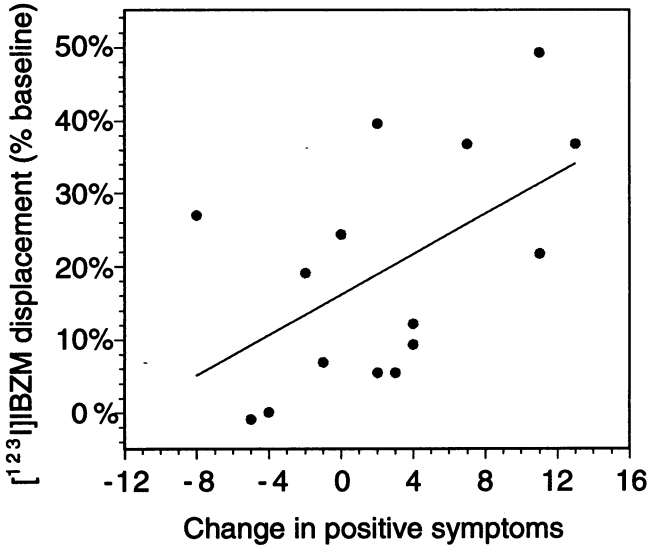

FIG. 3. Relationship between amphetamine-induced changes in positive symptoms and amphetamine-induced relative decrease in [^{123}I]IBZM binding potential in the schizophrenic group ($n = 15$, $r = 0.53$, $P = 0.038$).

DISCUSSION

This study represents the first attempt to measure directly *in vivo* striatal dopamine transmission in patients with schizophrenia. The data indicate that more D_2 receptors are occupied by dopamine following amphetamine challenge in schizophrenic patients than in matched healthy controls. This increased response to amphetamine could not be attributed to differences in peripheral amphetamine disposition, since amphetamine plasma levels were similar in both groups and not related to the amphetamine effect on [^{123}I]IBZM binding potential. Furthermore, blood pressure response to amphetamine was similar between the groups. The increased effect of amphetamine on [^{123}I]IBZM binding potential in the schizophrenic group did not appear to be a remote effect of prior neuroleptic exposure, as the effect was not associated with lifetime neuroleptic exposure or duration of the neuroleptic-free period prior to the scan. Furthermore, chronic treatment with typical neuroleptic does not affect amphetamine-induced dopamine release as measured with microdialysis in rodents (34). Similarly, the exaggerated response observed in the schizophrenic group did not appear to be due to lorazepam administration. Patients who did not receive lorazepam during the drug-free period displayed the same level of response as the five patients who did receive lorazepam. Therefore, it is plausible that the increased effect of amphetamine on [^{123}I]IBZM binding potential reflects an abnormal response of the dopaminergic system associated with the disease process *per se*.

The increased displacement of [^{123}I]IBZM binding following dopamine release observed in the schizophrenic group could reflect either an increased affinity of D_2 receptors for dopamine or an increased concentration of dopamine in the vicinity of the receptors, or some combination of both factors. Available data do not support the existence of an increased affinity of D_2 receptors for agonists in schizophrenia: the sequence of the D_2 receptor gene is not altered (35) and the binding of dopamine agonists in postmortem striata is not increased in schizophrenia (36, 37). Nevertheless, a decreased dopamine concentration at baseline would result in an effective increased affinity of the unoccupied D_2 receptors (for both agonists and antagonists). Again, available data do not support the existence of a marked reduction in baseline dopamine in schizophrenia, since the *in vivo* affinity of [^{11}C]raclopride is not elevated in patients with this condition (8, 10). Therefore, while a contribution of the affinity factor cannot be definitively excluded, an increased concentration of dopamine in the vicinity of the receptors is likely to be the predominant mechanism underlying the observed effect.

Amphetamine increases extracellular dopamine concentration by various mechanisms: facilitation of transporter-mediated release of cytoplasmic dopamine (38), redistribution of dopamine from vesicular to cytoplasmic pool (39), inhibition of uptake (40), inhibition of monoamine oxidase activity (41), and calcium-dependent stimulation of dopamine synthesis (42). Any of these factors could be implicated in the exaggerated response observed in the patients. Interestingly, a recent positron emission tomography study has reported increased accumulation of the dopamine precursor 6-[^{18}F]fluoro-L-dopa in the striatum of patients with schizophrenia (43). An increase in enzymatic activity associated with dopamine synthesis might lead to the constitution of larger cytoplasmic and/or vesicular pool and to a larger amphetamine-induced dopamine release.

The mechanism of this putative increased dopaminergic neuronal reactivity remains to be elucidated. Corticofugal glutamatergic projections that increase the responsiveness of dopaminergic subcortical systems are inhibited by dopaminergic prefrontal projections, both directly and indirectly via GABAergic interneurons (44, 45). This glutamatergic cortical control occurs primarily through projections to the dopamine cell body area rather than the terminal region (46). In nonhuman primates, selective destruction of dopamine terminals in dorsolateral, medial, and orbital regions of the prefrontal cortex does not affect striatal baseline dopamine concentration but induces a long-lasting increase in striatal potassium-induced dopamine release (47). Since potassium, like amphetamine, stimulates both dopamine synthesis and release (48), this observation is potentially relevant to the present finding. Thus, the increased responsiveness of subcortical dopamine neurons observed in this study might be secondary to prefrontal dopaminergic or GABAergic deficits as both deficits have been proposed as constituents of the "cortical pathology" in schizophrenia (49, 50).

A large variability in the amphetamine effect was evident in the schizophrenic group, and three patients showed lower amphetamine-induced [^{123}I]IBZM displacement than their matched controls. This heterogeneity in the biochemical response to amphetamine matched the heterogeneity of the clinical response well and indicated that the abnormality revealed by this study is not present in all patients with schizophrenia. The correlation between [^{123}I]IBZM displacement and the emergence or exacerbation of positive symptoms supports the role of increased dopamine transmission in the genesis of these symptoms. Yet, this correlation was relatively weak, and two schizophrenic subjects experienced a psychotic reaction despite [^{123}I]IBZM displacement values overlapping with control values. Therefore, increased dopamine transmission at the D_2 receptors is not the only factor contributing to a psychotic response to amphetamine in schizophrenic subjects.

Several limitations of this study should be mentioned. (*i*) Because of the lack of placebo control, we could not assess the respective contribution of amphetamine and of the stress associated with the experimental setting to the induction of psychotic reactions. However, this limitation does not affect the observation that a psychotic response (whether due to amphetamine or stress or both) was associated with increased dopamine release. (*ii*) Patients included in this study were able to provide informed consent and to comply with this demanding protocol. Thus, patients devoid of insight about their illness or with major psychotic symptoms were excluded. The impact of this selection bias on the results is not known. (*iii*) While we failed to document that increased amphetamine-induced dopamine release was associated with previous neuroleptic exposure, potential impact of prior medication on the observed effect could not be definitively ruled out. Studies in neuroleptic-naive patients are needed to address this issue. (*iv*) Considerable preclinical evidence supports the hypothesis that antipsychotic drug action is associated with dopamine antagonism in the mesolimbic rather than the nigrostriatal dopaminergic projections (for review, see ref. 45). The limited resolution of the camera precluded evaluation in humans of the respective contributions of limbic versus sensorimotor striatal dopamine release in the production of psychotic symptoms. However, the results of this study might support the contention that dopamine hyperactivity in schizophrenia is not limited to the mesolimbic system (51). (*v*) This study measured only the relative increase in dopamine release following amphetamine challenge and did not provide information about dopamine release at baseline. We are currently developing a dopamine depletion paradigm which, coupled with SPECT imaging, might provide absolute measurement of baseline dopamine concentration in the vicinity of D_2 receptors.

In conclusion, this study used a newly developed noninvasive method to measure amphetamine-induced dopamine release in patients with schizophrenia and suggested the existence of a dysregulation of dopamine neurons in schizophrenia, leading to an increased dopamine transmission in response to amphetamine. If independently replicated, this observation would support the time-honored dopaminergic hypothesis of schizophrenia.

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