Modulation of Amphetamine-Induced Striatal Dopamine Release by Ketamine in Humans: Implications for Schizophrenia

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Background: Recent brain imaging studies have indicated that schizophrenia is associated with increased amphetamine-induced dopamine release in the striatum. It has long been hypothesized that dysregulation of subcortical dopamine systems in schizophrenia might result from a failure of the prefrontal cortex (PFC) to adequately control subcortical dopaminergic function. The activity of midbrain dopaminergic neurons is regulated, in part, by glutamatergic projections from the PFC acting via glutamatergic N-methyl-D-aspartate (NMDA) receptors. The goal of this study was to test the hypothesis that a pharmacologically induced disruption of NMDA transmission leads to an increase in amphetamine-induced dopamine release in humans.

Methods: In eight healthy volunteers, we compared striatal amphetamine-induced (0.25 mg/kg) dopamine release under control conditions and under sustained disruption of NMDA transmission induced by infusion of the noncompetitive NMDA antagonist ketamine (0.2 mg/kg intravenous bolus followed by 0.4 mg/kg/hour intravenous infusion for 4 hours). Amphetamine-induced dopamine release was determined with single photon emission computed tomography, as the reduction in the binding potential (BP) of the radiolabeled D2 receptor antagonist [123I]IBZM.

Results: Ketamine significantly enhanced the amphetamine-induced decrease in [123I]IBZM BP, from 25.5% ± 3.5% under control conditions to 212.8% ± 8.8% under ketamine pretreatment (repeated-measures analysis of variance, p = .023).

Conclusions: The increase in amphetamine-induced dopamine release induced by ketamine (greater than twofold) was comparable in magnitude to the exaggerated response seen in patients with schizophrenia. These data are consistent with the hypothesis that the alteration of dopamine release revealed by amphetamine challenge in schizophrenia results from a disruption of glutamatergic neuronal systems regulating dopaminergic cell activity. Biol Psychiatry 2000;48:627–640 © 2000 Society of Biological Psychiatry

Key Words: Schizophrenia, SPECT, dopamine, [123I]IBZM, glutamate, ketamine, amphetamine

Introduction

The classic dopamine hypothesis of schizophrenia proposes that hyperactivity of dopamine transmission is responsible for positive symptoms of the disorder (Carlsson and Lindqvist 1963). This hypothesis was traditionally supported by the correlation between clinical doses of antipsychotic drugs and their potency to block dopamine D2 receptors (Creese et al 1976; Seeman and Lee 1975), and by the psychotogenic effects of dopamine-enhancing drugs (for reviews, see Angrist and van Kammen 1984; Lieberman et al 1987). More recently, the hypothesis of a dysregulation of dopamine function in schizophrenia received additional support from the observation of increased striatal dopamine transmission following acute amphetamine challenge in untreated patients with schizophrenia. Amphetamine-induced increase in D2 receptor occupancy by dopamine was measured as the reduction in the binding potential (BP) of the single photon emission computed tomography (SPECT) radiotracer [123I]IBZM or the positron emission tomography radiotracer [11C]raclopride. In three independent cohorts, the amphetamine-induced reduction in radiotracer BP was found to be significantly increased in patients with schizophrenia, as compared with matched healthy control subjects (Abi-Dargham et al 1998; Breier et al 1997b; Laruelle et al 1996). This exaggerated response was also documented in first-episode patients never previously exposed to antipsychotic drugs (Laruelle et al 1997). Together, these data support the hypothesis of a dysregulation of dopamine release in schizophrenia (Laruelle and Abi-Dargham 1999).
The pathophysiologic mechanism underlying this dysregulation remains to be elucidated. The amphetamine challenge might reveal a primary abnormality of dopaminergic neurons in schizophrenia or, more likely, a deficiency in the feedback mechanisms that modulate dopaminergic cell activity following amphetamine exposure. Abnormalities of the glutamatergic afferents from the prefrontal cortex (PFC) to the midbrain dopaminergic cell areas are likely to be implicated in this deficient regulation, given the evidence for deficits in PFC function in schizophrenia (for reviews, see Goldman-Rakic and Selemon 1997; Weinberger and Berman 1996).

Since positive symptoms are more sensitive than negative symptoms to direct manipulation of the dopamine system, hyperactivity of dopamine transmission is more relevant to positive symptoms than to cognitive and negative ones (Crow 1980). The hypothesis that schizophrenia might be associated with a deficiency of N-methyl-D-aspartate (NMDA) receptor function addresses some of the limitations of the dopamine hyperactivity model (Javitt and Zukin 1991; Olney and Farber 1995; Tamminga et al 1995). In contrast to dopamine agonists, noncompetitive NMDA antagonists such as phencyclidine (PCP) and ketamine induce positive and negative symptoms in healthy and schizophrenic subjects (Allen and Young 1978; Collier 1972; Krystal et al 1994; Lahti et al 1995). Thus, NMDA receptor hypofunction might offer a more compelling pharmacologic model of the illness than the dopamine hyperactivity model. In addition, it has been postulated that the dysregulation of dopamine transmission in schizophrenia might itself be secondary to an alteration in NMDA transmission (Grace 1991; Olney and Farber 1995).

Recent data in rodents offer a putative direct link between the NMDA hypofunction hypothesis and the alteration of dopamine transmission actually observed in patients with schizophrenia (i.e., increased response to amphetamine). Miller and Abercrombie (1996) reported that pretreatment with the noncompetitive antagonist MK-801 resulted in a large potentiation of amphetamine-induced dopamine release measured under conditions of impaired NMDA transmission would be significantly elevated compared with amphetamine-induced dopamine release measured under control conditions.

**Methods and Materials**

**Subjects**

Eight healthy subjects (three female, five male; ages 27 ± 2 years), never previously exposed to psychostimulants, were studied twice (control and ketamine conditions), at intervals of 8 ± 4 days (range, 5–16 days). Two subjects were African American, four white, and two Hispanic. Inclusion criteria were 1) absence of past or present neurologic or psychiatric illness, 2) no current or past cardiovascular conditions, 3) no pregnancy, and 4) no prior exposure to psychostimulants.

Intravenous (IV) administration of both $^{123}$IIBZM and amphetamine were approved by the Food and Drug Administration under an Investigational New Drug protocol. The protocol was approved by the institutional review boards of Columbia Presbyterian Medical Center and New York State Psychiatric Institute. All subjects gave written informed consent for the study, after detailed explanation of the nature and risks of the study. The information relative to the risks of the study included discussion of risks associated with radioactivity exposure, cardiovascular side effects (transient increase in blood pressure), expected side effects of the challenges (transient induction of dissociative or psychotic state), and exposure to drugs of abuse potential (ketamine and amphetamine).

**Radioligand Preparation**

$^{123}$IIBZM was prepared by direct electrophilic radiiodination of the phenolic precursor BZM \([\delta(\sim)-N-[(1-ethyl-2-pyrroldinyl)methyl]-2-hydroxy-6-methoxybenzamide]\) as described (Kung and Kung 1990; Zea-Ponce and Laruelle, in press). Labeling yield was 69% ± 7%, the radiochemical yield 63% ± 8%, and the radiochemical purity 98% ± 0.6% \((n = 16)\).

**Study Design**

A schematic representation of the study protocol is presented in Figure 1. The previously described $^{123}$IIBZM constant infusion technique was used to perform the experiments under sustained equilibrium binding conditions (Laruelle et al 1994b, 1995). Two
of this information avoided the necessity of a counterbalanced design for this study.

**Imaging Protocol**

**CONTROL DAY.** To decrease radiation exposure to the thyroid gland, subjects received 0.6 g potassium iodide 60 min before \([^{123}I]IBZM\) injection. Four fiducial markers each containing 3 μCi of \([^{99m}Tc]NaTcO_4\) were attached to each side of the subject’s head at the level of the cantho-meatal line. An IV catheter was inserted in each arm, one for blood sampling and the other for amphetamine injection and ligand administration. \([^{123}I]IBZM\) was administered as a priming bolus, immediately followed by a continuous infusion at a constant rate (bolus/infusion ratio, 3.92 : 1) for the duration of the experiment (360 min). The total injected dose was 10.1 ± 0.7 mCi, decay corrected to the beginning of the experiment. We previously established that, using this administration schedule, stable activity levels in plasma and brain are observed from 150 min until the end of the infusion. During the first 180 min of the infusion subjects were allowed to relax in a comfortable setting, in a room adjacent to the camera room. The first scanning session (preamphetamine) was initiated at 180 min and lasted 60 min (Figure 1A). Single photon emission computed tomography data were obtained with the triple-head PRISM 3000 (Picker, Cleveland), equipped with low-energy ultra–high-resolution fan beam collimators (full width at half maximum 8–10 mm). Scanning was performed with the following acquisition parameters: continuous acquisition mode; matrix, 64 × 64 × 32; angular range, 120; angular steps, three; seconds per step, 18; frame duration, 12 min; number of frames, five; and radius of rotation, 13.5 cm. Amphetamine (0.25 mg/kg, IV) was injected shortly after completion of the first scanning session (240 min). Amphetamine injection was followed by 60 min of behavioral ratings, as detailed below, and vital signs monitoring. The second scan session (postamphetamine) was obtained using similar parameters from 300 to 360 min.

**KETAMINE DAY.** The only change to the above protocol consisted of the use of one additional IV catheter for ketamine administration. Ketamine hydrochloride (S-R-racemic) was administered as a bolus (0.2 mg/kg) at 60 min before the first SPECT scan (i.e., 120 min after beginning the radioligand infusion), followed by a constant infusion of 0.4 mg/kg/hour for the remaining 4 hours of the study (Figure 1B). All subjects were

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**Figure 1. Protocol timeline of the first study day (A, control day) and of the second study day (B, ketamine day).** The control day included two single photon emission computed tomography scans (60-min acquisition time) separated by 1 hour with intervening amphetamine bolus injection (0.25 mg/kg), all under sustained equilibrium administration of \([^{123}I]IBZM\). The ketamine experiment was conducted under the same experimental conditions except that, in addition, a bolus injection of ketamine (0.2 mg/kg) was given at 120 min, followed by a constant infusion of ketamine (0.4 mg/kg/hour) for the remaining 4 hours of the study.
admitted to the Irving Center for Clinical Research at Columbia Presbyterian Medical Center on the ketamine condition day and were discharged after an overnight stay and physician clearance the next morning.

**Image Analysis**

Image analysis was performed as previously described (Laruelle et al 1995). Briefly, frames were transferred into the MEDx software system (Sensor Systems, Sterling, VA) and corrected for attenuation assuming uniform attenuation (attenuation coefficient \( \mu = 0.10 \text{ cm}^2/\text{g} \)) within an ellipse drawn around the skull as identified by fiducial markers. Frames were realigned to each other, using a least-squares algorithm for within-modalities coregistration (automated image registration; Woods et al 1992).

Standard regions of interest of constant size were used to analyze all studies. Nonspecific activity was estimated as the average of the frontal and occipital regional activities, since these regions can be identified with greater reliability than the cerebellum. For each scanning session, the specific to nonspecific equilibrium ratio (\( V_s/V_n \)) was calculated as the ratio of striatal minus nonspecific to nonspecific activity (Laruelle et al 1994a). Under steady-state conditions, and assuming that amphetamine does not affect \([^{123}\text{I}]\text{IBZM}\) nonspecific binding, the percent decrease in \( V_s/V_n \) is equal to the percent decrease in BP (Laruelle et al 1995). Amphetamine-induced decrease in \([^{123}\text{I}]\text{IBZM}\) BP was expressed as a percentage of the preamphetamine value.

**Amphetamine and Ketamine Plasma Measurement**

Amphetamine plasma levels were measured in three venous samples obtained at 10, 20, and 40 min after amphetamine injection. Amphetamine was quantified as its N-heptafluorobutyl derivative via gas chromatography/mass spectroscopy utilizing a capillary column with mass spectrometer with simultaneous ion monitoring in the negative chemical ionization mode utilizing a capillary column with mass spectrometer with simultaneous ion monitoring in the negative chemical ionization mode. The method is essentially the same as described by Reimer et al (1993) with the following modifications: a 30-m DB-17 capillary column was substituted to improve separation and peak symmetry. Trideuterated amphetamine was used as the internal standard. Standard curves for both compounds are uniformly linear \((r > .99)\) over the range tested \((0.1–500 \text{ ng/mL})\) with negligible intercepts. Sensitivity is <0.1 ng/mL for each when 1 mL of plasma is extracted. Interassay RSD% is 5.2% at 5 ng/mL.

Ketamine plasma levels were measured in three venous samples obtained at 1, 2, and 3 hours after beginning the ketamine infusion. Plasma ketamine and norketamine were assayed using a validated liquid chromatographic (LC) procedure with UV detection. Following the addition of 500 ng of internal standard (2-phenylnormorpholinol, BW306U), ketamine and the metabolite norketamine were extracted from 1 mL of plasma, made alkaline with 0.5 mol/L NaOH, with 5.0 mL of 1.5% isoamyl alcohol in n-heptane. The organic extract was back-extracted with 0.25 mL of 0.01 mol/L HCl and transferred to inserts for injection on the LC. Chromatography was performed using a trimethylsilyl-bonded silica column (LC-1, Supelco, Bellefonte, PA) with a mobile phase consisting of 85% phosphoric acid and 15% acetonitrile, adjusted to pH of 2.4 with phosphoric acid, triethylamine, and heptane sulfonate. At a flow rate of 2.0 mL/min, ketamine, norketamine, and the internal standard were separated and detected at a UV wavelength of 210 nm in less than 12 min. Within-day coefficient of variation of ketamine and norketamine did not exceed 10.6% \((range, 2000–25 \text{ ng/mL}; n = 12 for each of seven concentrations). Day-to-day variation of ketamine and norketamine quality controls at 1250, 250, and 50 ng/mL did not exceed 5.6% and 5.8%, respectively \((n = 20 days).\) The minimum quantifiable limits were set at 10 ng/mL for both ketamine and norketamine. Plasma levels were not obtained in one subject on the ketamine day because of difficulties with IV access for blood sampling.

**Behavioral Responses**

Behavioral responses to the challenges were measured with the Clinician Administered Dissociative Symptom Scale (CADSS; Krystal et al 1994), the Brief Psychiatric Rating Scale (BPRS; Overall and Gorham 1962), and a modified version of the Amphetamine Interview Rating Scale (Laruelle et al 1995). The CADSS consists of 27 items rated from 0, “not at all,” to 4, “extremely,” and includes 19 subjective items and eight observer items \((range, from 0 to 108).\) The BPRS consists of 18 items rated from 1, “not present,” to 7, “extremely severe.” A positive symptom subscale \((Bowers et al 1980; Kane et al 1988; Krystal et al 1993, 1994)\) was evaluated consisting of the four symptoms conceptual disorganization, suspiciousness, hallucinatory behavior, and unusual thought content. This positive subscale ranges from 4 to 28. A negative symptom subscale \((Krystal et al 1994; Thiemann et al 1987)\) was also separately evaluated containing the three items blunted affect, emotional withdrawal, and motor retardation, ranging from 3 to 21.

The CADSS and BPRS were administered at six time points during the study \((times are relative to the beginning of the \([^{123}\text{I}]\text{IBZM}\) infusion; Figure 1): Time 0, 90 min; Time 1, 120 min; Time 2, 160 min; Time 3, 240 min; Time 4, 270 min; and Time 5, 360 min. On the control days, Times 0, 1, 2, and 3 corresponded to baseline ratings and Times 4 and 5 corresponded to postamphetamine ratings \((amphetamine was administered at 240 min). On the ketamine day, Time 0 corresponded to the baseline rating; Times 1, 2, and 3 corresponded to ratings under ketamine alone; and Times 4 and 5 corresponded to ratings under both amphetamine and ketamine.

**Statistical Analyses**

All values are expressed as mean ± SD. Normality of the distribution of the outcome measures was assessed with Kolmogorov–Smirnov tests before submission to analysis of variance (ANOVA). Homogeneity of variance between groups was assessed with F test of variance ratio. Repeated-measures ANOVA was used to test for significant aggregate changes between control and ketamine conditions and for significant changes over time of the behavioral ratings. For repeated measures, degrees of freedom and \(p\) values were adjusted for time points autocorrelation when needed (Hunyé–Feldt adjustments).
Results

Imaging Data

Table 1 lists \([123\text{I}]\text{IBZM} V_3^\prime\prime\) values for both pre- and postamphetamine scans on the control day and the ketamine day. Figure 2 shows the individual amphetamine-induced percent decrease in BP under control conditions and under ketamine pretreatment, with each subject’s two data points connected.

On the test day, amphetamine induced a significant 5.5% ± 3.5% reduction in \([123\text{I}]\text{IBZM} V_3^\prime\prime\) [repeated-measures ANOVA, \(F(7,1) = 18.4, p = .003\)]. On the ketamine day, amphetamine reduced \([123\text{I}]\text{IBZM} V_3^\prime\prime\) by 12.8% ± 8.8% [repeated-measures ANOVA, \(F(7,1) = 17.4, p = .004\)]. The amphetamine-induced decrease in \([123\text{I}]\text{IBZM} V_3^\prime\prime\) on the control day, as compared with the control day [repeated-measures ANOVA, \(F(7,1) = 8.3, p = .023\)]. The variance of the amphetamine effect was significantly higher on the ketamine day [
\(F\) test of variance ratio, \(F(7,7) = 0.16, p = .03\)]. Because of this difference in variance, we also analyzed the significance of the between-day difference in amphetamine effect using a nonparametric test (Wilcoxon signed rank test), with similar results (significant difference between amphetamine effect on test and retest day, \(p = .049\)).

No differences were found between preamphetamine \([123\text{I}]\text{IBZM} V_3^\prime\prime\) on the control day (0.74 ± 0.06) and ketamine day [0.74 ± 0.07; repeated measures ANOVA, \(F(7,1) = 0.26, p = .87\)]. Thus no effect of ketamine alone was detected on \([123\text{I}]\text{IBZM} V_3^\prime\prime\).

The ketamine modulation of the amphetamine effect on \([123\text{I}]\text{IBZM} V_3^\prime\prime\) was assessed by calculating the relative increase in the amphetamine effect due to ketamine. A larger amphetamine-induced reduction in \([123\text{I}]\text{IBZM} V_3^\prime\prime\) on the ketamine day, as compared with the control day, was observed in six out of eight subjects. Ketamine increased the amphetamine effect by 183% ± 171% (range, from −11% to 474%). Thus, a large between-subject variability was observed in the ketamine modulation of the amphetamine effect.

We observed a nonsignificant association between the amphetamine effect on \([123\text{I}]\text{IBZM} V_3^\prime\prime\) on control and ketamine days (\(r^2 = .38, p = .09\)). The amphetamine effect under control conditions was not predictive of the magnitude of the ketamine modulation of this effect (\(r^2 = .28, p = .17\)).

The increased effect of amphetamine on \([123\text{I}]\text{IBZM} V_3^\prime\prime\) on the ketamine day was not due to differences in amphetamine plasma levels. On the control day, plasma amphetamine levels averaged over the three measurements for each subject were 35 ± 10 ng/mL (n = 8); on the ketamine day they were 33 ± 5 ng/mL [n = 7; repeated-measures ANOVA, \(F(7,1) = 0.026, p = .87\)]. No relationships were observed between amphetamine plasma levels and amphetamine-induced decrease in \([123\text{I}]\text{IBZM} V_3^\prime\prime\), either on the control day (\(r^2 = .04, p = .61\)) or on the ketamine day (\(r^2 = .04, p = .66\)).

Ketamine plasma levels, measured 2 hours from beginning of ketamine infusion (before amphetamine injection) and at 3 hours (after amphetamine injection), were 191 ± 38 ng/mL (n = 6) and 197 ± 52 ng/mL (n = 7), respectively. These values were not significantly different by repeated-measures ANOVA [\(F(5,1) = 2.1, p = .20\)]. Thus, the injection of amphetamine did not affect ketamine plasma levels. Ketamine plasma levels were not significantly correlated with the ketamine modulation of

Table 1. Amphetamine Effect on D2 Receptor Availability on Control and Ketamine Days

<table>
<thead>
<tr>
<th>Experimental day</th>
<th>Preamphetamine ([123\text{I}]\text{IBZM} V_3^\prime\prime) (unitless)</th>
<th>Postamphetamine ([123\text{I}]\text{IBZM} V_3^\prime\prime) (unitless)</th>
<th>Amphetamine-induced decrease in ([123\text{I}]\text{IBZM} BP) (% baseline)</th>
<th>Amphetamine plasma level (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control day</td>
<td>0.74 ± 0.06</td>
<td>0.70 ± 0.06</td>
<td>−5.5% ± 3.6%</td>
<td>35 ± 10</td>
</tr>
<tr>
<td>Ketamine day</td>
<td>0.74 ± 0.07</td>
<td>0.65 ± 0.07</td>
<td>−12.8% ± 8.8%</td>
<td>33 ± 5</td>
</tr>
</tbody>
</table>

BP, binding potential.

*p = .02 (repeated-measures analysis of variance).
repeated-measures ANOVA, \( F \) and postamphetamine levels of 169
6
ed-measures ANOVA, administration by repeated-measures ANOVA [Figure 3; repeat-
showed no significant changes with amphetamine admin-
dissociation. On control day, CADSS mean total scores
Behavioral Responses
the ketamine metabolite norketamine increased with time,
the amphetamine effect (\( r^2 = .28, p = .22 \)). The levels of
the ketamine metabolite norketamine increased with time,
with preamphetamine levels of 134 ± 39 ng/mL (n = 6)
and postamphetamine levels of 169 ± 52 ng/mL [n = 7;
repeated-measures ANOVA, \( F(5,1) = 12.7, p = .02 \)].

**Behaviors Responses**

**DISSOCIATION.** Amphetamine alone did not induce
dissociation. On control day, CADSS mean total scores
displayed no significant changes with amphetamine ad-
ministration by repeated-measures ANOVA [Figure 3; repeat-
ed-measures ANOVA, \( F(7,5) = 1.2, p = .28 \)]. In contrast,
ketamine produced significant levels of dissociation. On
ketamine day, CADSS mean total scores displayed signifi-
cant changes [repeated-measures ANOVA, \( F(7,5) = 3.9, p = .06 \)]. Post hoc analysis revealed that CADSS scores
were significantly elevated relative to baseline score (0 ± 0)
at every time point during ketamine administration,
with the exception of the first score obtained following
amphetamine bolus injection (Time 4): Time 0, 0 ± 0;
Time 1, 21 ± 20, \( p = .002 \); Time 2, 14 ± 14, \( p = .013 \);
Time 3, 12 ± 15, \( p = .034 \); Time 4, 5 ± 9, \( p = .33 \); Time
5, 12 ± 21, \( p = .031 \). Figure 3 illustrates the average
changes in dissociation over time in the control and
ketamine days. Thus, amphetamine injection resulted in a
transient improvement of ketamine-induced dissociation,
which, as noted, was not due to an impact of amphetamine
on ketamine plasma levels.

**POSITIVE SYMPTOMS.** Amphetamine alone did not
elicit positive symptoms: no significant changes in the
total of four positive items of the BPRS were observed on
the control day [repeated-measures ANOVA, \( F(7,5) = 1.0, p = .43 \)]. On the ketamine day, ketamine administra-
tion was followed by a mild increase in positive symptoms
that did not reach significance [repeated-measures
ANOVA, \( F(7,5) = 1.3, p = .28 \)]; Time 0, 4.0 ± 0; Time
1, 5.2 ± 1.5; Time 2, 5.2 ± 1.6; Time 3, 5.6 ± 3.1; Time
4, 5.1 ± 2.2; Time 5, 4.6 ± 1.7.

**NEGATIVE SYMPTOMS.** Amphetamine alone did not
elicit negative symptoms: on the control day, the sum of
the three negative symptoms of the BPRS remained at its
minimum of 3 ± 0 at every time point. On the ketamine
day, significant changes were observed [repeated-meas-
ures ANOVA, \( F(7,5) = 3.74, p = .008 \)]. Post hoc analysis revealed that negative scores were significantly
elevated relative to baseline scores (3 ± 0) during ket-
amine administration before the amphetamine injection
(Times 1, 2, and 3), but not after amphetamine (Times
4 and 5): Time 1, 4.1 ± 1.6, \( p = .03 \); Time 2, 5.0 ± 1.8, \( p = .0004 \); Time 3, 4.1 ± 1.6, \( p = .03 \); Time 4, 3.8 ± 1.0, \( p = .15 \);
Time 5, 3.4 ± 0.7, \( p = .46 \).

In summary, infusion of low-dose ketamine induced a
significant increase in dissociative and negative symp-
toms, and these symptoms were transiently improved
following the bolus injection of amphetamine.

**SAFETY INFORMATION.** No synergy between am-
phetamine and ketamine was observed on the cardiovas-
cular response (data not shown). The subjective effects of
ketamine vanished within minutes of discontinuation of
the infusion. Subjects were observed overnight in a re-
search-dedicated inpatient unit and were discharged the
next day after medical evaluation, which was unremark-
able in all cases. Subjects were contacted by phone 3
months after completion of the study. None of the subjects
reported any side effects of the study during this time
interval.

**Discussion**

This study indicates that, in humans, acute administration
of the noncompetitive NMDA antagonist ketamine in-
duces a significant increase in the effect of amphetamine
on \([^{123}\text{I}]\text{IBZM} \) BP. On the test day, amphetamine (0.25
mg/kg) induced a small but significant 5.5% ± 3.5%

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**Figure 3.** Dissociative symptoms: dissociative state measured by
the Clinician Administered Dissociative Symptom Scale (CADSS) mean total score on the control day and on the
ketamine day as a function of time (n = 8). No significant
dissociation was observed on the control day, either before or
after amphetamine challenge (arrow). On the ketamine day,
dissociation was rapidly induced by the bolus of ketamine. This
dissociation stabilized at lower levels during the ketamine
constant infusion. Amphetamine bolus administration was fol-
dowed by a brief improvement in dissociation. As the effect of the
amphetamine bolus waned, the ketamine-induced dissociative
state reemerged. *Rating significantly different from baseline; repeated-measures analysis of variance followed by post hoc test,
\( p = .05 \).
reduction in [123]IBZM BP. This response was smaller
than reported previously for healthy volunteers (−7.6% ±
8.0%, n = 15 [Laruelle et al 1996]: −7.11% ± 6.3%, n =
15 [Abi-Dargham et al 1998]; and −8.2% ± 7.7%, n = 6
[Kegeles et al 1999]), presumably because of the lower
dose of amphetamine injected in our study (0.25 mg/kg),
as compared with the previous studies (0.3 mg/kg). On the
ketamine day, amphetamine (0.25 mg/kg) reduced
[123]IBZM BP by 12.8% ± 8.8%, an effect significantly
larger than on the test day. This result suggests that
disruption of NMDA transmission significantly increases
amphetamine-induced dopamine release.

This conclusion is supported by several considerations.
First, the larger amphetamine effect observed on the
second study day (ketamine day) was not due to sensiti-
zation to amphetamine induced by the first amphetamine
injection (control day), since we previously observed an
absence of sensitization or tolerance to amphetamine-
induced dopamine release in healthy volunteers studied
tswe times at a 2-week interval under similar conditions (Kege-
les et al 1999). Second, this effect was mediated by
disruption of NMDA transmission. The selectivity of
racemic ketamine for the NMDA receptor (Kd = 0.6
μmol/L; Tam and Zhang 1988) is only relative, since
racemic ketamine also displays affinities for other recep-
tors known to affect dopamine release such as mu opioid
receptors (Kd = 27 μmol/L), sigma receptors (Kd = 66
μmol/L), or dopamine transporters (Kd = 63 μmol/L; Nishimura
et al 1998; Smith et al 1987). Yet, at the
steady-plasma level achieved in this study (195 ± 45
ng/mL) the intracerebral free ketamine concentration is
expected to be in the range of 0.38 ± 0.09 μmol/L (given
a molecular weight of 274.19 and a plasma free fraction of
53%; Dayton et al 1983). At this concentration, ketamine
should affect mainly, if not exclusively, NMDA receptors.
Third, the larger amphetamine-induced reduction in
[123]IBZM BP stimulated by ketamine could result from a
larger amphetamine-induced dopamine release, an
increased affinity of D2 receptors for dopamine induced by
ketamine, or some combination of both factors (for a
review, see Laruelle 2000a). Yet, we did not observe any
effect of ketamine alone on [123]IBZM binding, suggest-
ing that ketamine does not significantly alter the affinity of
D2 receptors for dopamine, the endogenous competitor of
[123]IBZM. Therefore, the data reported in this study
suggest that, in humans, alteration in NMDA transmission
results in a significant increase in amphetamine-induced
dopamine release.

Ketamine as a Pharmacologic Model of Cortical–
Subcortical Dysconnectivity
This observation confirms in humans a regulation of
dopaminergic responses by glutamatergic inputs previ-
ously observed in rodents (Miller and Abercrombie 1996)
and indicates that the acute response of dopaminergic
neurons to amphetamine exposure in humans is not solely
determined by the availability of dopamine stores in the
terminals, but also by the regulation of dopaminergic
neurons through glutamatergic transmission. The enhance-
ment of striatal amphetamine-induced dopamine release
induced by ketamine was comparable to the greater
response to amphetamine challenge (more than twofold
higher release; Table 2) observed in patients with schizo-
phrenia (Abi-Dargham et al 1998; Breier et al 1997b;
Laruelle et al 1996). The SD of the amphetamine effect
was larger under ketamine conditions, as compared with
the control conditions, a finding also observed in patients
with schizophrenia compared with control subjects. These
results suggest the increased amphetamine-induced dopa-
mine release measured in patients with schizophrenia
might result from an abnormal glutamatergic regulation of
dopaminergic neurons, rather than from a primary pathol-
ogy of these neurons (Weinberger 1999).

It has long been hypothesized that dysregulation of
subcortical dopamine function in schizophrenia may be
secondary to a failure of the PFC to adequately control
subcortical dopaminergic function (Grace 1991; Wein-
berger et al 1986). The PFC regulates subcortical dopa-
mine via, in part, glutamatergic projections to the midbrain
dopaminergic neurons. This regulation is mediated, among
others, by NMDA receptors. Therefore, pharmacologically

\begin{table}
\centering
\caption{Amphetamine Effect on [123]IBZM Binding Potential}
\begin{tabular}{|l|c|c|c|}
\hline
Study (reference) & Amphetamine dose & Percent change in binding potential (condition) & Relative increase \\
\hline
Test/retest in healthy control subjects (Kegeles et al 1999) & 0.30 mg/kg & −8.2% ± 7.7% (test) & −9.2% ± 7.2% (retest) & 1.12 \\
 & & (n = 6) & (n = 6) & \\
Control/NMDA deficiency in healthy control subjects (this study) & 0.25 mg/kg & −5.5% ± 3.6% (test) & −12.8% ± 8.8% (NMDA deficiency) & 2.32 \\
 & & (n = 8) & (n = 8) & \\
Healthy control/patients with schizophrenia (Laruelle et al 1999) & 0.30 mg/kg & −7.5% ± 7.1% (test-control subjects)−17.1% ± 13.2% (test-patients) & & 2.28 \\
 & & (n = 36) & (n = 34) & \\
\hline
\end{tabular}
\end{table}
induced disruption of NMDA receptor function provides a model for cortical–subcortical dysconnectivity potentially relevant to the pathophysiology of the illness. The demonstration that disruption of cortical–subcortical connectivity by ketamine in healthy individuals induces a dysregulation of dopaminergic function (i.e., increase in amphetamine-induced dopamine release) similar to the one observed in patients with schizophrenia supports the relevance of these models (Grace 1991; Weinberger et al. 1986).

Glutamatergic Control of Dopaminergic Cell Response to Amphetamine

Modulation of dopamine cell activity and dopamine release by glutamatergic projections is complex. Stimulation of NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate receptors localized on A9 and A10 midbrain dopaminergic cells by direct application of glutamate or glutamate agonists enhances dopaminergic cell-firing rate and burst firing (Chergui et al. 1993; Christoffersen and Meltzer 1995; Overton and Clark 1992; Scarnati and Pacitti 1982; Suaid-Chagny et al. 1992), leading to increased dopamine release in the terminal regions as measured by voltametry (Suaid-Chagny et al. 1992) and microdialysis (Enrico et al. 1998; Kalivas et al. 1989; Schilistrom et al. 1998; Westerink et al. 1998). Stimulation of glutamatergic afferents from the PFC to the ventral tegmental area (VTA) and substantia nigra (SN; Christie et al. 1985; Sesack and Pickel 1992) results in the same effect. For example, an increase in burst-firing activity of midbrain dopaminergic neurons and an increase in striatal dopamine release are elicited by electrical stimulation of the PFC (Gariano and Groves 1988), prefrontal application of glutamate (Murase et al. 1993a), or blockade of the inhibitory effects of γ-aminobutyric acid (GABA) on prefrontal neurons by local application of the GABA_A antagonist bicuculline (Karreman and Moghaddam 1996). Conversely, inhibition of prefrontal cell activity by prefrontal application of the sodium channel blocker tetrodotoxin or the monoamine releaser amphetamine decreases dopamine release in the striatum, an effect demonstrated both in rats (Karreman and Moghaddam 1996) and in rhesus monkeys (Kolachana et al. 1995). This activation of dopamine transmission by the PFC is mediated by activation of glutamatergic projections to midbrain dopaminergic neurons rather than to the dopaminergic terminals in the striatum, since the increased striatal dopamine release elicited by prefrontal stimulation is inhibited by local injection of glutamate antagonists in the VTA but not in the striatum (Karreman and Moghaddam 1996).

One of the apparent paradoxes of glutamatergic modulation of dopamine release is that systemic administration of noncompetitive NMDA antagonists also stimulates dopamine cell activity. A large number of studies reported that systemic administration of noncompetitive NMDA antagonists such as PCP or MK-801 increases the firing rate of midbrain dopaminergic neurons (Freeman and Bunney 1984; French 1986; French and Ceci 1990; French et al. 1993; Murase et al. 1993b; Pawlowski et al. 1990; Rouillard et al. 1990; Steinfels et al. 1989; Svensson et al. 1995; Zhang et al. 1992) and increases dopamine release and utilization in the terminal fields (Carboni et al. 1989; Deutch et al. 1987; Doherty et al. 1980; Hertel et al. 1995; Hondo et al. 1994; Jentsch et al. 1997; Mathe et al. 1996; Miller and Abercrombie 1996; Steinpreis and Salamone 1993; Verma and Moghaddam 1996; Wedzony et al. 1993). These stimulatory effects of systemic injections of noncompetitive NMDA antagonists are more prominent on the VTA cells than on the SN cells (Zhang et al. 1992), and the increase in dopamine release elicited by these drugs is more pronounced in the mesolimbic system (PFC, hippocampus, and nucleus accumbens) than in the nigrostriatal system (dorsal striatum; Carboni et al. 1989; Deutch et al. 1987; Kashihara et al. 1990; Verma and Moghaddam 1996; Wedzony et al. 1993; Weihmuller et al. 1991; Whitton et al. 1992). Three recent brain imaging studies (Breier et al. 1998; Smith et al. 1998; Vollenweider et al. 2000) demonstrated that, in healthy humans, ketamine administration alone induced a slight reduction of [11C]raclopride striatal BP (an effect not detected in this study, possibly due to the lower vulnerability to endogenous competition of [123I]IBZM binding relative to [11C]raclopride binding). Importantly, the stimulatory effect of systemic noncompetitive NMDA antagonists on dopaminergic activity is not observed when these drugs are applied directly into the VTA (Freeman and Bunney 1984; French 1986; Rouillard et al. 1990; Westerink et al. 1998; Zhang et al. 1992, 1994), suggesting that the activating effects of systemic noncompetitive antagonists on dopamine systems are mediated by indirect mechanisms.

Essentially two mechanisms have been recently proposed to account for the apparently contradictory observations that both NMDA agonists (applied locally in the VTA) and antagonists (given systemically) stimulate dopamine activity. One hypothesis is that systemic noncompetitive NMDA antagonists would affect GABA-ergic inhibition of dopaminergic midbrain cells. Midbrain dopaminergic cells are under tonic GABA-mediated inhibitory influence (Scarnati and Pacitti 1982; Westerink et al. 1998), provided by GABA-ergic projections from subthalamic nuclei and by local midbrain GABA-ergic interneurons (for a review, see Kalivas 1993). Supporting this mechanism is the observation that systemic administration of PCP and MK-801 inhibits activity of local, nondopa-
minergic, presumably GABA-ergic interneurons in the SN (Ceci and French 1989; Zhang et al. 1993, 1994). Thus, the activating effect of NMDA noncompetitive antagonists might result from inhibition of glutamatergic cortical projections to GABA-ergic midbrain interneurons or to GABA-ergic striatomesencephalic projections (Carlsson and Carlsson 1990; Carlsson 1993).

According to this model (Carlsson et al. 1999b), activity of midbrain dopaminergic neurons is under dual influence of the PFC via an activating pathway (the “accelerator”) and an inhibitory pathway (the “brake”), allowing fine tuning of dopaminergic activity by the PFC (Figure 4). The activating pathway is provided by direct glutamatergic projections onto the dopaminergic cells, and this stimulatory influence is mediated by NMDA, AMPA, and kainate receptors. The inhibitory pathway is provided by glutamatergic projections to GABA-ergic interneurons or striatomesencephalic GABA neurons. Since systemic administration of AMPA–kainate antagonists do not stimulate dopamine release (Bubser et al. 1995), this inhibitory pathway is most likely mainly mediated by NMDA transmission.

The inhibition of dopaminergic cell firing following amphetamine is an important feedback mechanism by which the brain reduces the effect of amphetamine on dopamine release. The inhibition of dopaminergic cell firing induced by amphetamine is mediated both by stimulation of presynaptic D2 autoreceptors and by stimulation of this inhibitory pathway (Bunney and Aghajanian 1978). The model depicted in Figure 4 predicts that, following administration of amphetamine (i.e., under conditions in which the inhibitory pathway should be activated), NMDA receptor blockade would result in a failure of activation of the inhibitory pathway, resulting in exaggerated amphetamine-induced dopamine release (Carlsson et al. 1999b). This prediction is consistent with observations in rodents (Miller and Abercrombie 1996) and in humans (this study).

More recently, a second mechanism has been proposed to explain the paradoxical increase in dopaminergic cell activity following systemic administration of noncompetitive NMDA antagonists. The basic observation leading to this hypothesis is that systemic administration and local VTA application of AMPA and kainate antagonists, though having no effect on striatal dopaminergic output per se, effectively block the increase in extracellular dopamine induced by systemic injection of MK-801 and ketamine (Mathe et al. 1998; Moghaddam et al. 1997; Svensson et al. 1998). This observation suggested that MK-801–induced dopamine release is mediated by activation of AMPA and kainate receptors in the VTA. This hypothesis is consistent with the recent demonstration that systemic administration of noncompetitive NMDA antag-

![Figure 4. Simplified circuitry diagram showing control of dopamine (DA) cell activity in the midbrain (ventral tegmental area [VTA] and substantia nigra [SN]) by glutamatergic (GLU) projections from the prefrontal cortex (PFC). The PFC control of DA activity is mediated by an activating pathway (‘accelerator,’ left) and an inhibitory pathway (‘brake,’ right), allowing constant fine tuning of DA activity by the PFC. The activating pathway consists of direct glutamatergic projection to the dopaminergic cell bodies. Stimulation of DA activity by this pathway is mediated by N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and kainate (KAI) receptors. The inhibitory pathway is mediated by glutamatergic projections to γ-aminobutyric acid–ergic (GABA-ergic) interneurons and striatomesencephalic GABA-ergic projections, not depicted) that inhibit DA cell activity. Activation of these GABA-ergic projections by glutamate is postulated to be mainly mediated by NMDA receptors. Activation of this inhibitory pathway is one of the mechanisms by which DA cell firing is inhibited following amphetamine administration, and failure to activate this pathway because of NMDA receptor blockade is one mechanism that might account for the enhancement of ketamine of amphetamine-induced DA release. In addition, acute blockade of NMDA receptors results in increased glutamate release in the midbrain. In turn, this increased glutamate availability activates AMPA–kainate receptors located on the dopaminergic cells, resulting in stimulation of DA release. The activation of this mechanism might also contribute to the increased amphetamine-induced DA release observed following ketamine administration. Thus, this diagram represents two different and possibly complementary mechanisms by which NMDA blockade increases the dopaminergic response to amphetamine. See text for references.](Image 314 to 546x705)
Figure 4. The model depicted in Figure 4 emphasizes the afferents from the PFC to the VTA, yet does not rule out the role of other glutamatergic projections, such as afferents from the amygdala or hippocampus, whose disruption by ketamine might also contribute to the observed effect.

Implication for the NMDA Deficiency Model of Schizophrenia

Although results from this study are consistent with an NMDA receptor deficiency model of schizophrenia, the direct evidence for such a deficiency is still lacking. Two important limitations of this study should be kept in mind regarding the relevance of these imaging data to the NMDA deficiency hypothesis of schizophrenia.

First, the NMDA receptor hypofunction hypothesis of schizophrenia assumes a chronic and possibly neurodevelopmental deficit (Olney and Farber 1995) rather than an acute deficit such as induced in this study. Acute and chronic NMDA receptor hypofunctions have different consequences for brain function (for a review, see Jentsch and Roth 1999); however, two recent studies in rodents indicate that striatal amphetamine-induced dopamine release is also increased following chronic PCP exposure (D.C. Javitt, personal communication, October 1999; Jentsch et al 1998). These data suggest that both acute and chronic NMDA hypofunction might be associated with increased responsivity of subcortical dopaminergic systems.

Second, the failure of glutamatergic control of dopamine release might stem from mechanisms other than NMDA hypofunction. For example, glutamatergic projections from the PFC to the VTA are under tonic inhibition by prefrontal GABA and dopamine activity (see Karrman and Moghaddam 1996 and references therein). It follows that deficits in GABA-ergic or dopaminergic function in the PFC are expected to have consequences similar to an NMDA deficiency on the subcortical dopamine response to amphetamine. Interestingly, both deficits have also been implicated in schizophrenia (Benes 1991; Deutch et al 1990; Weinberger 1987). Thus, in patients with schizophrenia various or multiple mechanisms (NMDA receptor hypofunction, GABA-ergic or dopaminergic deficits in the PFC) may lead to the dysregulation of subcortical dopamine revealed by the amphetamine challenge.

Dopamine and Psychotomimetic Effects of Low-Dose Ketamine

Besides investigating in humans neuromodulatory effects previously demonstrated in rodents, brain imaging enables direct assessment of the subjective effects of pharmacologic manipulations in relation to neurotransmission events. Whether the psychotomimetic effects of acute administration of noncompetitive NMDA antagonists are mediated entirely, partly, or not at all by an increase in dopamine activity is a long-standing debate (Carlsson et al 1999a). Our study clearly demonstrates that the acute dissociative state induced by this low dose of ketamine is not caused by an increase in dopamine activity, since this dissociative state was, if anything, reduced following amphetamine administration. The decrease in ketamine-induced dissociative state following amphetamine was an interesting observation but should be viewed with caution, given the absence of a comparison group with ketamine-only administration. Nevertheless, functional imaging studies suggested that the dissociative state induced by subanesthetic ketamine might be due to perturbation of intracortical connectivity at the level of the PFC (Breier et al 1997a; Vollenweider et al 1997). Amphetamine-induced dopamine release in the PFC might actually partially compensate for this disorganized activity, since prefrontal dopamine has been shown to augment the coherence of cognitive processing in the PFC (Daniel et al 1991). According to this view, the increase in PFC dopamine following acute (but not chronic) exposure to noncompetitive NMDA antagonists might be viewed as a compensatory reaction (i.e., part of the solution rather than part of the problem). In this regard, it is interesting to note that clozapine, a drug shown to antagonize some of the psychotomimetic effects of ketamine (Malhotra et al 1997), also augments dopamine release in the PFC (Youngren et al 1999).

At this low dose, ketamine alone failed to elicit a significant increase in the BPRS positive subscale, an observation consistent with a previous report of lack of significant induction of positive symptoms following this low dose of ketamine in healthy subjects (Krystal et al 1994). In healthy volunteers, positive symptoms are typically observed following higher doses of ketamine (Krystal et al 1994; Newcomer et al 1999). Neither amphetamine alone nor the combination of amphetamine and ketamine elicited significant positive symptoms, despite the marked elevation of dopamine release measured by SPECT. This result is consistent with the well-established observation that acute stimulation of dopamine release in otherwise healthy subjects is not sufficient to provoke positive psychotic symptoms (for a review, see Lieberman et al 1987 and references therein). Rather, sustained stimulation of dopamine release is needed for the emergence of positive symptoms in healthy subjects and, presumably, in patients with schizophrenia (Angrist and Gershon 1970; Laruelle 2000b; Lieberman et al 1990).

Conclusion

In conclusion, this study demonstrated that acute alteration of NMDA transmission is associated with an increased
amphetamine-induced dopamine release in humans. This observation supports the hypothesis that, in schizophrenia, a deficiency of glutamatergic control of dopamine neuronal activity might underlie the increase in amphetamine-induced dopamine release previously reported (Abi-Dargham et al 1998; Breier et al 1997b; Laruelle et al 1996). The mechanism(s) responsible for this deficient glutamatergic regulation of subcortical dopamine activity in schizophrenia remain to be elucidated. If an alteration of NMDA receptor function is implicated, pharmacologic augmentation of NMDA transmission might restore the ability of the PFC to control dopamine release and offer an antipsychotic strategy potentially devoid of the morbidity associated with chronic D₂ receptor blockade.

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