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Role of GABA Deficit in Sensitivity to the Psychotomimetic Effects of Amphetamine

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Some schizophrenia patients are more sensitive to amphetamine (AMPH)-induced exacerbations in psychosis–an effect that correlates with higher striatal dopamine release. This enhanced vulnerability may be related to gamma-aminobutyric acid (GABA) deficits observed in schizophrenia. We hypothesized that a pharmacologically induced GABA deficit would create vulnerability to the psychotomimetic effects to the 'subthreshold' dose of AMPH in healthy subjects, which by itself would not induce clinically significant increase in positive symptoms. To test this hypothesis, a GABA deficit was induced by intravenous infusion of iomazenil (IOM; 3.7 μg/kg), an antagonist and partial inverse agonist of benzodiazepine receptor. A subthreshold dose of AMPH (0.1 mg/kg) was administered by intravenous infusion. Healthy subjects received placebo IOM followed by placebo AMPH, active IOM followed by placebo AMPH, placebo IOM followed by active AMPH, and active IOM followed by active AMPH in a randomized, double-blind crossover design over 4 test days. Twelve healthy subjects who had a subclinical response to active AMPH alone were included in the analysis. Psychotomimetic effects (Positive and Negative Syndrome Scale (PANSS)), perceptual alterations (Clinician Administered Dissociative Symptoms Scale (CADSS)), and subjective effects (visual analog scale) were captured before and after the administration of drugs. IOM significantly augmented AMPH-induced peak changes in PANSS positive symptom subscale and both subjective and objective CADSS scores. There were no pharmacokinetic interactions. In conclusion, GABA deficits increased vulnerability to amphetamine-induced psychosis-relevant effects in healthy subjects, suggesting that pre-existing GABA deficits may explain why a subgroup of schizophrenia patients are vulnerable to AMPH.
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INTRODUCTION

Psychostimulants such as amphetamine and methylphenidate when administered at high doses and/or repeatedly produce transient psychosis characterized by positive symptoms and thought disorder in healthy individuals (Griffith *et al*, 1968; Angrist and Gershon, 1970; Bell, 1973; Janowsky and Risch, 1979; Bartlett *et al*, 1991). However, schizophrenic patients are more vulnerable to the effects of psychostimulants. Schizophrenic patients experience psychotic exacerbations with single, modest doses of psychostimulants (Lieberman *et al*, 1987b), and the magnitude of the psychotomimetic effects of amphetamine is greater in schizophrenic patients than in healthy subjects (Janowsky *et al*, 1973; Koreen *et al*, 1997; Lieberman *et al*, 1987b). However, the amphetamine response in schizophrenia is heterogenous. While some patients showed worsening of symptoms (~40%), improvement of symptoms and no effect of amphetamine were also observed in others (Lieberman *et al*, 1987b).

Consistent with the amphetamine-induced symptom exacerbation in some schizophrenia patients observed in earlier psychopharmacological challenge studies, more recent imaging studies have revealed significantly higher amphetamine-induced striatal dopamine (DA) release in schizophrenic patients relative to healthy controls (Abi-Dargham *et al*, 1998; Abi-Dargham *et al*, 2009; Breier *et al*, 1997; Laruelle *et al*, 1996). Furthermore, DA release correlated with the amphetamine-induced increases in psychosis. One possible explanation for the enhanced amphetamine sensitivity in some schizophrenia patients might be related to the well-documented gamma-aminobutyric acid (GABA) deficits observed in the disorder (Lewis *et al*, 2005), and the important interplay between the DA and GABA systems (Carr and Sesack, 2000; Sesack *et al*, 2003; Tam and Roth, 1990; Tzschentke, 2001; Van Bockstaele and Pickel, 1995).

Release of DA is under GABAergic influence. Fifteen to twenty percent of cells in the ventral tegmental area (VTA) contain GABA (Kalivas, 1993). GABAergic interneurons exert an inhibitory tone on midbrain dopaminergic neurons via several different pathways. First, DA neurons in VTA receive two major glutamatergic excitatory inputs, one from

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the prefrontal cortex and the other from the brain stem lateral dorsal/pendunculopontine tegmentum (Sesack *et al*, 2003; Tzschentke, 2001). Dopamine neurons in the substantia nigra receive an additional glutamatergic input from the subthalamic nucleus (Sesack *et al*, 2003; Tzschentke, 2001). GABA neurons in the prefrontal cortex dampen the activity of glutamatergic projections to the tegmental pedunculopontine nucleus (Sesack *et al*, 2003; Tzschentke, 2001), and thus indirectly inhibit midbrain DA cells. Second, there exist both GABA-A and GABA-B receptors in VTA DA neurons, which exert direct inhibitory input (Tam and Roth, 1990). Third, in VTA and substantia nigra, there are small number of GABA neurons that project to the same brain area with DA neurons (Carr and Sesack, 2000; Van Bockstaele and Pickel, 1995), which implicates their modulatory effects on the target areas of these dopaminergic projections.

Converging lines of evidence, including postmortem (Benes, 2000; Benes and Berretta, 2001; Hashimoto *et al*, 2003; Lewis *et al*, 2005; Ohnuma *et al*, 1999; Volk *et al*, 2000; Volk and Lewis, 2002a; Volk *et al*, 2002b; Woo *et al*, 1998), genetic (reviewed by Cherlyn *et al* (2010)), and brain imaging studies (Ball *et al*, 1998; Busatto *et al*, 1997; Kegeles *et al*, 2012; Ongur *et al*, 2010; Schroder *et al*, 1997; Verhoeff *et al*, 1999; Yoon *et al*, 2010), suggest that the dysfunction of the GABA system may contribute to the pathophysiology of schizophrenia. The results of *in vivo* magnetic resonance spectroscopy studies are mixed with some studies reporting elevations (Kegeles *et al*, 2012; Ongur *et al*, 2010), reductions (Rowland *et al*, 2013; Yoon *et al*, 2010) or no differences (Goto *et al*, 2009; Tayoshi *et al*, 2010) in GABA levels in specific brain regions of schizophrenia patients relative to controls. Some, but not all, *in vivo* receptor imaging studies suggest reduced benzodiazepine receptor binding in schizophrenia (Ball *et al*, 1998; Busatto *et al*, 1997; Schroder *et al*, 1997; Verhoeff *et al*, 1999). Several post-mortem studies have revealed evidence of pre- and postsynaptic abnormalities in specific GABAergic interneurons, namely the parvalbumin-positive basket cells, resulting in a reduction in the inhibitory control of pyramidal cells (Lewis *et al*, 2012; Lewis *et al*, 2005).

This study tested the hypothesis that inducing GABA deficits in healthy subjects would increase the psychotomimetic effects of amphetamine. More specifically, we hypothesized that in healthy individuals who do not experience clinically significant positive symptoms in response to a low dose of amphetamine would do so in the presence of a pharmacologically modeled GABAergic deficit.

MATERIALS AND METHODS

In a four test day, double-blind, placebo-controlled, randomized, crossover and counterbalanced study, healthy volunteers received active iomazenil followed by active amphetamine, active iomazenil followed by placebo amphetamine, placebo iomazenil followed by active amphetamine, and placebo iomazenil followed by placebo amphetamine. The study was conducted in the Neurobiological Studies Unit (VA Connecticut Healthcare System (VACHS), West Haven, CT).

Approvals

The study was approved by the institutional review boards of the Veterans Affair Health Care System, West Haven, CT (VAHCS-WH) and Yale University School of Medicine and was carried out in accordance with the Helsinki Declaration of 1975. The study was carried out under Investigational New Drug applications (75 099). Subjects were informed about the potential for adverse effects of amphetamine, iomazenil, and the combination.

Participants

Healthy male subjects aged between 18 and 55 were recruited by word of mouth, and public advertisement, and compensated $250 per test day for participating, for a total of $1000 for the four test days. Female subjects were excluded from this study because the teratogenic potential of iomazenil has not been studied. Potential subjects underwent a thorough medical and psychiatric history, complete physical examination, and a battery of laboratory tests including electroencephalogram (EEG), electrocardiogram (ECG), blood chemistry (CBC, BUN, creatinine, fasting blood glucose, electrolytes, liver and thyroid function tests, and VDRL), and urinalysis. Subjects underwent a structured psychiatric interview for DSM-IV and were screened for any DSM-IV Axis I or Axis II lifetime psychiatric or substance abuse disorder. Those who were treated with any psychotropic medication were excluded. The history provided by subjects was confirmed by a telephone interview conducted with an individual (spouse or family member) who was identified by the subject before screening. Given the proconvulsant potential of iomazenil, a baseline EEG was obtained and evaluated by a qualified neurologist (RAS). Those with an abnormal EEG at screening and a personal or family history of seizure disorder were excluded from the study.

As defined elsewhere (D'Souza *et al*, 2005), clinically significant positive symptoms were operationalized as higher than 3-point increase in the positive-symptom subscale in Positive and Negative Syndrome Scale (PANSS). The use of a threshold score has been reported by us in several other studies (Abi-Saab *et al*, 2002; D'Souza *et al*, 2006; D'Souza *et al*, 2004) and was used here to only include subjects who were not responsive to amphetamine alone.

Study Drugs

Both amphetamine and iomazenil solution were prepared by the VACHS-WH Research Pharmacy Service. Iomazenil solution was prepared as described elsewhere (D'Souza *et al*, 2006). Before use, product concentration was verified using high-performance liquid chromatography and tested for sterility and pyrogenicity.

Amphetamine

The most commonly utilized intravenous dose of amphetamine in human psychopharmacologic infusion studies is ~0.3 mg/kg or ~20 mg (Lieberman *et al*, 1987a). At this dose, (Laruelle *et al*, 1996) have demonstrated an estimated 7.6% and 19.5% increase in striatal DA release in healthy subjects and schizophrenia patients, respectively.

Furthermore, the increase in striatal DA release also correlated with an increase in PANSS positive symptom subscale scores. On the basis of the study hypothesis that iomazenil would potentiate the effects of amphetamine, a dose of amphetamine in this study (0.1 mg/kg) that was not expected to produce clinically significant psychosis (>3 point PANSS-positive-subscale score; Abi-Saab *et al*, 2002; D'Souza *et al*, 2006; D'Souza *et al*, 2004) was chosen.

Iomazenil

Iomazenil has high affinity and selectivity for benzodiazepine receptors ($K_d = 0.5$ nM; Johnson *et al*, 1990). Iomazenil (Ro 16-0154) is an iodine analog of the benzodiazepine receptor-competitive antagonist flumazenil. Some of its pharmacologic properties are comparable to those of flumazenil (Beer *et al*, 1990). However, unlike the competitive antagonist flumazenil, which blocks the effects of benzodiazepine agonists but lacks intrinsic pharmacological effects (Hunkeler *et al*, 1981), inverse agonists have intrinsic pharmacological effects opposite to those of benzodiazepines (Tallman and Gallager, 1985). In preclinical studies iomazenil has been shown to behave as a benzodiazepine receptor-competitive antagonist with inverse agonist effects (Abel *et al.*, 2003; Beer *et al*, 1990; Schubiger and Hasler, 1989). Similarly, clinical studies demonstrate that iomazenil has anxiogenic effects and at higher doses has proconvulsant effects (Randall, personal communication, 1995) that are consistent with inverse agonist activity at benzodiazepine receptors. Iomazenil produces a net deficit in GABA function. Consistent with a role of GABA deficits in the pathophysiology of psychosis, iomazenil has been shown to increase the psychotomimetic effects of the 5-HT-2 partial agonist, 1-(m-chlorophenyl)piperazine (*m*-CPP) in healthy subjects (D'Souza *et al*, 2006), and schizophrenia patients are more vulnerable to the propsychotic effects of iomazenil (Ahn *et al*, 2011). Iomazenil was administered intravenously at a dose of 3.7 μg/kg over 10 min. At this dose, iomazenil causes an estimated 25% benzodiazepine receptor occupancy in nonhuman primates (Innis *et al*, 1991). This has also been shown to be safe and well-tolerated in both healthy subjects and to enhance vulnerability to drug-induced psychotomimetic effects (D'Souza *et al*, 2006).

Experimental Design

Subjects completed four test days in randomized, double-blind, crossover, and counterbalanced design. Each test day was separated by at least 72 h in order to minimize any carryover effects. Subjects were instructed to refrain from using alcohol, street drugs, psychotropic medications, or caffeinated beverages for 2 weeks before testing and throughout the entire study. Urine toxicology on each test day ruled out recent drug use and a positive screen resulted in exclusion from the study. On each day, subject received either Iomazenil (3.7 μg/kg) or placebo infusion over 10 min, which was followed by either amphetamine (0.1 mg/kg) or placebo bolus infusion over 1 min (see Table 1: Schedule of procedures). After amphetamine infusion, ECG was monitored by a physician up to 1 h. Vital signs were monitored at −90, −20, −11, −1, 1, 5, 30, 70, 130, and 190 min after amphetamine bolus. All subjects who complete the study were contacted at 1 week, and 3 and 6 months after completing the study to detect any changes in their physical or mental health.

Measures

The primary outcomes included the psychotic symptoms measured using the positive-symptom subscale of the PANSS (Kay *et al*, 1989) and perceptual alterations measured using the subject-rated and clinician-rated subscales of the Clinician Administered Dissociative Symptoms Scale (CADSS; Bremner *et al*, 1998) that were administered at baseline, 5, 70, 130, and 190 min after amphetamine infusion. On the morning of each test day, the rater used the PANSS to assess the past 3 days in order to establish a baseline. Following that every subsequent assessment is for the time period since the last assessment. Furthermore, one item of the PANSS (passive/apathetic social withdrawal) that is not relevant to the context of an acute psychopharmacological challenge was dropped.

Anxiety, drowsiness, high, irritability, sadness, energy level, depressed mood, fearfulness, anger, and tiredness were assessed using a visual analog scale. The same research coordinators rated all four test days for each subject. Inter-rater reliability sessions were conducted every 1–2 months over the time period (~4 years) that this study was conducted and intraclass correlation coefficients for the PANSS were consistently greater than 0.85.

Statistical Analysis

Initially, data were examined descriptively using the means, SDs, and graphs. As per the study protocol, subjects with higher than 3-points increase in PANSS-positive-symptom subscale induced by amphetamine alone were excluded. Normal probability plots and Kolmogorov–Smirnov test statistics revealed that PANSS, CADSS, and VAS outcomes were not normally distributed. Furthermore, the absence of sufficient variance during the placebo administration necessitated the use of a nonparametric approach for repeated measures data (Brunner *et al*, 2002), where the data were first ranked, and then fitted using a mixed effects model with an unstructured variance–covariance matrix and *P*-values adjusted for ANOVA-type statistics (ATS). For each outcome, we analyzed the peak change from baseline, given that drug-induced behavioral and subjective changes occurred primarily at the +5 min time point (see Figure 1) with limited variability elsewhere. Each model included drug condition as a 4-level, within-subject factor: (1) amphetamine and iomazenil, (2) amphetamine and placebo, (3) iomazenil and placebo, and (4) placebo and placebo. However, unlike behavioral and subjective effects, heart rate and blood pressure effects were not restricted to a single time point, the analysis included all time points. Analyses of other subscales of the PANSS were subjected to adjustment for multiple comparisons. Bonferroni correction was applied within but not across hypotheses. For example, for PANSS general and negative symptom subscales, a cutoff of $0.05/2 = 0.025$ was used to declare significant effects. Vital signs were normally distributed and analyzed using repeated measure ANOVA. All statistical analyses were performed in SAS, version 9.3 (Cary, NC).

Table 1 Schedule of Procedures

Time (minutes)	Drug	Behavioral ratings (PANSS, CADSS, and VAS)	ECG, EEG	Vital signs	Blood level
~4 Weeks before test date	-Interview: Medical and Psychiatric History, SCID, drug/alcohol use -Confirmation of history with collateral -Physical examination and Lab tests: Chemistry, hematology, urine toxicology		Baseline EEG and ECG		
−90 min				×	
−60 min		×			×
−20 min				×	
−11 min	Iomazenil Infusion (over 10 min)			×	
−1 min	Amphetamine infusion (over 1 min)			×	
1 min			ECG, EEG monitoring	×	
5 min		×		×	
30 min				×	×
70 min		×		×	×
130 min		×		×	
190 min		×		×	
End	Exit interview, mini-mental state examination, discharge instruction				
1,3, and 6 months after the last test day	Safety follow-up				

Abbreviations: CADSS, Clinician Administered Dissociative Symptoms Scale; ECG, echocardiogram; EEG, electroencephalography; PANSS, Positive and Negative Syndrome Scale; SCID, Structured Clinical Interview for DSM Disorders; VAS, Visual Analog Scale.

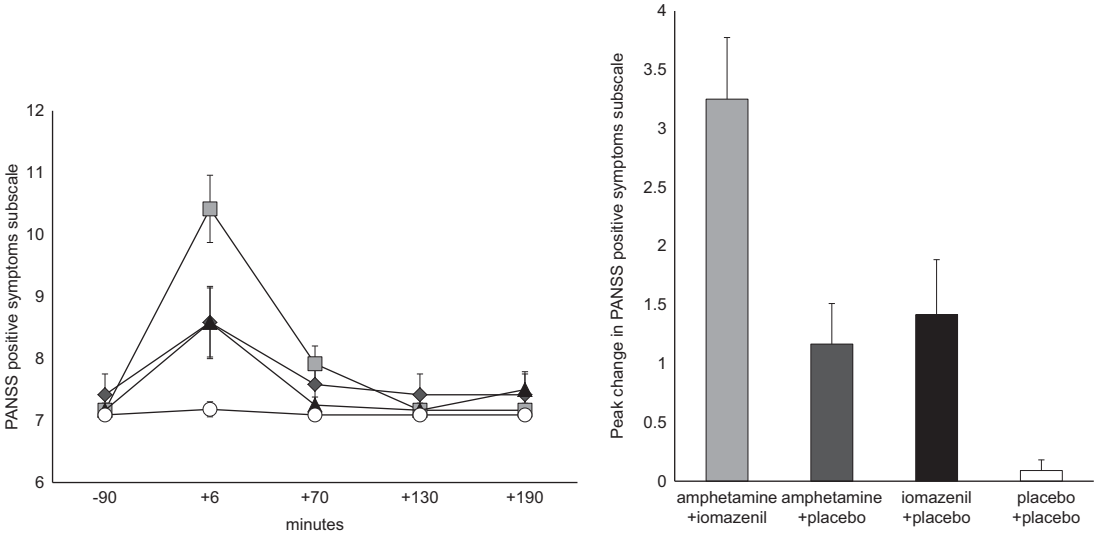

Figure 1 Effects of amphetamine and iomazenil on Positive and Negative Syndrome Scale (PANSS)-positive symptoms subscale. □ amphetamine+iomazenil, ◆ amphetamine+placebo, ▲ iomazenil+placebo, and ○ placebo+placebo.

RESULTS

Among 18 male subjects who were recruited, 12 subjects met all the study criteria. One subject dropped out on the first day after iomazenil/placebo infusion and was excluded. Five subjects showed higher than 3-point increase in PANSS-positive symptom subscale score and was excluded. One subject dropped out after three test days without completing the fourth test day, which was the placebo–placebo condition. The subjects were 26.2 ± 6.5 years old and weighed 187.9 ± 22.7 pounds. Ten were Caucasians and two were Asians.

Amphetamine and Iomazenil Levels

There was no significant difference in the amphetamine plasma level between amphetamine+placebo condition (19.4 ± 3.48 ng/ml) and amphetamine+iomazenil condition

Table 2 Iomazenil and Amphetamine Effects on Behavioral Measures

				Post hoc contrast between amphetamine+iomazenil and amphetamine+placebo		
Dose	***N***	**Mean**	**s.d.**	**df**	**ATS**	***P*-value**
PANSS						
Positive-symptom subscale						
Amphetamine+iomazenil	12	3.25	1.82	1	8.07	0.0045
Amphetamine+placebo	12	1.17	1.19			
Iomazenil+placebo	12	1.42	1.62			
Placebo+placebo	11	0.45	1.21			
Negative-symptom subscale						
Amphetamine+iomazenil	12	0.83	2.41	1	1.09	0.2969
Amphetamine+placebo	12	−0.17	0.94			
Iomazenil+placebo	12	0.75	1.22			
Placebo+placebo	11	0.09	0.30			
General symptom subscale						
Amphetamine+iomazenil	12	4.17	3.76	1	10.33	0.0013
Amphetamine+placebo	12	0.75	1.91			
Iomazenil+placebo	12	3.58	5.30			
Placebo+placebo	11	0.55	1.29			
CADSS						
Subject-rated subscale						
Amphetamine+iomazenil	12	3.83	4.69	1	13.48	0.0002
Amphetamine+placebo	12	0.83	2.08			
Iomazenil+placebo	12	2.25	3.14			
Placebo+placebo	11	1.09	3.30			
Clinician-rated subscale						
Amphetamine+iomazenil	12	1.92	2.15	1	3.86	0.0493
Amphetamine+placebo	12	1.33	2.50			
Iomazenil+placebo	12	1.42	2.47			
Placebo+placebo	11	0.27	0.90			

Mixed effects model with an unstructured variance–covariance matrix and *P*-values adjusted for ANOVA-type statistics. *Post hoc* contrasts performed only if dose effect is significant.

(21.2 ± 5.76 ng/ml, $t = 0.920$, df = 10, $P = 0.279$) at 30 min when the primary outcome measures were collected (Supplementary Figure 1A). There was no significant difference in the iomazenil plasma level between iomazenil+placebo condition (5.11 ± 2.02 ng/ml) and amphetamine+iomazenil condition (6.03 ± 2.80 ng/ml, $t = -1.257$, df = 6, $P = 0.256$) at 30 min (Supplementary Figure 1B).

Behavioral Measures

As the primary hypothesis was that the combination of iomazenil and amphetamine would produce greater changes than amphetamine alone, for parsimony, only the contrasts between the two are reported.

Positive Symptoms

There was a significant effect of dose on the peak change in PANSS positive symptom subscale score (ATS = 8.78, df = 2.41, $P < 0.0001$). The peak increase in PANSS positive symptom subscale score induced by amphetamine+iomazenil (3.25 ± 1.82) was greater than the effect of amphetamine+placebo (1.16 ± 1.19) or iomazenil+placebo (1.41 ± 1.62; Figure 1, Table 2). *Post hoc* analysis revealed significant differences between the amphetamine+iomazenil and amphetamine+placebo conditions (ATS = 8.07, df = 1, $P = 0.0045$, Table 2). Furthermore, 50% of subjects scored higher than a 3-point increase (PANSS-positive-symptom subscale) on the iomazenil+amphetamine condition.

Analysis of the interactions between iomazenil (placebo and active) and amphetamine (placebo and active) on the peak change in PANSS-positive-symptom scores was not significant

Negative Symptoms

There was no significant effect of dose on the peak change in PANSS-negative-symptom subscale score.

General Psychopathology Symptoms

There was a significant effect of dose on the peak change in the PANSS general psychopathology subscale score (ATS = 6.22, df = 2.15, $P = 0.0015$). *Post hoc* analysis revealed significant differences between the amphetamine+iomazenil combination and amphetamine+placebo condition (ATS = 10.3, df = 1, $P = 0.0013$).

Perceptual Alterations

There were significant effects of dose on the peak changes in both CADSS subject-rated and clinician-rated subscale scores (ATS = 6.77, df = 2.45, $P = 0.0005$; ATS = 4.3, df = 2.53, $P = 0.0078$, respectively). *Post hoc* analysis revealed significant differences between the amphetamine+iomazenil and amphetamine+placebo conditions for both the subject-rated (ATS = 13.48, df = 1, $P = 0.0002$) and clinician-rated subscales (ATS = 3.86, df = 1, $P = 0.0493$).

Visual analog scale. There were significant effects of dose on the peak changes in drowsiness (ATS = 3.44, df = 2.23, $P = 0.0273$), high (ATS = 3.28, df = 2.02, $P = 0.0373$), and energy (ATS = 3.25, df = 2.44, $P = 0.0293$; Table 3). However, there were no significant differences between the amphetamine+iomazenil and amphetamine+placebo conditions in *post hoc* analyses.

Safety

Given the potential for iomazenil to lower seizure threshold, all subjects had a baseline EEG to rule out any evidence of seizure-like activity and the first five subjects had real-time continuous EEG monitoring by a qualified neurologist (RAS) during each test for ~1 h after iomazenil administration. As no seizure-like activity was observed, continuous EEG monitoring was no longer necessary. There were significant dose × time interaction in systolic blood pressure ($F(27,413) = 3.19$, $P < 0.001$), diastolic blood pressure ($F(27,413) = 1.85$, $P = 0.0065$), and heart rate ($F(27,413) = 2.32$, $P = 0.0003$), which was driven by amphetamine administration (Supplementary Figure 2). There were no significant difference between the amphetamine+iomazenil and amphetamine+placebo conditions in *post hoc* analyses. None of the subjects reported adverse effects when questioned 1 week, and 3 and 6 months after the last participation date.

DISCUSSION

To our knowledge, this is the first study in humans examining the interactions between the GABA and DA systems on psychosis-relevant outcomes using a pharmacological approach. In summary, in healthy subjects without any obvious risk of psychosis, iomazenil unmasked the psychotomimetic and perceptual altering effects of a dose of amphetamine that on its own did not produce these effects. The unmasking effect of iomazenil cannot be explained by a simple pharmacokinetic interaction since there were no differences in amphetamine blood levels across the two conditions. In addition, the unmasking effect of iomazenil on amphetamine effects was specific to positive symptoms (PANSS positive symptom subscale) and quasi-positive symptoms (CADSS) as evidenced by a lack of such effects on a range of other measures that are known to be sensitive to amphetamine effects including anxiety, euphoria ('high'), energy level, and cardiovascular effects (systolic and diastolic blood pressures and heart rate).

As described earlier, iomazenil produces a net GABA deficit. Studies investigating iomazenil's effects on psychosis have consistently shown that iomazenil-induced GABA deficits can create vulnerability to psychosis, but does not directly induce psychotic symptoms. The administration of iomazenil alone showed no significant effects on PANSS and CADSS in this study, which is consistent with our previous studies in healthy subjects (Ahn *et al*, 2011; D'Souza *et al*, 2006) with the same dosing regimen of iomazenil. Collectively, these three studies ($n = 48$) clearly demonstrate that iomazenil alone does not induce significant increases in measures of psychosis and perceptual alterations in healthy subjects. These data suggest that a GABA deficit alone, as modeled by iomazenil, is not sufficient to induce psychosis. However, when administered to stable, antipsychotic-treated schizophrenia patients iomazenil produced small increases in psychotic symptoms. Furthermore, as observed in this study, iomazenil increased the psychotic symptoms induced by amphetamine and, as shown previously by us, iomazenil increased the psychotic symptoms induced by m-CPP in healthy subjects (D'Souza *et al*, 2006). Similarly, iomazenil has been shown to increase psychosis induced by delta-9-tetrahydrocannabinol (in review). Collectively, these studies suggest that GABA deficits confer vulnerability to psychosis related to perturbations of several receptor systems relevant to psychosis including DA, cannabinoid, and serotonin.

Whereas this study was not designed to inform the mechanism of the precise interactions between iomazenil and amphetamine, several lines of preclinical data suggest potential mechanisms. GABA-A receptor antagonists, including picrotoxin (Theile *et al*, 2011) and bicuculline (Westerink *et al*, 1996), disinhibit VTA-DA release. Similarly, GABA-A receptor inverse agonist, FG7142, has been shown to activate VTA neurons (Murphy *et al*, 1996). FG7142-induced VTA activation was reversed by DA receptor antagonists. Although admittedly speculative, administration of GABA-A receptor antagonists or inverse agonists would be predicted to increase DA release by drugs such as amphetamine. This would be consistent with the findings of the current study showing that iomazenil increased the DA-related symptomatology induced by amphetamine. It will be interesting to follow-up this behavioral study with an imaging study of whether iomazenil increases amphetamine-induced DA release as indexed by displacement of [11C]-raclopride.

Table 3 Iomazenil and Amphetamine Effects on Subjective Effects

Dose	*N*	Mean	s.d.	*Post hoc* contrasts
Anxiety				
Amphetamine+iomazenil	12	8.48	11.34	
Amphetamine+placebo	12	3.98	10.25	
Iomazenil+placebo	12	9.21	15.06	
Placebo+placebo	11	2.85	7.07	
*Drowsiness**				
Amphetamine+iomazenil	12	3.47	26.13	Amphetamine+iomazenil *versus* amphetamine+placebo, P = n.s.
Amphetamine+placebo	12	−5.22	21.50	
Iomazenil+placebo	12	7.68	24.22	
Placebo+placebo	11	0.86	6.62	
*High**				
Amphetamine+iomazenil	12	12.45	18.47	amphetamine+iomazenil *versus* amphetamine+placebo, P = n.s.
Amphetamine+placebo	12	8.34	17.34	
Iomazenil+placebo	12	7.85	19.74	
Placebo+placebo	11	1.51	4.65	
Irritability				
Amphetamine+iomazenil	12	4.00	22.90	
Amphetamine+placebo	12	−2.91	8.91	
Iomazenil+placebo	12	6.71	12.38	
Placebo+placebo	11	3.28	7.35	
Sadness				
Amphetamine+iomazenil	12	6.21	21.35	
Amphetamine+placebo	12	−0.08	0.29	
Iomazenil+placebo	12	2.38	7.31	
Placebo+placebo	11	0.14	0.32	
*Energy**				
Amphetamine+iomazenil	12	8.12	18.65	Amphetamine+iomazenil *versus* amphetamine+placebo, P = n.s.
Amphetamine+placebo	12	12.62	23.95	
Iomazenil+placebo	12	−2.32	18.01	
Placebo+placebo	11	3.23	10.24	
Depression				
Amphetamine+iomazenil	12	−7.74	33.48	
Amphetamine+placebo	12	−14.07	21.44	
Iomazenil+placebo	12	−3.81	22.94	
Placebo+placebo	11	−3.55	11.32	
Fearful				
Amphetamine+iomazenil	12	5.31	18.48	
Amphetamine+placebo	12	0.04	0.14	
Iomazenil+placebo	12	2.71	6.03	
Placebo+placebo	11	−0.13	0.85	
Anger				
Amphetamine+iomazenil	12	1.95	6.95	
Amphetamine+placebo	12	0.09	0.32	
Iomazenil+placebo	12	0.13	0.31	
Placebo+placebo	11	0.28	0.66	
Tiredness				
Amphetamine+iomazenil	12	−7.74	33.48	
Amphetamine+placebo	12	−14.07	21.44	
Iomazenil+placebo	12	−3.81	22.94	
Placebo+placebo	11	−3.55	11.32	

Mixed effects model with an unstructured variance–covariance matrix. *Post hoc* contrasts conducted only if initial analysis showed significance *$P < 0.05$.

Strengths and Limitations

This study has a number of strengths, including the double-blind, randomized, crossover within subjects design, the use of well-validated measures, and the inclusion of only those subjects with subclinical response to amphetamine.

Although the observation that the largest increases in psychosis-like phenomena occurred with the combination of iomazenil and amphetamine provides some support for the mechanistic hypothesis, the lack of statistically significant interactive effects weakens that support. It is likely that the absence of statistically significant interactive effects ($P=0.12$) is likely related to the small sample size. Furthermore, as only men were studied, the results may not generalize to women. Future studies with a larger sample size, which includes women, may permit a better assessment of interactive effects and also effects on individual symptoms.

CONCLUSION

In conclusion, the findings of the current study suggest that GABA deficit can increase vulnerability to amphetamine-induced psychosis-like phenomena. The precise anatomical and neurochemical mechanisms underlying the interactive effects of iomazenil and amphetamine are beyond the scope of current study. Future studies employing neuroreceptor imaging may provide more direct *in vivo* evidence whether iomazenil's enhancement of amphetamine-induced psychosis-like phenomena is related to its capacity to enhance amphetamine-induced DA release.

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