Synergistic Interactions between Ampakines and Antipsychotic Drugs

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Accepted for publication November 19, 1998 This paper is available online at http://www.jpet.org

ABSTRACT
Tests were made for interactions between antipsychotic drugs and compounds that enhance synaptic currents mediated by \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid-type glutamate receptors ("ampakines"). Typical and atypical antipsychotic drugs decreased methamphetamine-induced hyperactivity in rats; the effects of near or even subthreshold doses of the antipsychotics were greatly enhanced by the ampakines. Interactions between the ampakine CX516 and low doses of different antipsychotics were generally additive and often synergistic. The ampakine did not exacerbate neuroleptic-induced catalepsy, indicating that the interaction between the different pharmacological classes was selective. These results suggest that positive modulators of cortical glutamatergic systems may be useful adjuncts in treating schizophrenia.

Schizophrenia is a chronic mental illness in which affected individuals have a range of symptoms, including chronic cognitive dysfunction, disordered thought, emotional withdrawal, and episodic delusions and hallucinations. Although the clinical efficacy of dopamine \( D_2 \) receptor blockers suggests a dopamine imbalance is important in the disease, it has become clear that several other neurotransmitter systems, including the glutamatergic system, are also involved in the pathophysiology of the schizophrenic brain. Glutamate is the major excitatory neurotransmitter in the brain, especially in neocortical and limbic regions, and glutamatergic transmission is known to play a fundamental role in cognitive processes. Accumulating evidence suggests that reduced excitatory (glutamatergic) activity, especially involving select neocortical areas, could underlie some, if not many, symptoms of the disease (Coyle, 1996; Tamminga, 1998). Imaging and postmortem morphometry studies of schizophrenic brains have found abnormalities in a number of brain regions, such as prefrontal, temporal and anterior cingulate cortices, hippocampus, amygdala, and striatum, that are connected by glutamatergic circuits (Andreasen et al., 1992; Carpenter et al., 1993; Weinberger and Berman, 1996). Phencyclidine, ketamine, and other noncompetitive antagonists at \( N \)-methyl-\( \omega \)-aspartate (NMDA)-type glutamate receptors exacerbate symptoms in patients (Lahti et al., 1995) and produce a range of psychotic symptoms in volunteers that are similar to those of schizophrenic patients (Krystal et al., 1994).

This and other evidence suggests that drugs that enhance glutamatergic transmission might offset the postulated imbalance between ascending midbrain monoaminergic systems and descending cortical glutamatergic systems in the schizophrenic brain (Carlsson and Carlsson, 1990). One approach has centered on enhancing NMDA receptor activity with glycine or related agonists (\( \gamma \)-cycloserine) of the strychnine-insensitive glycine coagonist site. Some beneficial effects of \( \gamma \)-cycloserine on negative symptoms in patients coadministered a typical antipsychotic have been reported (Goff et al., 1995; Heresco-Levy et al., 1996). Studies using large doses of glycine, or a glycine prodrug to overcome the limited ability of glycine to penetrate the blood-brain barrier, did not demonstrate clinical benefit (Rosse et al., 1989, 1991). However, coadministration of glycine with neuroleptics improved negative symptoms (Javitt et al., 1994).

Because the NMDA receptor is voltage regulated (via voltage-sensitive magnesium blockade of the associated ion channel), increased coagonist (glycine) may not be as effective as further membrane depolarization via \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor facilitation. Enhancement of glutamatergic transmission via allosteric modulation of AMPA receptors has not been tried. Tests of this hypothesis became possible, in principle at least, with the development of a family of drugs, ampakines, that freely cross the blood-brain barrier and positively modulate AMPA receptors (Staubli et al., 1994a; Rogers et al., 1997). Ampakines enhance excitatory (glutamatergic) transmission.

ABBREVIATIONS: NMDA, \( N \)-methyl-\( \omega \)-aspartate; AMPA, \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; LMA, locomotor activity.
(Arai et al., 1994), facilitate long-term potentiation (Staubli et al., 1994a), and enhance learning and memory in rodents (Staubli et al., 1994b) and humans (Ingvar et al., 1997), suggesting the drugs may improve cognitive dysfunction in patients. Consonant with the general idea of competitive glutamatergic/dopaminergic systems, an ampakine has been shown to reduce the aberrant behaviors induced in rats by methamphetamine (Larson et al., 1996), a common and often predictive test of antipsychotic drug activity.

Implicit in the hypothesis that schizophrenia arises from an imbalance between opposing neurotransmitter systems (Carlsson and Carlsson, 1990) is the prediction that antagonists of one of the systems and positive modulators of the other should be at least additive and probably synergistic. This is of considerable clinical significance because it suggests a novel therapeutic strategy involving low levels of two completely different classes of drugs. Reducing the dose of commonly used antipsychotics should reduce their often treatment-limiting side effects. Here we report that ampakines can interact synergistically with antipsychotic drugs with regard to antagonism of the behavioral disturbances induced by acute methamphetamine.

**Materials and Methods**

**Antagonism of Psychostimulant Activity.** Male Sprague-Dawley rats (250–300 g; Harlan, San Diego, CA) were given ad libitum food and water and maintained on a 12:12-h light/dark cycle with lights on at 6:00 a.m. Behavioral studies that quantified the antagonistic effects of AMPA-R-modulating compounds (ampakines) and/or various antipsychotic compounds on psychostimulant-induced (amphetamine or methamphetamine) hyperactivity used a computerized PhotoBeam Activity System (San Diego Instruments, San Diego, CA). Each of 10 test cages [standard polycarbonate animal cage; 26 × 48 × 20 cm (width × length × height)] were surrounded by two photobeam arrays, placed to detect locomotor behavior with a lower array and rearing behavior with an upper array. Locomotor and rearing activities were continuously monitored by computer for all test cages. Test cages (with photobeam arrays) were placed in a partially darkened room with room ventilation as background noise. On test day, naive rats were initially placed in the test cages, and baseline behavioral activity in the novel environment was monitored during a 30-min acclimation period. The rats (8–10 rats were randomly assigned to each experimental group) were then injected (i.p.) with vehicle or drug(s) dissolved in vehicle, immediately returned to the test cage, and monitored undisturbed for 90 min. Photobeam breaks were summed by the computer into 10-min periods for analysis. Group mean and S.E.M. values are reported in the figures; statistical significance was determined by one-way ANOVA (Kruskall-Walliss test, with difference between each group determined by Dunn’s multiple comparison test using Prism, GraphPad Software, San Diego, CA).

**Catalepsy.** Sprague-Dawley rats (male, 300–350 g) were tested for cataleptogenic activity of CX516 by two standard tests, here referred to as the bar test and the grid test (Hoffman and Donovan, 1995). For the bar test, a bar was mounted horizontally across the width of a standard animal cage, 10 cm above a thick layer of bedding. The bar was a metal rod inserted into tygon tubing, with a final diameter of 1 cm. Rats were injected with vehicle or test compounds and tested thereafter at 30-min intervals for 3 h. The test consisted of placing the rat in the cage with its front paws on the rod and its rear paws on the bedding and measuring the time, in seconds, that the rat kept its front paws on the bar. The maximum time recorded was 180 s for any 30-min interval. A score of zero was given if after three attempts, the rat would not allow the investigator to place the animal’s front paws on the bar. The investigators were blind to the drug treatment the rats received at the beginning of the test. The bar test was run immediately after the conclusion of the bar test (i.e., after the rat removed its paws from the bar) at each 30-min interval. The grid consisted of an inclined (50 degrees from horizontal) wire mesh (15 × 22 inches; 0.5-cm-mesh size) enclosed on three sides with a 4-inch strip of black Plexiglas. The rat was placed approximately in the middle of the grid and timed until it moved or for a maximum of 180 s. As with the bar test, the grid test was performed at 30-min intervals for 3 h. Scores (number of seconds immobile) from each 30-min interval were summed for each rat. Independent experiments were composed of 6 to 10 rats per experimental group. The bar and grid test scores were combined for a total catalepsy score for each experimental group. Mean and S.D. values were calculated and compared by two-tailed t test assuming unequal variance. Experimental groups were (1) vehicle, (2) CX516 (10 mg/kg), (3) haloperidol (0.12, 0.25 mg/kg), and (4) CX516 (10 mg/kg) plus haloperidol (0.12, 0.25 mg/kg).

**Membrane Patch Electrophysiology.** Patch electrophysiology was used to determine the EC50 value of the up-modulation of AMPA receptor-gated ion currents by several different ampakines. Membrane patches were excised from pyramidal neurons in cultured hippocampal slices prepared from 10-day-old rats. Electrophysiological recordings were performed as described previously (Arai et al., 1996).

**Drugs.** Amphetamine sulfate (1.0 mg/kg salt), methamphetamine HCl (2.0 mg/kg salt), haloperidol (0.06 or 0.12 mg/kg), fluphenazine HCl (0.2 mg/kg), clozapine (1 or 10 mg/kg), and risperidone (0.1 mg/kg) were from Research Biochemicals (Natick, MA). Amphetamine and methamphetamine were dissolved in saline. Antipsychotic compounds were dissolved in 1% lactic acid and titrated to pH 5.0 with 0.1 N NaOH before injection. CX516 (1-quinoloxaline-6-ylcarbonyl)piperidine) and other ampakines were synthesized at Cortex Pharmaceuticals, Inc. and dissolved in saline or hydroxypropyl-β-cyclodextrin (Aldrich) in saline.

**Results**

**Ampakines Reduce Psychostimulant Hyperactivity.** Previous results have shown that AMPA receptor facilitation can block aberrant behavior due to a moderate dose of (-)-methamphetamine (2.0 mg/kg) (Larson et al., 1996). Subsequent characterization of a number of different Ampakines demonstrates a good correlation (R² = 0.86) between hippocampal patch EC50 and AMPA receptor modulation (EC50 value from hippocampal pyramidal membrane patch electrophysiology) and in vivo potency (ED50) to reverse the effects of low dose (S)-amphetamine (1.0 mg/kg) on locomotor activity (LMA) (Fig. 1A). A similar correlation (R² = 0.80) between hippocampal patch EC50 and ED50 values for amphetamine rearing is also shown (Fig. 1B). These correlative data support the proposal that ampakines inhibit amphetamine hyperactivity through facilitation of the AMPA receptor.

**Synergy Between CX516 and Antipsychotics.** The ampakine CX516 has no significant effects on exploratory activity in a novel environment, response latencies, or other arousal-dependent measures at doses up to 50 mg/kg (Larson et al., 1995; Davis et al., 1997). A dose of 10 mg/kg decreased methamphetamine (2 mg/kg)-induced LMA and rearing activity by 27 ± 9% and 41.0 ± 6%, respectively (mean ± S.E.M. for seven experiments; 8–10 rats per experiment). The effects of clozapine (1 mg/kg), CX516 (10 mg/kg), and clozapine plus CX516 on methamphetamine rearing activity are summarized in Fig. 2. Clozapine alone, as expected for the low dose used, had no influence on methamphetamine-induced rearing activity but nevertheless markedly potenti-
ated the suppressive effects of the ampakine. That is, CX516 (10 mg/kg) reduced the effect of methamphetamine on rearing activity relative to saline injections by about 35%, but combined with the otherwise ineffective dose of clozapine (5% increase), it caused a 90% reduction (Fig. 2, Table 1). Statistical comparisons between the “methamphetamine + drug combination” group and “methamphetamine + either drug alone” groups using the Kruskal-Wallis test (nonparametric one-way ANOVA) followed by Dunn’s multiple comparison test were highly significant (Kruskal-Wallis: $P < .0001$; Dunn’s tests: methamphetamine + clozapine versus methamphetamine + CX516; $P < .001$; methamphetamine + CX516 versus methamphetamine + CX516 + clozapine: $P < .01$). In separate experiments, the combination of CX516 (10 mg/kg) and risperidone (0.1 mg/kg) decreased methamphetamine-induced rearing to the vehicle (control) level (106% reduction; $P < .05$ versus methamphetamine versus either CX516 or risperidone by Dunn’s test) compared with 25% and 50% reductions, respectively, for each drug alone over the 90-min period (Table 1). Other dose combinations of CX516 and risperidone were not investigated. Results similar to these have been obtained with another ampakine.

CX516 was also tested in combination with the typical antipsychotic (neuroleptic) haloperidol. In the experiment summarized in Fig. 3, a low dose of haloperidol (0.06 mg/kg) reduced the effect of methamphetamine on rearing by 16%, and CX516 (30 mg/kg) caused a 23% decrease. The combination of haloperidol and CX516 was synergistic in that it reduced rearing activity more completely (71%) than would be expected from the sum of the separate effects of the drugs (39%) (Table 1). Analysis of the differences between the effects of the haloperidol/CX516 combination and haloperidol alone on methamphetamine were significant ($p < .01$; Dunn’s test after Kruskal-Wallis test of multiple groups; $p = 0.0005$). Similar results were obtained for LMA (Table 1): 9% or 10% reduction for haloperidol or CX516, respectively, compared with a 60% reduction for the ampakine/haloperidol drug combination ($p < .05$ for combination versus either haloperidol or CX516 alone). A higher dose of haloperidol (0.12 mg/kg) was more effective by itself in counteracting hyperactivity. Although the ampakine/haloperidol combination further reduced rearing and LMA versus haloperidol (0.12 mg/kg) alone, the difference between these groups did not reach significance by Dunn’s test (Table 1).

**CX516 and Haloperidol-Induced Catalepsy.** CX516 did not produce catalepsy in rats at doses up to 256 mg/kg over a period of 6 h (not shown). Production of catalepsy in rats predicts the propensity for induction of extrapyramidal side effects (e.g., akathisia, drug-induced parkinsonism) in patients (Hoffman and Donovan, 1995). Subsequent tests for an effect of CX516 (10 mg/kg) on haloperidol-induced cata-
The present results provide the first evidence that enhancement of AMPA receptor-mediated currents can potentiate, sometimes synergistically, the activity of typical and atypical antipsychotics in blocking methamphetamine-induced LMA or rearing, a behavioral test often used to detect potential antipsychotic activity. The present findings thus point to the potential use of AMPA receptor-enhancing drugs alone, or as adjuncts to antipsychotic drugs, in treating schizophrenia.

The potentiating effects of CX516 with antipsychotic drugs could have been due to the effects of CX516 on serum protein binding interactions or metabolism of the antipsychotic with which it was coadministered. Both clozapine and risperidone are highly protein bound (97% and 90%, respectively), and therefore displacement by CX516 could rapidly produce higher plasma levels. This drug/drug interaction could serve to raise a subeffective level of antipsychotic drug to an effective level and yield results indistinguishable from those observed here. However, CX516 is less than 3% bound to rat plasma protein at the dose used in these experiments (data not shown) and therefore is unlikely to affect binding of low doses of other compounds. It is also unlikely that CX516 at 10 mg/kg and the more potent ampakine CX691 at only 0.1 mg/kg would both interfere with the metabolism of three different antipsychotic drugs reported in this study (preliminary data not shown here suggest that 0.1 mg/kg CX691 is also able to enhance antipsychotic antagonism of methamphetamine hyperactivity). Nevertheless, we tested the possible effect of CX516 (10 mg/kg) on the plasma pharmacokinetic profile of clozapine in rats. The drugs were coinjected (i.p.) with methamphetamine (2 mg/kg) in three rats per dose combination (methamphetamine + clozapine, n = 3, versus methamphetamine + clozapine + CX516, n = 3), and plasma samples were collected at multiple time points from each rat for HPLC/mass spectrometry analysis of clozapine concentration. We found no significant difference in the mean plasma clozapine concentration between the two groups of rats, suggesting that CX516 did not raise plasma clozapine concentration. Furthermore, in the case of risperidone, the point is actually most because of the active metabolite (9-hydroxyrisperidone) that contributes significantly to its pharmacodynamic properties in both rat and humans. Whether metabolism was blocked would not matter. Last, a decrease in the rate of plasma elimination of an antipsychotic drug due to CX516 should become more noticeable at longer time periods.

**TABLE 1**

<table>
<thead>
<tr>
<th>Antipsychotic</th>
<th>Activity</th>
<th>Antipsychotic Alone</th>
<th>CX516 Alone</th>
<th>CX516 plus Antipsychotic</th>
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<tbody>
<tr>
<td>Haloperidol</td>
<td>Rearing</td>
<td>81</td>
<td>74</td>
<td>28&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>(0.06 mg/kg)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Crossing</td>
<td>105</td>
<td>104</td>
<td>47&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
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<td>Rearing</td>
<td>31</td>
<td>57</td>
<td>16&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>(0.12 mg/kg)</td>
<td>Crossing</td>
<td>34</td>
<td>57</td>
<td>24&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Clozapine</td>
<td>Rearing</td>
<td>105</td>
<td>66</td>
<td>10&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
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<td>106</td>
<td>132</td>
<td>65&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>Risperidone</td>
<td>Rearing</td>
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<td>75</td>
<td>-6&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>(0.1 mg/kg)</td>
<td>Crossing</td>
<td>53</td>
<td>96</td>
<td>42&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> The dose of CX516 was 10 mg/kg in all experiments except 30 mg/kg in this case.
<sup>b</sup> P < .05 versus methamphetamine plus antipsychotic by Dunn’s test after Kruskal-Wallis test (one-way ANOVA).
<sup>c</sup> P < .05 versus methamphetamine plus CX516 (Dunn’s test after Kruskal-Wallis test).
<sup>d</sup> P < .01 versus methamphetamine plus antipsychotic (Dunn’s test after Kruskal-Wallis test).
<sup>e</sup> P < .001 versus methamphetamine plus antipsychotic (Dunn’s test after Kruskal-Wallis test).
<sup>f</sup> Not significant by Dunn’s test after Kruskal-Wallis test.

**Discussion**

The present results provide the first evidence that enhancement of AMPA receptor-mediated currents can potentiate, sometimes synergistically, the activity of typical and atypical antipsychotics in blocking methamphetamine-induced rearing activity. A, behavioral activity was monitored with a computerized photobeam system as described in the text. Each point represents the mean cumulative rearing score for the previous 10-min interval. There was a large induction of rearing activity by 2.0 mg/kg methamphetamine (squares) compared with saline vehicle (dotted line with open circles). Low-dose clozapine (1.0 mg/kg; circles) had no significant effect on methamphetamine rearing. CX516 (10 mg/kg; diamonds) had a modest, but statistically insignificant, antagonism in this experiment. However, CX516 (10 mg/kg) and clozapine (1 mg/kg) together produced a synergistic interaction, reducing methamphetamine rearing to nearly that of vehicle-treated rats (triangles). The arrow indicates when vehicle or drug or drugs were administered. B, bar graph shows total cumulative rearing activity during the 90-min period after methamphetamine administration. Mean ± S.E.M. and number of animals for the experimental groups are as follows: saline, 56 ± 9, n = 12; methamphetamine (2 mg/kg), 724 ± 136, n = 20; methamphetamine + clozapine (1.0 mg/kg), 780 ± 146, n = 18; methamphetamine + CX516 (10 mg/kg), 495 ± 78, n = 19; and methamphetamine + CX516 + clozapine, 125 ± 22, n = 17 (**P < .001 versus methamphetamine + clozapine (1.0 mg/kg); P < .01 versus methamphetamine + CX516 by Dunn’s multiple comparison test after a Kruskal-Wallis test (one-way ANOVA)).
but not necessarily immediately on administration. However, the synergistic interaction between CX516 and clozapine or haloperidol was apparent immediately after coadministration (Figs. 2 and 3). This result agrees with the expected rapid effect of an allosteric modulator of the fast ion channel of the AMPA-type glutamate receptor.

Examination of data presented in Fig. 1 and Table 1 suggests that ampakines, either alone or combined with a threshold antipsychotic dose, are somewhat more potent against amphetamine-induced rearing than LMA. In our hands, this is also true for several atypical antipsychotics, such as clozapine, risperidone, or olanzapine, when tested alone against methamphetamine. Although several studies have anatomically dissected amphetamine stereotopy (striatum) from amphetamine LMA (nucleus accumbens), there is little information on the neurochemistry and neuronal circuitry that differentiate amphetamine LMA and rearing, making it difficult to speculate on the greater effects of ampakines on rearing.

Studies of the activity-dependent gene c-fos suggest that antipsychotics activate striatopallidal (indirect) cells, whereas amphetamine increases activity in striatal cells that project directly to the output stations of the basal ganglia (Robertson et al., 1992; Jaber et al., 1995). Selective activation of these two functionally antagonistic systems could thus account for the antagonistic behavioral effects of amphetamines and neuroleptics. Ampakines presumably counteract the effects of methamphetamine by a route different from neuroleptics. Analyses of c-fos mRNA levels demonstrated that methamphetamine shifted the balance of aggregate activity in cortex versus striatum to favor striatum, whereas ampakines shifted it to favor cortex (Palmer et al., 1997). Given that removal of cortical telencephalon enhances amphetamine-induced hyperactivity (Lynch et al., 1969), it is not surprising that increasing cortical activity depresses it. This could be achieved by enhancing the excitatory drive 1) from superficial cortical layers to the indirect path or 2) from the deeper cortical layers to the inhibitory (GABAergic) striatonigral projections. The former effect would offset the imbalance created by amphetamine between the opposing striatal outputs, whereas the latter would reduce the ascending dopaminergic activity and thus the substrate on which methamphetamine acts.

Positive interactions between neuroleptics and ampakines
are expected outcomes from the above proposed mechanisms. The neurons in the striatum in which convergence of drug effects is postulated to occur have classic firing thresholds and thus provide the opportunity for positively interacting manipulations to have nonlinear outcomes. For example, partial suppression of the mesostriatal dopaminergic projections and partial blockade of dopamine receptors could result in a greater-than-additive increase in the number of “indirect path” cells that cross their firing thresholds in response to their excitatory inputs. Predictions from these hypotheses are reasonably straightforward. If ampakines counteract their excitatory inputs, then they should reduce methamphetamine-induced increases in c-fos mRNA. If ampakines and antipsychotics converge on indirect pathway cells, then the former should enhance the response to the latter. Experiments directed at these arguments are pertinent to the drug interactions reported here and to evaluating a combination drug therapy in treating schizophrenia.

Acknowledgments

We thank Dr. John Larson for advice during the initial stages of this work and Dr. Vincent Simmon for review of the manuscript.

References