ACP-103, a 5-Hydroxytryptamine 2A Receptor Inverse Agonist, Improves the Antipsychotic Efficacy and Side-Effect Profile of Haloperidol and Risperidone in Experimental Models


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ABSTRACT

Dopamine D₂ receptor antagonism contributes to the therapeutic action of antipsychotic drugs (APDs) but also produces undesirable side effects, including extrapyramidal motor deficits, cognitive dulling, and prolactinemia. The introduction of atypical APDs was a significant advancement in the treatment of schizophrenia. Whereas these agents are D₂ receptor antagonists, they are also potent 5-hydroxytryptamine (5-HT)₂A receptor inverse agonists, a feature that may explain their improved efficacy and tolerability. Recently, we reported that N-(4-fluorophenylmethyl)-N-(1-methylpiperidin-4-yl)-N’-(4-(2-methylpropoxy)phenylmethyl) carbamide (2R,3R)-dihydroxybutanedioate (2:1) (ACP-103), a novel selective 5-HT₂A receptor inverse agonist that fails to bind D₂ receptors, is active in several models predictive of antipsychotic activity. Using ACP-103, we tested the hypothesis that combining high levels of 5-HT₂A inverse agonism with low levels of D₂ antagonism would result in a favorable interaction, such that antipsychotic efficacy could be achieved with reduced D₂ receptor-related adverse effects. Here we show that ACP-103 1) potently inhibited head-twitching produced by the 5-HT₂₂₃,₄ receptor agonist (±)-2,5-dimethoxy-4-iodoamphetamine, 2) increased the potency of haloperidol against amphetamine-induced hyperactivity, 3) interacted synergistically with haloperidol or risperidone to suppress hyperactivity induced by the N-methyl-D-aspartate receptor antagonist (5R,10S)-(−)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate (MK-801), and, by contrast, 4) attenuated haloperidol- or risperidone-induced prolactinemia. ACP-103 also attenuated catalepsy produced by haloperidol or risperidone. However, the doses that were required for this effect were higher than would be expected for a 5-HT₂A receptor-mediated mechanism. These data indicate that utilizing ACP-103 as an adjunctive therapy to currently used APDs may result in enhanced antipsychotic efficacy while reducing adverse effects including those attributable to D₂ receptor antagonism.

Antagonism of dopamine D₂ receptors, a property shared by first-generation or “typical” antipsychotic drugs (APDs), contributes to the therapeutic action of these agents (Creese et al., 1976; Snyder, 1976). This property, however, has also been linked to undesirable side effects, including extrapyramidal motor deficits, cognitive dulling, and hyperprolactinemia. The introduction of second-generation, or “atypical,” APDs was a significant development, as these drugs achieve efficacy with significantly less D₂ receptor blockade. Despite their advantages, recent findings from the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study reported that 74% of patients treated with olanzapine, quetiapine, ziprasidone, or risperidone discontinued treatment within a period of 18 months because of either a lack of efficacy or intolerability of side effects (Lieberman et al., 2005). In a subsequent study, it was shown that after treat-
ment discontinuation, patients who were switched to clozapine were less apt to discontinue their medication and demonstrated improved efficacy compared with patients who were given a different atypical APD (McEvoy et al., 2006). Of the atypical APDs assessed in the CATIE studies, clozapine possesses the highest affinity ratio for 5-HT2A relative to D2 receptors (Meltzer et al., 1989; Stockmeier et al., 1993; Weiner et al., 2001).

ACP-103, a 5-HT2A receptor inverse agonist that is devoid of D2 receptor binding (Vanover et al., 2006), is currently being developed as an adjunctive therapy for schizophrenia. ACP-103 demonstrates a behavioral profile consistent with that of an atypical APD as it 1) inhibits head-twitching behavior elicited by the 5-HT2A receptor agonist (±)-2,5-dimethoxy-4-iodoamphetamine (DOI), 2) attenuates hyperlocomotor activity produced by the NMDA receptor antagonist MK-801, and 3) restores DOI-induced disruption of prepulse inhibition (Vanover et al., 2006). Furthermore, preliminary studies have shown that ACP-103 does not adversely affect cognition (L. R. Gardell, unpublished data) nor does it elicit catalepsy (present study). Moreover, consistent with an atypical APD profile, Meltzer and colleagues (Li et al., 2005), have recently shown that ACP-103 preferentially increases dopamine release in the medial prefrontal cortex (mPFC) compared with that in the accumbens nucleus (NAC) and potentiates haloperidol-induced dopamine release in the mPFC while inhibiting that in the NAC, actions that are believed to contribute to an atypical APD profile. However, in contrast with the APDs with D2 blocking activity, ACP-103 does not effectively reverse amphetamine-induced hyperactivity (present study).

The purpose of the present investigation was to test whether antipsychotic-like efficacy can be achieved by combining ACP-103 with low levels of D2 receptor antagonism, as provided by haloperidol or risperidone. To this end, we assessed ACP-103, alone and in combination, with either haloperidol or risperidone against hyperactivity induced by amphetamine and MK-801. Furthermore, to determine whether an improved safety profile could be achieved, the effects of ACP-103 on catalepsy and prolactinemia induced by haloperidol and risperidone were assessed.

Materials and Methods

Animals. Male non-Swiss albino mice and Sprague-Dawley rats (Harlan, San Diego, CA) were housed in climate-controlled rooms on a 12:12 light/dark cycle with on lights at 6:00 AM. Rats were housed in groups of two, and mice were housed in groups of eight. Food and water were available ad libitum except during experimental procedures. At the time of testing, mice weighed 20 to 30 g, and rats weighed between 275 and 325 g. All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health (National Research Council, 1996) and with the Institutional Animal Care and Use Committee of ACADIA Pharmaceuticals.

Drugs. Amphetamine, DOI, MK-801, and haloperidol were obtained from Sigma-Aldrich (St. Louis, MO). Risperidone was obtained from Toronto Research Chemicals (North York, ON, Canada). ACP-103 was synthesized by ACADIA Pharmaceuticals. Drugs were administered either in a volume of 0.1 ml/10 g b.wt. or 1.0 ml/kg b.wt. to mice and rats, respectively. The vehicle used for amphetamine, MK-801, DOI, and ACP-103 was saline. Amphetamine, MK-801, and DOI were administered i.p. The vehicle used for haloperidol and risperidone was 10% Tween 80 in saline unless otherwise specified.

Haloperidol and risperidone were administered s.c., unless otherwise noted. The doses of ACP-103 are expressed as free base and were administered by the s.c. route.

DOI-Induced Head Twitch Assay. Head-twitching behavior was produced in mice and rats by administration of 2.5 mg/kg DOI, a 5-HT2A receptor agonist. Vehicle or various doses of ACP-103 were administered 60 min before DOI. Immediately after DOI injection, animals were placed in individual chambers for an 8-min session. Across the session the number of head twitches was recorded. To generate dose-response curves, head twitch counts (HTCs) were converted to percent maximum possible inhibition (%MPI): %MPI = [(HTC for drug – HTC for DOI control)/HTC for DOI control] × 100. The dose of each compound that elicited 50% inhibition (ID50) and the corresponding 95% confidence interval (95% CI) were determined using linear regression analysis (Tallarida and Murray, 1987). Each dose was tested in separate groups of animals.

Hyperlocomotor Activity Assays. Vehicle or ACP-103 was given 60 min before the animal entered the motor activity chambers (AccuScan Instruments, Columbus, OH). Vehicle or APD (haloperidol or risperidone) was injected 30 min before the animal entered the activity chambers (i.e., 30 min after vehicle or ACP-103 administration). Hyperlocomotion was produced in mice by administration of either amphetamine (3 mg/kg) or MK-801 (0.3 mg/kg) 15 min before the animal entered the activity chambers (i.e., 45 min after vehicle or ACP-103 administration). Immediately before the mice were placed into the activity chambers, the presence of ataxia and muscle incoordination was determined using the horizontal wire test. Once inside the chambers, the total distance traveled in centimeters was determined during a 15-min session. To generate dose-response curves, the distance traveled was converted to %MPI: %MPI = [distance traveled for drug or drug combination – distance traveled for amphetamine or MK-801 control]/distance traveled for vehicle control × 100. The ID50 value and the corresponding 95% CI were determined as mentioned previously. Mice had no prior exposure to the chambers, and each dose combination was tested in separate groups of mice. After testing, brain samples were collected and stored at −80°C until assayed for drug exposure.

Drug Interaction Studies. To assess the interaction of ACP-103 with haloperidol on amphetamine-induced hyperactivity, dose-response curves were constructed for haloperidol in the presence of vehicle or a fixed dose of ACP-103 (0.03 mg/kg) according to the protocol mentioned in the previous section. Hyperlocomotion was produced in mice by administration of amphetamine (3 mg/kg) 15 min before they entered the activity chambers (i.e., 45 min after vehicle or ACP-103 administration).

To assess the interaction of ACP-103 with haloperidol or risperidone on MK-801-induced hyperactivity, an isobolographic analysis was completed. This approach was necessary as ACP-103, haloperidol, and risperidone are all efficacious in this model. This method is based on the comparison of dose combinations in which the doses of each individual agent are determined to be equiphasic. In this case, dose-response curves were generated after coadministration of ACP-103 with either haloperidol or risperidone in a fixed dose ratio based on the individual calculated ID50 values of each drug. Therefore, separate groups received ACP-103 ID50 + haloperidol or risperidone ID50, (ACP-103 ID50 + haloperidol or risperidone ID50)V2, (ACP-103 ID50 + haloperidol or risperidone ID50)V4, and (ACP-103 ID50 + haloperidol or risperidone ID50)V8. Based on the dose-response curves obtained for the combined agents (i.e., ACP-103 + haloperidol or ACP-103 + risperidone), the ID50 value and 95% CI for each drug combination were obtained. These values served as the basis for plotting the isobolograms as described elsewhere (Tallarida et al., 1997).

Exposure Analysis. Liquid chromatography/mass spectrometry detection and quantification of test compounds were carried out using a Micromass Quattro Ultima Pt mass spectrometer interfaced with a 1525u high-performance liquid chromatograph (Waters, Mil-
ford, MA). Positive ion electrospray was used as the means of ionization. Data collection and processing was performed using MassLynx software (version 4.0, Waters). High-performance liquid chromatography was performed on a Synergi Polar RP column (30 × 2 mm, 4-mm particles; Phenomenex, Torrance, CA). Compounds were eluted from the column using a solvent gradient at a flow rate of 0.5 ml/min. The mobile phase consisted of A (95% purified water and 5% acetonitrile + 0.5% acetic acid) and B (95% acetonitrile and 5% purified water + 0.5% acetic acid). The initial condition of 0% B was gradually ramped to 100% B in 2 min and maintained at 100% B for 1.2 min. The gradient was then returned to 0% B in 0.1 min and maintained in 2.2 min for equilibration of the column. The total runtime was 5.5 min.

Brains were homogenized using 3 volumes of purified water per weight of tissue. To precipitate proteins, acetonitrile was added to the homogenized brain tissue followed by centrifugation. An aliquot of the supernatant was withdrawn, diluted 3-fold with purified water, and injected (10 μl) onto column for chromatographic separation. The multiple reaction monitoring mode was used for selective detection of the following compounds: ACP-103, transition 428.5 → 223.3; haloperidol, transition 376.2 → 165.1; and risperidone, transition 411.3 → 191.2. The dwell times were set at 0.2 s and cone voltage at 52 V. Standard curves for quantification of the compounds of interest were constructed over ranges of 10 to 6250 nM.

**Prolactin Assay.** Dose-response curves were generated for haloperidol, risperidone, and ACP-103 on serum prolactin levels. Rats were given vehicle (100% dimethyl sulfoxide), haloperidol, or risperidone i.p. ACP-103 or vehicle (saline) was given s.c. Blood samples were collected 30 min after vehicle, haloperidol, or risperidone administration or 60 min after ACP-103 administration. Rats were deeply anesthetized with isoflurane, and blood samples were obtained by cardiac puncture, allowed to clot, and then centrifuged for 10 min to yield serum for analysis. Serum prolactin levels were quantified using a commercially available enzyme immunoassay kit (ALPCO Diagnostics, Windham, NH).

To explore the potential interaction between haloperidol or risperidone and ACP-103 on serum prolactin levels, rats were given vehicle or various doses of ACP-103 s.c., and then 30 min later were given either vehicle or a fixed dose of haloperidol or risperidone i.p. Blood samples were collected 30 min after vehicle, haloperidol, or risperidone administration (i.e., 60 min after vehicle or ACP-103 administration). The time point for sample collection was chosen on the basis of pilot work in which we established 30 min as the time at which peak prolactin levels can be detected after risperidone or haloperidol treatment. The fixed doses of haloperidol (0.1 mg/kg) and risperidone (0.01 mg/kg) were chosen because they elicited statistically significant and reproducible, but submaximal, increases in prolactin, thus allowing for the detection of potential increases as well as decreases.

**Catalepsy Assessment.** Rats were positioned with their forepaws on a horizontal bar (diameter 10 mm) elevated 10 cm above the bench top. The duration of the cataleptic bout was recorded up to a maximum value of 120 s. Catalepsy values were obtained at 30 and 60 min after i.p. administration of risperidone or haloperidol, respectively. Doses of ACP-103 were administered s.c. 60 min before either haloperidol or risperidone. To generate dose-response curves, catalepsy values were converted to percent maximum possible catalepsy (%MPC): %MPC = [(catalepsy value for drug or drug combination − catalepsy value for vehicle control)/120 − catalepsy value for vehicle control] × 100. The dose that elicits 50% of maximum catalepsy (CD_{50}) and the corresponding 95% CI as previously mentioned. Each dose or dose combination was assessed in separate groups of rats.

**Statistical Analysis.** Linear regression analysis of the log dose-response curves was used to calculate the ID_{50} and CD_{50} values and the 95% CIs. Relative potency was determined as a ratio of the ID_{50} and CD_{50} values. Significant shifts in dose-response curves were determined when the 95% CIs of the relative potency of the dose-response curves did not include unity (Tallarida and Murray, 1987).

The significance of the relative potency and the 95% CIs was determined by applying a t test. These evaluations were performed with the pharmacological statistics package FlashCalc (Dr. Michael H. Ossipov, University of Arizona, Tucson, AZ). One-way analysis of variance followed by post hoc Dunnett’s multiple comparison test was performed where appropriate (GraphPad Prism 4.0; GraphPad Software Inc., San Diego, CA). p < 0.05 was considered statistically significant.

**Results**

**Effects of ACP-103 on Suppression of Head-Twitching Behavior Produced by DOI.** Administration of 2.5 mg/kg DOI, a 5-HT_{2A} receptor agonist, elicited robust and reproducible head-twitching behavior in mice and rats. In the presence of vehicle, DOI treatment produced "0.3 ± 0.9 and 12.8 ± 1.3 twitches in mice and rats, respectively. Pretreatment with ACP-103 dose-dependently attenuated head-twitching behavior in both mice and rats with ID_{50} values of 0.04 mg/kg (95% CI 0.03–0.06) and 0.03 mg/kg (0.02–0.04), respectively (Fig. 1). These values served as guides for selective 5-HT_{2A} receptor-blocking doses in our subsequent studies.

**Effects of Haloperidol Alone and in Combination with ACP-103 on Suppression of Amphetamine-Induced Hyperlocomotion in Mice.** Amphetamine significantly increased distance traveled to 2764 ± 230 cm from 876 ± 42 cm obtained in vehicle controls (Fig. 2A). Haloperidol dose-dependently inhibited amphetamine-induced hyperlocomotion with an ID_{50} value of 0.012 mg/kg (0.009–0.016) (Fig. 2B). In contrast, ACP-103, at a dose that is the approximate ID_{50} against DOI-induced head twitches (0.03 mg/kg), did not significantly alter amphetamine-induced hyperlocomotor activity (2538 ± 109 versus 2764 ± 230 cm for amphetamine-treated controls) (Fig. 2A). However, when this fixed dose of ACP-103 was combined with various doses of haloperidol, the dose-response curve for haloperidol was significantly displaced to the left, having a calculated ID_{50} value of 0.0013 mg/kg (0.0005–0.0031). The combination of ACP-103 and haloperidol resulted in a 9.5-fold (3.8–23.8) shift in potency (Fig. 2B).
presence of ACP-103 was not due to alterations in the pharmacokinetics of haloperidol as brain levels for each compound were not significantly different across dosing conditions (Table 1).

Effects of Haloperidol and ACP-103 Alone and in Combination on Suppression of MK-801-Induced Hyperlocomotion in Mice. MK-801 significantly increased distance traveled to 2227 ± 116 cm from 792 ± 40 cm obtained in vehicle controls. Haloperidol or ACP-103 elicited a dose-dependent attenuation of MK-801-induced hyperlocomotion, achieving ID_{50} values of 0.07 mg/kg (95% CI 0.063–0.087) and 0.09 mg/kg (0.067–0.12), respectively (Fig. 3A). Given that haloperidol and ACP-103 were equipotent in this

<table>
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<th>Treatment Conditions</th>
<th>ACP-103 Brain Level (nmol/kg)</th>
<th>Haloperidol Brain Level (nmol/kg)</th>
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</thead>
<tbody>
<tr>
<td>ACP-103 + vehicle</td>
<td>23 ± 6</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Vehicle + haloperidol (0.003)</td>
<td>17 ± 6</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Vehicle + haloperidol (0.01)</td>
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<td>11 ± 3</td>
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<tr>
<td>Vehicle + haloperidol (0.03)</td>
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<td>25 ± 5</td>
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Fig. 2. A, relative to vehicle (Veh) controls (○), amphetamine (△) significantly increases hyperlocomotor activity in mice. ACP-103 at a dose of 0.03 mg/kg (●) failed to suppress hyperlocomotion produced by amphetamine. In contrast, haloperidol (□) dose-dependently attenuated hyperactivity produced by amphetamine. However, haloperidol, when combined with a fixed dose of ACP-103 (0.03 mg/kg, ■), demonstrated an enhanced suppression of amphetamine-induced hyperlocomotory activity. B, the raw data contained in A were converted to %MPI to generate the dose-response curves contained herein. Haloperidol (□) produced a dose-dependent attenuation of hyperactivity elicited by amphetamine. However, when combined with a fixed dose of ACP-103 (0.03 mg/kg, ■), the dose-response curve for haloperidol was significantly shifted to the left by a factor of approximately 10. Each data point represents a minimum n of 8.

ACP-103 Is Synergistic with Haloperidol or Risperidone 865

Fig. 3. A, dose-response curves for haloperidol (□), ACP-103 (■), and the combination of haloperidol with ACP-103 in a 1:1 fixed dose ratio (●) on the suppression of MK-801-induced hyperactivity. Each data point represents a minimum n of 16. B, isobologram generated from data in A. The calculated ID_{50} (and 95% CI) values for ACP-103 and haloperidol when administered alone (□) are plotted on the x- and y-axes, respectively. The dashed line connecting these two points represents the line of theoretical additivity. The experimental ID_{50} (●, B) for the dose combination was significantly less than the theoretical ID_{50} (■, A), indicating a synergistic interaction.
MK-801, achieving a %MPI of 82. Coadministration of risperidone and ACP-103 dose-dependently attenuated hyperlocomotor activity induced by MK-801 administered alone (0.067–0.12 mg/kg). Coadministration of haloperidol and ACP-103 dose-dependently attenuated hyperlocomotor activity induced by MK-801, achieving a %MPI of 103 ± 6% (Fig. 3A). Isobolographic analysis revealed a synergistic interaction between haloperidol and ACP-103 (Fig. 3B). The experimental ID₅₀ for the dose mixture was significantly less than the theoretical ID₅₀ with values of 0.04 mg/kg (0.03–0.05) and 0.08 mg/kg (0.68–0.93), respectively (Fig. 3B). The synergy observed between ACP-103 and haloperidol was not the result of alterations in the pharmacokinetics of either compound as brain levels were not significantly different across dosing conditions (Table 2).

**Effects of Risperidone and ACP-103 Alone and in Combination on Suppression of MK-801-Induced Hyperlocomotion in Mice.** As in the previous experiment, MK-801 treatment significantly increased total distance traveled to 2020 ± 223 cm from 649 ± 67 cm obtained in vehicle controls. Either risperidone or ACP-103 dose-dependently attenuated MK-801-induced hyperlocomotion with ID₅₀ values of 0.0045 mg/kg (95% CI 0.003–0.006) and 0.09 mg/kg (0.067–0.12), respectively (Fig. 4A). Given that risperidone was more potent than ACP-103 in this assay, a 1:18 fixed dose ratio (risperidone/ACP-103) was administered in fractions of the approximated ID₅₀ dose combinations of 0.06 + 0.06 mg/kg (ID₅₀/2 = 0.03 + 0.03 mg/kg; ID₅₀/4 = 0.015 + 0.015 mg/kg; ID₅₀/8 = 0.0075 + 0.0075 mg/kg). Coadministration of haloperidol and ACP-103 dose-dependently attenuated hyperlocomotor activity induced by MK-801, achieving a %MPI of 103 ± 6% (Fig. 3A). Isobolographic analysis revealed a synergistic interaction between haloperidol and ACP-103 (Fig. 3B). The experimental ID₅₀ for the dose mixture was significantly less than the theoretical ID₅₀ with values of 0.04 mg/kg (0.03–0.05) and 0.08 mg/kg (0.68–0.93), respectively (Fig. 3B). The synergy observed between ACP-103 and haloperidol was not the result of alterations in the pharmacokinetics of either compound as brain levels were not significantly different across dosing conditions (Table 2).

**Effects of Haloperidol and Risperidone Alone and in Combination on Serum Prolactin Levels in Rats.** Serum prolactin levels obtained in vehicle-treated controls were 24 ± 3 and 31 ± 3 ng/ml after 30 and 60 min, respectively. Haloperidol or risperidone, but not ACP-103, dose-dependently increased serum prolactin levels. Rather, rats treated with ACP-103 demonstrated a significant reduction in serum prolactin concentrations with all ACP-103-

### Table 2

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<th>Treatment Conditions</th>
<th>ACP-103 Brain Level</th>
<th>Haloperidol Brain Level</th>
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<td>ACP-103 (0.015) + vehicle</td>
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<tr>
<td>ACP-103 (0.03) + vehicle</td>
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<td>Vehicle + haloperidol (0.06)</td>
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<td>ACP-103 (0.06) + haloperidol (0.06)</td>
<td>19 ± 3</td>
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![Fig. 4. A, dose-response curves for risperidone (□), ACP-103 (■), and the combination of risperidone with ACP-103 in a 1:18 fixed-dose ratio (○) on the suppression of MK-801-induced hyperactivity. Each data point represents a minimum n of 16. B, isobologram generated from data in A. The calculated ID₅₀ (and 95% CI) values for ACP-103 and risperidone when administered alone (□) are plotted on the x- and y-axes, respectively. The dashed line connecting these two points represents the line of theoretical additivity. The experimental ID₅₀ (●, B) for the dose combination was significantly less than the theoretical ID₅₀ (□, A), indicating a synergistic interaction.](image-url)
treated animals having serum prolactin concentrations below the limit of detection (15 ng/ml) (Fig. 5A).

To determine whether ACP-103 altered prolactinemia produced by haloperidol or risperidone, rats were pretreated with various doses of ACP-103 before receiving a fixed dose of haloperidol (0.1 mg/kg) or risperidone (0.01 mg/kg). This dose of haloperidol significantly increased serum prolactin levels from 31 ± 3 to 102 ± 12 ng/ml. Similarly, risperidone significantly increased serum prolactin levels from 24 ± 3 to 102 ± 12 ng/ml. However, in the presence of ACP-103, the magnitude of prolactinemia induced by either haloperidol or risperidone was significantly attenuated (Fig. 5B).

**Effects of ACP-103 on Haloperidol- and Risperidone-Induced Catalepsy in Rats.** Vehicle treatment elicited a maximum catalepsy value of 6.8 ± 0.9 s. ACP-103 did not elicit catalepsy at doses up to 10 mg/kg, achieving a maximum catalepsy value of 10.5 ± 2.4 s (not significantly different from that of vehicle controls). In contrast, both haloperidol and risperidone produced marked increases in catalepsy with CD50 values of 0.24 mg/kg (95% CI 0.19–0.39) (Fig. 6A) and 1.1 mg/kg (0.79–1.62) (Fig. 6B), respectively.

ACP-103 did not potentiate the catalepsy induced by either haloperidol or risperidone. The combination of 1 or 3 mg/kg ACP-103 with haloperidol did not significantly alter haloperidol-induced catalepsy with CD50 values of 0.24 mg/kg (95% CI 0.16–0.36) and 0.38 mg/kg (0.24–0.61), respectively. However, the addition of 10 mg/kg ACP-103 to haloperidol significantly increased the observed CD50 value from 0.27 mg/kg (0.19–0.39) to 0.53 mg/kg (0.31–0.91) (Fig. 6A), indicating a reduction of catalepsy.

ACP-103 at all doses tested resulted in a dose-dependent and significant rightward displacement of the risperidone dose-response curve for catalepsy. The calculated CD50 values for risperidone in the presence of 1, 3, or 10 mg/kg ACP-103 were 2.0 mg/kg (95% CI 1.3–3.0), 4.4 mg/kg (2.6–7.5), and 5.1 mg/kg (3.2–8.3), respectively (Fig. 6B), indicating a reduction of catalepsy.

**Discussion**

The major finding of the present investigation was that ACP-103 increased the potency of haloperidol and risperidone against amphetamine and MK-801-induced hyperlocomotor activity without increasing the propensity of these drugs to produce catalepsy or prolactinemia. This represents the first clear demonstration that 5-HT2A receptor antagonism can produce a significant dose-sparing effect for both typical and atypical APDs. This dose-sparing action of ACP-103 may allow for improved antipsychotic efficacy while concomitantly reducing unwanted side effects attributable to high levels of D2 receptor antagonism.

Head-twitching behavior elicited by DOI is a response that is dependent upon the activation of 5-HT2A receptors. Previous studies have shown that DOI-induced head twitches are completely abolished in mice lacking the 5-HT2A receptor (González-Maeso et al., 2003) and are dose-dependently attenuated by the selective 5-HT2A antagonist, M100907 (Kehne et al., 1996). Therefore, this assay was used to estimate doses of ACP-103 required for functional 5-HT2A receptor blockade. The ID50 values for ACP-103 in mice and rats, after s.c. administration, were 0.04 and 0.03 mg/kg, respectively. These doses served as guides for the subsequent experiments.

Consistent with a previous report (Vanover et al., 2006), ACP-103 potently attenuated hyperlocomotor activity produced by the NMDA receptor antagonist MK-801. However, at a dose expected to block 5-HT2A receptors, ACP-103 did not reverse hyperactivity elicited by amphetamine. In the present study we extended these findings by examining the interactions between ACP-103 and either haloperidol or risperidone using behavioral models of hyperdopaminergia and hypoglutamatergia. ACP-103, at a dose that does not suppress amphetamine-induced hyperactivity, when combined with haloperidol, increased the potency of haloperidol approximately 10-fold. Furthermore, we demonstrate that ACP-103 interacted synergistically with haloperidol and with risperidone to reduce MK-801-induced hyperactivity. The actions of ACP-103 were not achieved by simply altering the pharmacokinetics of either haloperidol or risperidone, as brain exposures for these agents were not significantly altered in the presence of ACP-103.

These effects of ACP-103 were probably mediated by antagonism of 5-HT2A receptors. ACP-103 is a relatively selective 5-HT2A antagonist/inverse agonist with approximately 50-fold higher affinity for the 5-HT2A than the 5-HT2C receptor (Vanover et al., 2006). Also, the doses used in these studies were chosen to correspond to those that produced a 5-HT2A receptor blockade-mediated inhibition of DOI-induced head twitching. Furthermore, it is unlikely that antagonism of 5-HT2C receptors contributed to the synergistic interaction of ACP-103 with these antipsychotic drugs on amphetamine- or MK-801-induced hyperactivity because...
5-HT₂C antagonists increase, rather than inhibit, stimulant-induced hyperactivity. It has been recently reported that 5-HT₂C agonists can inhibit MK-801- or amphetamine-induced hyperactivity (Marquis et al., 2007). However, there is no indication that ACP-103 or other selective 5-HT₂A antag-

Fig. 5. A, prolactin levels obtained in rats after various doses of risperidone (▲), haloperidol (■), and ACP-103 (●). Both risperidone and haloperidol produced robust and dose-dependent elevations of plasma prolactin in rats. In contrast, ACP-103 up to a dose of 3 mg/kg did not significantly alter plasma prolactin levels. B, prolactin levels obtained in rats after a fixed dose of haloperidol (0.1 mg/kg) in the presence of vehicle or various doses of ACP-103. ACP-103 significantly attenuated prolactinemia produced by haloperidol. C, prolactin levels obtained in rats after a fixed dose of risperidone (0.01 mg/kg) in the presence of vehicle or various doses of ACP-103. ACP-103 significantly attenuated prolactinemia produced by risperidone. Each data point represents a minimum n of 12. **, p < 0.01; *, p < 0.05, compared with vehicle controls.

Fig. 6. A, as expected, haloperidol (○) produced a dose-dependent increase in catalepsy time in rats. ACP-103 failed to potentiate haloperidol-induced catalepsy at any of the doses tested. Whereas ACP-103 at doses of 1 mg/kg (●) or 3 mg/kg (□) failed to significantly alter haloperidol-induced catalepsy, 10 mg/kg (▲) of ACP-103 produced a small, but statistically significant, attenuation of catalepsy produced by haloperidol. B, as expected, risperidone (○) produced a dose-dependent increase in catalepsy time in rats. ACP-103 failed to potentiate risperidone-induced catalepsy at any of the doses tested. However, ACP-103 at doses of 1 mg/kg (●), 3 mg/kg (□), and 10 mg/kg (▲) significantly attenuated catalepsy produced by risperidone. Each data point represents a minimum n of 12.
onists (such as M100907) that reverse MK-801-induced hyperactivity have 5-HT$_{2C}$ agonist activity. Thus, in all likelihood, neither antagonism nor agonism at 5-HT$_{2A}$ receptors accounted for these actions of ACP-103. Rather these actions are most probably the consequence of 5-HT$_{2A}$ receptor blockade.

The mechanism by which 5-HT$_{2A}$ receptor blockade enhances the action of APDs in these models is unclear; however, microdialysis and other studies suggest that 5-HT$_{2A}$ inverse agonists may have regionally specific effects on DA transmission. Previous studies have shown that DOI increases DA release (Bowers et al., 2000) and potentiates amphetamine-induced DA release in the NAC, an effect that is blocked by M100907, suggesting that 5-HT$_{2A}$ receptor inverse agonists are more apt to modulate evoked, rather than basal, DA release (Kuroki et al., 2003). Haloperidol, which potently inhibits amphetamine hyperactivity, has been shown to paradoxically increase DA release in the NAC, an effect blocked by ACP-103 (Li et al., 2005). These data suggest that ACP-103 may potentiate the actions of haloperidol via direct or indirect modulation of evoked DA release in the NAC. Another possibility is that 5-HT$_{2A}$ inverse agonists may block a “pro- psychotic” drive associated with APD-induced enhanced serotonergic transmission in limbic or cortical structures (Meltzer, 1991; Carlsson et al., 1999). After systemic administration of NMDA antagonists, extracellular DA and 5-HT concentrations rise in the NAC (Schmidt and Fadayel, 1996; Yan et al., 1997) and mPFC (Yan et al., 1997). High doses of atypical APDs, such as clozapine and olanzapine, but not typical APDs, such as haloperidol, produce preferential increases in DA release in the mPFC compared with the NAC (Moghaddam and Bunney, 1990; Li et al., 1998; Kuroki et al., 1999), a property that may explain how atypical APDs improve cognition in schizophrenia (Moghaddam and Bunney, 1990; Kuroki et al., 1999; Meltzer and McGurk, 1999; Ichikawa et al., 2001; Li et al., 2004). ACP-103 has recently been shown to inhibit DA release induced by a low dose of haloperidol in the NAC while enhancing this effect in the mPFC (Li et al., 2005). And finally, the principal action of 5-HT$_{2A}$ inverse agonists may be on the glutamatergic system, with a converging effect of D$_2$ blockade and enhanced glutamatergic transmission converging on GABAergic systems in the striatum (Carlsson, 2006). Regardless of the mechanism, these findings indicate that ACP-103 has dose-sparing actions for APDs in models predictive of antipsychotic action.

However, this dose-sparing action will only be advantageous if ACP-103 does not similarly potentiate the adverse effects of APDs. To this end, we assessed the effects of ACP-103 alone, and in combination with haloperidol or risperidone, on two common undesired end points of APDs, namely, hyperprolactinemia and extrapyramidal motoric effects as reflected in animals as catalepsy. Antagonism of D$_2$ receptors produces robust prolactinemia both experimentally (Clemens et al., 1974) and clinically (Markianos et al., 2001; Zhang et al., 2005). Similarly, risperidone, an atypical APD, has also been shown to elicit prolactinemia as severe as haloperidol in humans (Markianos et al., 2001; Zhang et al., 2005). Here we demonstrate that although both haloperidol and risperidone produced robust increases in serum prolactin, ACP-103 did not elevate, and indeed slightly reduced, serum prolactin levels. Importantly, ACP-103 did not potentiate rather attenuated the hyperprolactinemia produced by these APDs. This finding is in agreement with another study in which it was reported that ICI 169,369 and other 5-HT$_{2A}$ antagonists modulate the effects of D$_2$ receptor blockade (Saller et al., 1990).

Despite the anatomical evidence supporting expression of 5-HT$_3$ receptors in the pituitary gland (De Souza, 1986), the preponderance of data suggests that the regulation of prolactin secretion mediated by 5-HT$_{2A}$ receptors occurs at the level of the hypothalamus (Bagdys, 1996; Zhang et al., 2002). Pituitary D$_2$ receptors, which lie outside of the blood-brain barrier (BBB), exert tonic inhibition of prolactin secretion, whereas activation of 5-HT$_{2A}$ receptors in the hypothalamus inhibits DA release, resulting in prolactin elevation. Thus, pure D$_2$ antagonists elicit prolactinemia by direct actions in the pituitary, whereas highly brain-penetrating APDs, especially those that possess high 5-HT$_{2A}$/D$_2$ affinity ratios (i.e., olanzapine and clozapine), do not elicit marked hyperprolactinemia because these drugs achieve sufficient 5-HT$_{2A}$ receptor blockade in the hypothalamus to counteract the effects of D$_2$ receptor blockade in the pituitary gland. This finding is critical with respect to risperidone, which has been shown to preferentially occupy D$_2$ receptors in the pituitary gland, compared with the striatum, at doses up to 2.5 mg/kg in rats (Kapur et al., 2002). If risperidone does indeed cross the BBB poorly, then the profile of this drug, with respect to prolactinemia, is more consistent with a typical, rather than an atypical APD, as the direct effects at D$_2$ in the pituitary are not likely to be counteracted by 5-HT$_{2A}$ receptor blockade inside the BBB. Consistent with this idea is the observation that risperidone elevates prolactin at doses much lower than those required to produce catalepsy. Furthermore, by combining ACP-103 with risperidone, a sufficient level of 5-HT$_{2A}$ receptor occupancy was reached inside the BBB to counteract risperidone-induced hyperprolactinemia. These findings may have significant clinical relevance, as hyperprolactinemia is correlated with numerous complications such as sexual dysfunction (for review, see Cutler, 2003), which is a prominent cause of noncompliance, particularly in men.

Finally, we demonstrate that although both haloperidol and risperidone produced dose-dependent catalepsy, ACP-103 alone did not elicit detectable catalepsy at doses as high as 10 mg/kg or 333-fold higher than the ID$_{50}$ in the DOI head twitch assay, consistent with its lack of affinity for D$_2$ receptors (Vanover et al., 2006). Importantly, we demonstrated that although ACP-103 potentiated the antipsychotic efficacy of haloperidol and risperidone, ACP-103 did not potentiate catalepsy produced by either drug. Instead, we observed a small but statistically significant reduction of haloperidol- or risperidone-induced catalepsy at a dose of ACP-103 that would be expected for supramaximal 5-HT$_{2A}$ receptor occupancy (i.e., 1 or 10 mg/kg). Others have shown that haloperidol-induced catalepsy was attenuated by a selective 5-HT$_{2B/2C}$ antagonist, SB228357, but not by M100907 or by a selective 5-HT$_{2A}$ antagonist, SB215505 (Reavill et al., 1999). This effect has been confirmed by our group as we have shown that haloperidol-induced catalepsy is significantly attenuated by a selective 5-HT$_{2C}$ antagonist, SB242084 (L. R. Gardell, unpublished observations). Taken together, these data suggest that ACP-103 may attenuate haloperidol-induced catalepsy via its relatively weaker 5-HT$_{2C}$ receptor antagonism.
In conclusion, our data suggest that ACP-103, via 5-HT2A receptor antagonism or inverse agonism, results in a significant dose-sparing effect for typical and atypical APDs such that antipsychotic efficacy can be maintained or improved at lower doses, thus concomitantly reducing the severity of unwanted side effects including those mediated via D2 receptor antagonism. The findings with risperidone suggest that the dose-sparing benefits of ACP-103 will be manifested even with those atypical APDs having an inherently high affinity for 5-HT2A receptors.

References


