Antipsychotic-Like Properties of Phosphodiesterase 4 Inhibitors: Evaluation of 4-(3-Butoxy-4-methoxybenzyl)-2-imidazolidinone (RO-20-1724) with Auditory Event-Related Potentials and Prepulse Inhibition of Startle

T. B. Halene and S. J. Siegel

International Research Training Group 1328 Schizophrenia and Autism, Department of Psychiatry and Psychotherapy, RWTH Aachen University, Aachen, Germany (T.B.H.); and Stanley Medical Research Institute Laboratory for Experimental Therapeutics in Psychiatry, Department of Psychiatry, University of Pennsylvania, Philadelphia (S.J.S.)

Received February 28, 2008; accepted April 16, 2008

ABSTRACT

Antipsychotic medications function through antagonism of D2 dopamine receptors. Blockade of D2 receptors causes an increase in intracellular cAMP, a ubiquitous second messenger. Inhibition of phosphodiesterase (PDE) activity, a family of enzymes that degrade cyclic nucleotides, causes the same effect. The conceptual linkage between dopamine D2 receptors and PDE activity via cAMP suggests a possible therapeutic potential for PDE inhibitors in schizophrenia. The limited number of studies in support of this hypothesis used rolipram, a specific inhibitor of the PDE4 family. In this study, we investigated the impact of 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (RO-20-1724), another PDE4-specific inhibitor, on auditory event-related potentials (ERPs), prepulse inhibition (PPI) of the startle reflex, and locomotor activity in mice. The ability to reverse amphetamine-induced alterations in ERPs and PPI was used as a model for psychosis. ERPs after RO-20-1724 revealed increased amplitude for the P20 and N40 ERP components. RO-20-1724 reversed the disruptive effect of amphetamines on ERPs and restored gating at a dose that did not impair locomotor activity. However, RO-20-1724 failed to reverse a amphetamine-induced decrease of PPI. Inconsistent results between these two psychosis models suggest that pure sensory processing, as measured with auditory ERPs, may be more sensitive to the effects of intracellular cAMP than sensorimotor effects as assessed with PPI. It remains unclear whether antipsychotic-like properties are a common feature of PDE4 inhibition, or if they are restricted to the pharmacological profile of rolipram. Future studies should examine how PDE4 subtype specificity might contribute to differences between rolipram and RO-20-1724 in sensorimotor gating.

Antipsychotic medications were introduced more than 50 years ago for the treatment of hallucinations, delusions, thought disorganization, and behavioral disorders. Although initially targeted for the treatment of schizophrenia, these medications act on psychotic symptoms irrespective of cause. Currently approved antipsychotic medications produce their beneficial effects through antagonism of the dopamine D2 receptor. Their potency is proportional to their affinity for this target (Kapur and Mamo, 2003). Blockade of D2 receptors causes an increase in intracellular cAMP, a ubiquitous second messenger. Inhibition of phosphodiesterase (PDE) activity, a family of enzymes with the ability to degrade cyclic nucleotides, causes the same effect. The conceptual linkage between the dopamine D2 receptor and PDE activity via cAMP suggests a possible therapeutic potential for PDE inhibitors in schizophrenia (Fig. 1).

No single antipsychotic substance has emerged as the gold standard for a first-line treatment in clinical trials or expert consensus guidelines, and with the notable exception of clozapine, all approved antipsychotics are of comparable efficacy. Antipsychotics vary in side effects including risk of extrapyramidal symptoms, weight gain, metabolic effects, sedation, hypotension, and cardiac complications. However, these differences seem to be quantitative rather than qualitative (Lieberman et al., 2005). Large clinical trials as well as systematic and critical reviews confirm that antipsychotics

ABBREVIATIONS: PDE, phosphodiesterase; RO-20-1724, 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone; DISC1, disrupted in schizophrenia 1; ERP, event-related potential; PPI, prepulse inhibition; S1, stimulus 1; S2, stimulus 2; ANOVA, analysis of variance; AC, adenylyl cyclase.
Among the most debilitating domain for affected patients is the experience of loss of effective responsiveness, verbal expression, delusions and hallucinations. Schizophrenia patients also experience loss of effective responsiveness, verbal expression, delusions and hallucinations. Schizophrenia patients also experience loss of effective responsiveness, verbal expression, delusions and hallucinations. Schizophrenia patients also experience loss of effective responsiveness, verbal expression, delusions and hallucinations. Schizophrenia patients also experience loss of effective responsiveness, verbal expression, delusions and hallucinations. Schizophrenia patients also experience loss of effective responsiveness, verbal expression, delusions and hallucinations.

Negative and manic symptoms. Although studies indicate that antipsychotics only partially relieve symptoms. Up to 30% of patients do not respond to first-line antipsychotic treatment (Kane et al., 1988). Furthermore, antipsychotic potency is mainly determined by the ability to improve positive symptoms such as delusions and hallucinations. Schizophrenia patients also experience loss of effective responsiveness, verbal expression, personal motivation, enjoyment, social drive, or attention to the environment, which are conceptualized as negative symptoms. Although studies indicate that antipsychotics provide minimal improvement in some cognitive and physiological abnormalities (Gonul et al., 2003; Keefe et al., 2004), it is unclear whether these improvements are due to medication or practice effects (Goldberg et al., 2007). Negative and cognitive symptoms remain largely untouched and emerge among the most debilitating domain for affected patients over time (Elvevåg and Goldberg, 2000).

Phosphodiesterase Inhibitors. Unmet medical needs, coupled with growing knowledge of the intracellular pathways after dopamine receptor activation, have identified cyclic nucleotides such as cAMP, cGMP, and the respective second messenger pathways as targets for drug development. Their possible manipulation by PDE inhibitors has received substantial interest in past years. Although the United States Food and Drug Administration-approved PDE inhibitors are still few and serve diverse indications, a growing number of preclinical in vitro and animal models illustrate the possibility of a receptor-independent manipulation of cell function (Lugnier, 2006).

PDE4 Inhibitors for the Treatment of Psychosis: Rationale for the Current Study. RO-20-1724 inhibits PDE4. Like rolipram, an extensively studied PDE4 inhibitor, it increases intensity and duration of cAMP-mediated signaling (Scuvée-Moreau et al., 1987). The widespread distribution of PDE4 isoenzymes in the brain suggests an opportunity for the development of PDE4 inhibitors as potential therapeutic targets (Zhang et al., 2005). PDE4 has been reported to influence mood and memory, modulate immune and inflammatory processes, and trigger nausea and emesis (O’Donnell and Zhang, 2004; Rutten et al., 2006). PDE4B has been identified as a risk factor for schizophrenia. It interacts in a complex manner with disrupted in schizophrenia 1 (DISC1), a genetic susceptibility factor for both depression and schizophrenia (Clapcote et al., 2007; Millar et al., 2007). We were surprised to find that only a limited number of preclinical studies have suggested a potential role for PDE4 in schizophrenia (Mori et al., 2000; Maxwell et al., 2004; Davis and Gould, 2005; Kanes et al., 2007; Murdoch et al., 2007; Siuciak et al., 2007).

Aim and Design of the Current Study. We previously investigated the PDE4-specific inhibitor rolipram in animal models used to predict antipsychotic efficacy (Maxwell et al., 2004; Kanes et al., 2007). Because previous studies mainly relied on rolipram as a PDE4 inhibitor, it is unclear whether the suggested antipsychotic-like effects are a general benefit of PDE4 inhibition, or whether they are restricted to the unique pharmacological properties or rolipram. This study uses an alternative PDE4 inhibitor, RO-20-1724, in both sensory (event-related potential; ERP) and sensorimotor (prepulse inhibition; PPI) models of psychosis in mice. The aim of the present study was to increase knowledge about the putative role of PDE4 inhibition for the treatment of psychosis. Therefore, ERP and PPI recording was performed after amphetamine challenge, which is an established animal model of psychosis.

Materials and Methods

To evaluate the performance of the PDE4 inhibitor RO-20-1724 on sensory processing, an initial group of 24 C57BL/6J mice underwent surgical electrode placement in the CA3 region of the hippocampus. Auditory ERPs were recorded after injection of vehicle followed by either 0.5 (n = 8), 1.0 (n = 8), or 2.5 (n = 8) mg/kg RO-20-1724. Measurement of locomotor activity after vehicle and drug injection followed. The lowest initial dose that was tested (0.5 mg/kg) had a significant effect on P20 and N40 amplitude. Therefore, tests were repeated with an additional set of 16 mice, applying a dose of 0.25 mg/kg (n = 8) and 0.1 mg/kg RO-20-1724 (n = 8). Auditory ERPs were recorded after d-amphetamine (n = 24) as well as simultaneous RO-20-1724 and d-amphetamine administration to assess its effectiveness in a disease model for psychosis (n = 24). For the assessment of sensorimotor performance using PPI, 16 animals received placebo, d-amphetamine, or d-amphetamine and RO-20-1724. To account for habituation and a possible order effect, mice were divided into three groups, order of treatment was balanced between groups, and there was a 48-h interval between test days 1, 2, and 3.

Ethics. All protocols were approved by the Institute of Laboratory Animal Resources (1996) and were conducted in accordance with the National Institutes of Health guidelines.

Mice. A total of 40 8-week-old C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and arrived at the University of Pennsylvania. Animals were maintained on a 12-h light/dark cycle (lights off at 7:00 PM) in a temperature-controlled facility with food and water available ad libitum. Mice were housed four to five per cage. Mice were acclimated to the housing facility for 1 week before surgery. After electrode placement, each mouse was housed individually. Surgery, behavioral, and electrophysiological training was performed during the light phase between 2:00 and 5:00.
PM. Electrode placement, electrophysiological, and behavioral testing was conducted between 9 and 19 weeks of age.

**Surgery.** Animals underwent stereotaxic implantation of tripolar electrode assemblies (Plastics One, Roanoke, VA) for nonanesthetized recording of auditory ERPs. Animals were anesthetized with isoflurane. Three stainless steel electrodes, mounted in a single pedestal, were aligned along the sagittal axis of the skull at 1-mm intervals with precut lengths of 3.0 mm (positive) and 1.0 mm (ground and negative). Positive electrodes were placed in the right CA3 hippocampal region, 1.4 mm posterior, 2.65 mm right lateral, and 2.75 mm deep relative to bregma (junction of sagittal and coronal sutures were used as surgical point of reference). Negative electrodes were placed adjacent to positive and ground electrodes on the ipsilateral cortex at 0.4 mm posterior, 2.75 mm lateral, and 0.75 mm deep to bregma. Ground electrodes were located between positive and negative electrodes on the ipsilateral cortex at 0.4 mm posterior, 2.75 mm lateral, and 0.75 mm deep to bregma. The electrode pedestal was secured to the skull with dental cement (Ortho Jet; Lang Dental, Wheeling, IL) and ethyl cyanoacrylate (Loctite; Henkel KGaA, Düsseldorf, Germany).

**Drugs.** RO-20-1724 was purchased from BIOMOL International (Plymouth Meeting, PA), and d-amphetamine was purchased from Sigma-Aldrich (St. Louis, MO). For injections, d-amphetamine was dissolved in 0.9% sterile saline, and RO-20-1724 was dissolved in 20% dimethyl sulfoxide and 80% sterile saline solution (0.9% sodium chloride). RO-20-1724 and vehicle (20% dimethyl sulfoxide and 80% sterile saline solution) injections were administered s.c. 5 min before testing/recording of brain activity.

**Measurement of Locomotor Activity.** Animals were transferred from their housing facility to the locomotor activity testing room in their home cages for a habituation period of 15 min before testing. Animals were placed in automated locomotor activity frames that created a grid of infrared light beams throughout the transparent home cages (31-cm length, 19-cm width, and 16-cm height) (MED Associates Inc., St. Albans, VT). The number of breaks of light beams caused by the moving animal was equivalent to its locomotor activity. Data were collected at 5-min intervals over a total period of 30 min, using a personal computer. Locomotor activity was measured with doses of 0.5 and 0.25 mg/kg versus vehicle condition. Testing sessions were 14 days apart (total n = 24).

**Electrophysiology.** Recording of brain activity for auditory ERPs was performed 14 days after electrode implantation. Each animal was placed in its home cage in a sound-attenuated recording room in their home cages for a habituation period of 15 min before testing. Animals were placed in automated locomotor activity frames that created a grid of infrared light beams throughout the transparent home cages (31-cm length, 19-cm width, and 16-cm height) (MED Associates Inc., St. Albans, VT). The number of breaks of light beams caused by the moving animal was equivalent to its locomotor activity. Data were collected at 5-min intervals over a total period of 30 min, using a personal computer. Locomotor activity was measured with doses of 0.5 and 0.25 mg/kg versus vehicle condition. Testing sessions were 14 days apart (total n = 24).

**Recording of Auditory ERPs.** Every ERP testing session consisted of 3 runs after a 15-min acclimation period. There was no intervention on the 1st run, vehicle administration on the 2nd run, and the drug condition for the 3rd run. Mice received injections 5 min before testing. The drug conditions were 0.1, 0.25, 0.5, 1.0, and 2.5 mg/kg RO-20-1724 in groups of eight mice to determine the lowest effective dose for an effect on the amplitude of the first click response. One mouse in the 2.5 mg/kg group had to be excluded after it lost its electrode cap. Mice were tested with the lowest ERP-effective dose to determine whether RO-20-1724 reversed the effects of d-amphetamine on ERPs. For this purpose, a group of 24 mice received two vehicle injections on day 1, a dose of 0.5 mg/kg d-amphetamine and a vehicle injection on day 2, and 0.5 mg/kg d-amphetamine and 0.25 mg/kg RO-20-1724 on day 3.

**Startle Response and Prepulse Inhibition.** Startle responses and inhibition of startle response after presentation of a nonstartling prepulse were registered by an accelerometer in response to acoustic stimuli delivered by a white noise generator (4–19 kHz) in a four-chamber system (San Diego Instruments, San Diego, CA). After the mouse was placed in the test chamber, the sessions began with a 5-min acclimation interval to a background white noise of 60 db. This was followed by a block of five 120-db startle pulses in an effort to make the subsequent startle responses less variable. During the next 10-min block, startle responses were measured to 40-ms pulses of 0 (control), 90, 95, 100, 110, 115, and 120-db sound pressure. Each intensity was presented five times in random order with an interstimulus interval randomized from 10 to 20 s with a mean of 15 s. The startle portion of the session concluded with an additional block of five 120-db pulses to assess potential effects of habituation. Startle trials were followed by a 10-min block of PPI trials. Each prepulse trial consisted of a 20-ms prepulse 4, 8, or 16-db above background noise (60 db) followed by a 40-ms pulse of 120 db 100 ms later. Five trials of each prepulse intensity, along with 10 startle-only trials, were presented in random order. Startle responses were collected as 60, 1-ms voltage readings, which were averaged over the collection interval to obtain an average measure for each trial using San Diego Instruments Startle Reflex Software.

**Treatment Conditions for Startle Response and PPI.** Immediately before the test session, mice received injections with either saline, d-amphetamine (5 mg/kg), or RO-20-1724 (2.5 mg/kg) and d-amphetamine. Mice were tested during three consecutive weeks with three testing days each week. The repetition with different doses of d-amphetamine (0.5, 2.5, and 5 mg/kg) and RO-20-1724 was necessary to establish the dose of d-amphetamine that significantly reduces PPI for all three prepulse intensities. Doses of 0.25, 2.5, and 4.0 mg/kg RO-20-1724 were tested for reversal of amphetamine-induced disruption of PPI.

**Analysis.** Amplitude and gating of the P20 and N40 ERP components were assessed. The P20 (most positive deflection between 10 and 30 ms) and N40 (most negative deflection between 25 and 60 ms) after both 1st (S1) and 2nd (S2) stimulus were determined and analyzed using repeated-measures analysis of variance (ANOVA) as described previously (Connolly et al., 2003; Siegel et al., 2003). Drug condition was designated the independent variable with stimulus condition (first click versus second click) as the repeated measure factor. Two analyses were performed using repeated-measures ANOVA. Significant main effects were followed by Fisher LSD post hoc comparisons. Two analyses were performed for main effect of drug: a RO-20-1724 dose response and the amphetamine reversal group.

For PPI, the average startle response was used as the primary dependent measure of the startle reflex. Percentage of prepulse inhibition was calculated using the following formula: [100 – (startle + prepulse/startle alone) × 100], where “startle alone” was obtained from trials conducted in the absence of prepulse stimuli. Results were analyzed using repeated-measures ANOVA. Significant main effects were followed by Fisher LSD post hoc comparisons.

**Results**

**Recording of Auditory ERPs.** RO-20-1724 Increases Amplitude of P20 and N40. There were no differences between treatment groups on any component (P20 or N40 for either the amplitude of the 1st or 2nd click responses or gating) before drug exposure (Tables 1
Effects of RO-20-1724 on P20 and N40 amplitude

Analyses for the amplitude of 1st click responses are shown. Corresponding results for the 2nd click response values are not displayed because there were no significant findings in any comparisons for this measure.

**TABLE 1**

Effects of RO-20-1724 and d-amphetamine on P20 and N40 amplitudes

Results are shown for the 1st click responses in all conditions and for the interaction of 1st and 2nd click responses within drug and vehicle conditions (gating). However, comparisons of 2nd click responses between drug and vehicle conditions yielded no significant results and are therefore not displayed.

<table>
<thead>
<tr>
<th>P20</th>
<th>N40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall effect of drug</td>
<td>Overall effect of drug</td>
</tr>
<tr>
<td>F(3, 27) = 2.3799, p &lt; 0.001</td>
<td>F(3, 27) = 20.393, p &lt; 0.001</td>
</tr>
<tr>
<td>Drug* dose interaction</td>
<td>Drug* dose interaction</td>
</tr>
<tr>
<td>F(3, 27) = 2.5694, p = 0.075</td>
<td>F(3, 27) = 3.6555, p = 0.027</td>
</tr>
<tr>
<td>Effect of drug</td>
<td>Effect of drug</td>
</tr>
<tr>
<td>0.1 mg/kg drug vs. vehicle</td>
<td>0.1 mg/kg drug vs. vehicle</td>
</tr>
<tr>
<td>F(1, 7) = 0.10262, p = 0.758</td>
<td>F(1, 7) = 0.31775, p = 0.591</td>
</tr>
<tr>
<td>0.25 mg/kg drug vs. vehicle</td>
<td>0.25 mg/kg drug vs. vehicle</td>
</tr>
<tr>
<td>F(1, 7) = 25.134, p = 0.002</td>
<td>F(1, 7) = 12.73, p = 0.009</td>
</tr>
<tr>
<td>0.5 mg/kg drug vs. vehicle</td>
<td>0.5 mg/kg drug vs. vehicle</td>
</tr>
<tr>
<td>F(1, 7) = 18.941, p = 0.003</td>
<td>F(1, 7) = 14.944, p = 0.006</td>
</tr>
<tr>
<td>1.0 mg/kg drug vs. vehicle</td>
<td>1.0 mg/kg drug vs. vehicle</td>
</tr>
<tr>
<td>F(1, 7) = 16.109, p = 0.005</td>
<td>F(1, 7) = 5.4867, p = 0.052</td>
</tr>
<tr>
<td>2.5 mg/kg drug vs. vehicle</td>
<td>2.5 mg/kg drug vs. vehicle</td>
</tr>
<tr>
<td>F(1, 6) = 5.9121, p = 0.051</td>
<td>F(1, 6) = 15.152, p = 0.008</td>
</tr>
</tbody>
</table>

*, p < 0.05 for interaction.

Effects of RO-20-1724 on P20 and N40 amplitude

Analyses for the amplitude of 1st click responses are shown. Corresponding results for the 2nd click response values are not displayed because there were no significant findings in any comparisons for this measure.

<table>
<thead>
<tr>
<th>P20</th>
<th>N40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall effect of drug</td>
<td>Overall effect of drug</td>
</tr>
<tr>
<td>F(1, 23) = 2.3799, p &lt; 0.001</td>
<td>F(1, 23) = 20.393, p &lt; 0.001</td>
</tr>
<tr>
<td>Drug* dose interaction</td>
<td>Drug* dose interaction</td>
</tr>
<tr>
<td>F(3, 27) = 2.5694, p = 0.075</td>
<td>F(3, 27) = 3.6555, p = 0.027</td>
</tr>
<tr>
<td>Effect of drug</td>
<td>Effect of drug</td>
</tr>
<tr>
<td>0.1 mg/kg drug vs. vehicle</td>
<td>0.1 mg/kg drug vs. vehicle</td>
</tr>
<tr>
<td>F(1, 7) = 0.10262, p = 0.758</td>
<td>F(1, 7) = 0.31775, p = 0.591</td>
</tr>
<tr>
<td>0.25 mg/kg drug vs. vehicle</td>
<td>0.25 mg/kg drug vs. vehicle</td>
</tr>
<tr>
<td>F(1, 7) = 25.134, p = 0.002</td>
<td>F(1, 7) = 12.73, p = 0.009</td>
</tr>
<tr>
<td>0.5 mg/kg drug vs. vehicle</td>
<td>0.5 mg/kg drug vs. vehicle</td>
</tr>
<tr>
<td>F(1, 7) = 18.941, p = 0.003</td>
<td>F(1, 7) = 14.944, p = 0.006</td>
</tr>
<tr>
<td>1.0 mg/kg drug vs. vehicle</td>
<td>1.0 mg/kg drug vs. vehicle</td>
</tr>
<tr>
<td>F(1, 7) = 16.109, p = 0.005</td>
<td>F(1, 7) = 5.4867, p = 0.052</td>
</tr>
<tr>
<td>2.5 mg/kg drug vs. vehicle</td>
<td>2.5 mg/kg drug vs. vehicle</td>
</tr>
<tr>
<td>F(1, 6) = 5.9121, p = 0.051</td>
<td>F(1, 6) = 15.152, p = 0.008</td>
</tr>
</tbody>
</table>

*, p < 0.05 for interaction.

and 2). Figure 2A displays the mean wave form for the 1st and 2nd stimulus response to the paired click stimuli used in this study. In comparison, the mean wave form obtained after injection of RO-20-1724 (0.5 mg/kg) is shown below in Fig. 2B. Both the RO-20-1724 dose response and the d-amphetamine challenge analyses yielded main effects of drug, stimulus condition, and stimulus condition by drug interactions for each component (Tables 1 and 2). When comparing the data obtained after vehicle injection, a single injection of RO-20-1724 significantly increased the amplitude of the P20 component of the first click (Table 1; Fig. 3A). This increment was consistent at 0.25, 0.5, and 1.0 mg/kg RO-20-1724. There was no significant effect of RO-20-1724 at 0.1 mg/kg, and a dose of 2.5 mg/kg drug yielded only a trend toward an increment of peak amplitude (p = 0.051). Thus, RO-20-1724 exhibited an inverted U-shaped effect distribution, with significant effects between 0.25 and 1.0 mg/kg.

RO-20-1724 increased the N40 amplitude compared with the corresponding vehicle condition (Table 1; Fig. 3B). A significant increase in N40 amplitude occurred at doses of 0.25, 0.5, and 2.5 but not at 0.1 mg/kg RO-20-1724. A dose of 1.0 mg/kg RO-20-1724 showed an increment while not reaching significance (p = 0.052). No significant differences were found on any component with regard to the second stimulus in any comparison. Thus, the minimal effective dose for RO-20-1724 was 0.25 mg/kg for both the P20 and N40 components.

**Locomotor Activity**

**RO-20-1724 in Low but Effective Doses Has Small to No Effect on Locomotor Activity.** Mice seemed to be sedated and had qualitatively reduced locomotor activity after doses of 0.5, 1.0, and 2.5 mg/kg RO-20-1724. ERP analyses revealed a comparable effect for lower doses, suggesting that there was no benefit applying the dose of 1.0 mg/kg. Therefore, doses of 0.25 and 0.5 mg/kg were considered for further measurement of locomotor activity. Measurement of locomotor activity with 0.5 mg/kg supported the empiric impression of reduced locomotor activity (Fig. 4). Although not statistically significant, it revealed a trend (p = 0.053) toward a difference between drug and vehicle condition, which was
Effects of RO-20-1724 in Animal Models of Psychosis

RO-20-1724 Reverses the Effects of d-Amphetamine on the N40 Component. We assessed the potential of the lowest effective dose (0.25 mg/kg) of RO-20-1724 to reverse the effects of d-amphetamine on ERPs (Fig. 5). Amphetamine (0.5 mg/kg) significantly reduced N40 S1 amplitude and disrupted gating in comparison to vehicle. The disruption of gating is evidenced by the significant difference between 1st and 2nd stimulus amplitude after vehicle injection but not after injection of amphetamine alone. Injection of 0.25 mg/kg RO-20-1724 in combination with amphetamine restores gating (i.e., S1 significantly greater than S2) and increased S1 amplitude compared with amphetamine alone. The 0.5 mg/kg dose of d-amphetamine did not produce a significant decrease of amplitude for the P20 component. This may be due to a floor effect for the smaller P20 component, where differences tend to be more difficult to demonstrate. Alternatively, the simultaneous injection of d-amphetamine and RO-20-1724 did reveal a significant increase in amplitude for the P20 component.
component compared with vehicle, suggesting that RO-20-1724 is able to overcome the disruptive influences of amphetamine on ERPs.

**Startle Response and Prepulse Inhibition**

**RO-20-1724 Does Not Reverse Disruption of PPI by d-Amphetamine.** A dose of 5 mg/kg d-amphetamine significantly reduced PPI for each prepulse intensity tested compared with saline injection. Lower doses of d-amphetamine yielded no significant reduction of PPI. d-Amphetamine is a psychotomimetic known to disrupt PPI, and the ability of an antipsychotic to reverse this disruption is a predictor of clinical efficacy (Geyer et al., 2001). Therefore, we next tested the ability of RO-20-1724 to block amphetamine-induced disruption of PPI to evaluate the antipsychotic-like properties in a second established animal model of psychosis (Fig. 6). A dose of 5 mg/kg amphetamine disrupted PPI. Pretreatment with RO-20-1724 did not significantly increase PPI in trials at any prepulse intensity for any tested doses (0.25, 2.5, and 4.0 mg/kg). Analyses yielded only a trend toward a significant increase of PPI under the highest prepulse intensity and a dose of 2.5 mg/kg RO-20-1724. Thus, RO-20-1724 did not fulfill criteria for antipsychotic-like effects using the amphetamine-induced disruption of the PPI model.

**Discussion**

The present study revealed inconsistent effects of the PDE4 inhibitor RO-20-1724 using two different animal models thought to predict antipsychotic properties. Previous studies with the PDE4 inhibitor rolipram were consistent with the current study for ERPs but differed from the tested agent for PPI. This suggests that some behavioral properties of rolipram are not a general trait of PDE4 inhibition but may be limited to the specific PDE4 subtype or other relevant pharmacology of rolipram. Furthermore, inconsistent results between reversal of amphetamine-induced deficits for pure sensory (ERP) and sensorimotor (PPI) tasks suggest that each model offers a unique perspective on the neural and...
behavioral effects of compounds being considered as therapeutic approaches to schizophrenia.

Based on our ERP and locomotor data, we propose that PDE4 inhibition may achieve some antipsychotic-like effects with fewer extrapyramidal side effects than currently approved dopamine D2 antagonists. We demonstrated an increase in both P20 and N40 amplitude after administration of a dose of RO-20-1724 that did not yield any locomotor retardation. Furthermore, RO-20-1724 reversed the detrimental effects of amphetamine on the N40 component, also using a dose without locomotor effects. These ERP characteristics are shared by classic antipsychotics and at least one other PDE4 inhibitor (Maxwell et al., 2004).

Although RO-20-1724 caused significant enhancement of the response to the first click for both P20 and N40, no significant differences were found on any component with regard to the second stimulus response in any comparison. This is consistent with previous studies demonstrating that gating is primarily a function of the first stimulus response in mice (Connolly et al., 2003; Maxwell et al., 2006). Likewise, several studies suggest that P50 abnormalities in schizophrenia are primarily related to the amplitude of the first response, rather than inhibition of the second (Jin et al., 1997; Patterson et al., 2000). This finding also complements previous studies with rolipram, another PDE4-specific inhibitor, and demonstrates parallel effects on P20 and N40 in comparison with currently used antipsychotics. These data suggest that augmentation of evoked potentials may be a general feature of PDE4 inhibitors. In contrast, PPI results in the current study differed from those for ERPs as well as previ-
ous results for PPI using rolipram. Here, RO-20-1724 did not reverse the PPI decrement caused by amphetamine. These findings complement numerous studies that suggest the beneficial effects of PDE4 inhibition on learning, memory, and mood and indicate that PDE4 inhibition may have potential as a novel approach to receptor-independent antipsychotic effects (Zhang et al., 2002; Rutten et al., 2006).

**Importance of PDE4 and Previous Studies with PDE4 Inhibitors.** Degradation by PDEs is the main adaptive controller for cAMP/cGMP second messengers and therefore mediates an extensive contribution to cell function (Conti and Beavo, 2007). Previous efforts to achieve antipsychotic effects using receptor-independent mechanisms have relied mainly on the inhibition of the PDE4 family. Several isoforms of the PDE4 family are widely distributed in the brain and specifically catalyze the hydrolysis of cAMP (Zhang et al., 2005). Likewise, activity at different PDE4 subfamilies may impart functional specificity (Murdoch et al., 2007). For example, PDE4D seems to mediate antidepressant-like effects, whereas PDE4B knockout mice are less sensitive to changes in conditioned avoidance, which is considered a model for antipsychotic effects (Zhang et al., 2002; Siuciak et al., 2007). In particular, PDE4B has been identified as a risk factor for schizophrenia (Clapcote et al., 2007). PDE4B activity is closely linked to its interaction with DISC1 (Millar et al., 2007). In comparison with rolipram, there has been little research on the PDE4 family in central nervous system diseases. Few studies indicate antidepressant-like effects for RO-20-1724, albeit at lower potency than rolipram (Wachtel, 1983). Potential antipsychotic effects of RO-20-1724 have been addressed before.

**Additional Benefits of PDE4 Inhibition.** Schizophrenia is associated with cognitive dysfunction, including impairments of learning, memory, and executive function (Eldevåg and Goldberg, 2000; Hill et al., 2002). Although not part of the formal diagnostic criteria, cognitive deficits, like negative symptoms, show small or no improvement on current treatments (Keefe et al., 2004). However, D2 antagonists have been shown to impair learning in several rodent models (Ploeger et al., 1992; Rosengarten and Quartermain, 2002). In contrast, a number of studies found improvement of memory function in rats using rolipram (Rutten et al., 2006; Rutten et al., 2007). In addition, comorbid conditions like depression or substance abuse may be linked to DISC1-PDE4 isoform interactions (Cheung et al., 2007; Polesskaya et al., 2007). Whether improvement of cognitive function, mood disorders, or substance dependence would be an additional benefit of PDE4 inhibition in schizophrenia requires further investigation.

**Limitations.** There were several limitations to the current study. Inconsistency between ERP and PPI results raises the question of whether antipsychotic-like properties are a general trait of PDE4 inhibition. Measurement of psychosis-like conditions with PPI and ERPs after an amphetamine challenge seems to yield different thresholds for hyperdopaminergic disruption. This was evident by the different doses required for a significant effect of amphetamine, with PPI requiring a 10-fold higher dose to show a significant decrease than ERPs. Only the 5.0 mg/kg dose led to a significant decrease in PPI, whereas a dose of 0.5 mg/kg was sufficient to reduce the N40 ERP and disrupt gating. However, it must be noted that the P20 component was not significantly decreased at this dose, possibly due to the lower peak values of this component and difficulty demonstrating a significant reduction. Alternatively, higher doses of amphetamine may have yielded a significant P20 reduction, leading to more consistent results among tasks. There is no unitary explanation for the inability of RO-20-1724 to re-establish normal PPI after amphetamine despite its ability to do so in ERP and locomotor tasks. Although the disruption of PPI in rodents and humans alike has been shown in several studies, there is not a consistent reversal of this effect by antipsychotics (Geyer et al., 2001; Nakai et al., 2008). In addition, we did not find any effect of the tested agent for the 2nd stimulus response in the measurement of ERPs. Although this is inconsistent with some models of schizophrenia in anesthetized...
mice, many recent studies demonstrate that the apparent loss of gating is due to a reduction in response to the first click (Jin et al., 1997; Patterson et al., 2000). In addition, current data are consistent with previous observations in awake mice and rats that show disruption of gating occurs by a decrease of the 1st click (N40) component rather than an increase of the 2nd click response (Siegel et al., 2003; Swerdlov et al., 2006). Our animal models do not mirror the complexity of the human condition. However, simplified designs under highly controlled conditions allow for conclusions based on clearly delimited questions, where previous studies have shown a validated and analogous response in both species. In addition, it is unclear whether certain PDE4 subfamilies, subtypes, and/or splice variants share a preferred interaction with RO-20-1724, which is of particular value when one seeks to determine the different effects in comparison with rolipram. In contrast to rolipram, RO-20-1724 has never been tested in humans. Studies with rolipram indicated that although antidepressant properties could be demonstrated, its clinical use was limited by nausea and emesis. It must be determined whether other PDE4 inhibitors share this same side effect liability or whether they are specific to rolipram due to a preference for certain PDE4 subtypes and splice variants. It seems that one of the greatest limitations of PDE inhibition is the lack of highly specific inhibitors. As shown with other drug classes, a more precise mode of action may induce fewer side effects. It important to note that other PDE4 inhibitors are likely to be clinically available in the near future because they are currently in clinical trials (Houslay et al., 2005). If these agents indicate that nausea and emesis are an obligatory side effect of PDE4 inhibition, it would limit their usefulness in clinical application for schizophrenia.

Conclusion

Current data complement previous studies by providing evidence for a subset of antipsychotic-like effects after PDE4 inhibition. However, inconsistencies in comparison to rolipram reduce the likelihood that inhibitors of all PDE4 isoforms will exhibit consistent antipsychotic-like effects. Inhibition of a limited group of isoforms may represent novel treatment options for schizophrenia if more specific inhibitors are identified. In addition, the side-effect profile of such selective PDE4 inhibitors will strongly influence their clinical potential.

References


Srucca-Moreau, Giesbers I, and Dresse A (1987) Effect of rolipram, a phosphodiesterase inhibitor and potential antidepressant, on the firing rate of central monoaminergic neurons in the rat. Arch Int Pharmacodyn Ther 288:45–59.


Address correspondence to: Dr. Steven J. Siegel, SMRI Laboratory for Experimental Therapeutics in Psychiatry, Department of Psychiatry, University of Pennsylvania, Translational Research Laboratories, 125 S. 31st Street, Philadelphia, PA 19104. E-mail: siegels@mail.med.upenn.edu