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Evaluating the antipsychotic profile of the preferential PDE10A inhibitor, papaverine

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Abstract

Rationale—Prepulse inhibition (PPI) is an operational measure of sensorimotor gating that is deficient in schizophrenia patients. In rats, PPI deficits induced by dopamine (DA) agonists are reversed by antipsychotics. Inhibition of the striatum-rich phosphodiesterase (PDE)10A may represent a novel antipsychotic mechanism. Previous studies were controversial, showing antipsychotic-like profiles in measures of PPI for the preferential PDE10A inhibitor papaverine (PAP) but not the novel PDE10A inhibitor TP-10.

Objective—The aim of the study was to evaluate the antipsychotic profile of PAP in rats using PPI.

Materials and methods—PPI deficits were induced in rats by apomorphine (APO; 0.1, 0.5 mg/kg) or D-amphetamine (AMPH; 4 mg/kg). PAP (3, 10, 30 mg/kg) or haloperidol (HAL; 0.1 mg/kg) was tested against these agonists in Sprague–Dawley (SD) or Wistar (WI) rats. Prepulse intervals ranged from 10 to 120 ms. Further tests evaluated the effects of PAP on spontaneous locomotion, AMPH (1 mg/kg)-induced hyperlocomotion, and core body temperature (T°).

Results—HAL reversed APO-induced PPI deficits but PAP failed to reverse APO- and AMPH-induced PPI deficits at all doses, strains, pretreatment times, and prepulse intervals. PAP (30 mg/kg) significantly reduced AMPH hyperlocomotion in SD rats, and a similar pattern was detected in WI rats. This PAP dose also strongly reduced spontaneous locomotion and T° in SD rats.

Conclusion—Our study does not support an antipsychotic-like profile of PAP in dopaminergic PPI models.

Keywords

D-Amphetamine; Apomorphine; Core body temperature; Dopamine; Locomotion; Papaverine; PDE10A; Prepulse inhibition

Introduction

Prepulse inhibition (PPI) of startle is a cross-species phenomenon that occurs when a weak lead stimulus inhibits the response to an intense, abrupt startling stimulus (Graham 1975). PPI is reduced in schizophrenia patients and their unaffected first-degree relatives (Braff et al. 1978; Cadenhead et al. 2000; Braff et al. 2001; Kumari et al. 2005). These studies suggest that deficient PPI may represent a useful schizophrenia endophenotype—i.e., an intermediate between genes and the more complex clinical disease manifestations (Gottesman and Gould 2003; Turetsky et al. 2007)—that can be studied under controlled laboratory conditions.

PPI deficits in schizophrenia are modeled in rats by dopamine (DA) receptor agonists and NMDA receptor antagonists, consistent with the dopaminergic and glutamatergic hypotheses of schizophrenia (Randrup and Munkvad 1972; Javitt and Zukin 1991). PPI deficits induced by DA agonists like the mixed D1/D2 agonist, apomorphine (APO), or the DA releaser, D-amphetamine (AMPH) are reversed by typical and atypical antipsychotics and are used in predictive models for antipsychotic drug discovery (Mansbach et al. 1988; Swerdlow et al. 1994).

All approved antipsychotics functionally antagonize neurotransmission at the DA D2 receptor, a G-protein-coupled receptor with inhibitory function on adenylate cyclase (AC; Senogles 1994). This suggests that one source of potentially novel targets for antipsychotic drug discovery may be downstream of D2 receptors, i.e., within the DA D2 signaling cascade. In particular, drugs that elevate cAMP within D2-expressing neurons in the striatum may yield a D2 antagonist-like effect. Such drugs may include certain inhibitors of phosphodiesterases (PDE), i.e., degrading enzymes of cAMP and/or cGMP (c.f. Halene and Siegel 2007).

Supporting evidence for this hypothesis comes from several PPI studies. Reduced PPI in postpartum rats is associated with reduced nucleus accumbens (NAC)—but not dorsal striatum—cAMP content (Byrnes et al. 2007). Similarly, studies in PDE-4B knock-out (KO) mice demonstrated marked PPI deficits together with increased startle amplitudes (Siuciak et al. 2008). Culm et al. (2004) demonstrated that D2 stimulation disrupts PPI by activating the inhibitory Gai/o proteins in the nucleus accumbens (NAC), thereby reducing cAMP signaling by inhibiting AC activity. Furthermore, PPI is impaired in mice by intracerebroventricular administration of Rp-cAMPS, a competitive inhibitor of cAMP protein kinase A types I and II, and is increased by the selective PDE4 inhibitor rolipram (Kelly et al. 2007). Notably, rolipram also blocks the PPI-disruptive effects of AMPH in mice (Kanes et al. 2007; Kelly et al. 2007). Thus, in both rat and mouse models, reduced forebrain cAMP activity appears to be associated with PPI deficits. However, a recent study with a RO-20-1724, another selective PDE4 inhibitor, demonstrated an antipsychotic-like profile of this compound in models of auditory event-related potentials, but not in measures of AMPH-induced PPI deficits (Halene and Siegel 2008).

Among all drug candidates that elevate cAMP, PDE10A inhibitors have stirred the most attention due to the highly localized expression levels of PDE10, particularly in the striatum (Seeger et al. 2003; Xie et al. 2006). PDE10A is a recently discovered PDE subtype that hydrolyzes both cAMP and cGMP (Fujishige et al. 1999). High levels of PDE10A protein and mRNA have been discovered in GABAergic medium spiny neurons of the NAC, caudate nucleus, and olfactory tubercle (Seeger et al. 2003). Medium spiny neurons are the predominant cell type of this brain region. They represent a convergence site for glutamatergic input from cortical and thalamic regions and dopaminergic input from the midbrain. A recent study in our laboratory has detected significant differences in PDE10A gene expression in the NAC between Sprague–Dawley (SD) and Long Evans (LE) rats. Compared to LE rats, SD rats had significantly higher PDE expression levels ($p < 10^{-6}$), and this corresponded to higher sensitivity to the PPI-disruptive effects of DA agonists in SD rats (Shilling et al. 2008). Recent studies with papaverine (PAP), a PDE10A inhibitor with ≥ 9 times lower IC_{50} values for PDE10A than for the other members of the PDE family (Siuciak et al. 2006), have demonstrated an antipsychotic-like profile of PAP in a paradigm of attentional set-shifting in rats designed to reflect frontal function in man (Rodefer et al. 2005). An antipsychotic-like profile of PAP was further observed in the conditioned avoidance (CAR) model and in D-amphetamine (AMPH)- and PCP-induced hyperlocomotion tests (Siuciak et al. 2006). Similar effects were observed with the novel, more selective PDE10A inhibitor TP-10. Importantly, this compound also displayed an antipsychotic-like profile in a model of AMPH-induced event-related potential (ERP) sensory gating deficits (Schmidt et al. 2008).

Data available on PDE10 inhibitors in models of sensorimotor gating are controversial at present. Bleickardt et al. (2007) and Lu et al. (2007) reported data in abstract form showing that PAP reverses MK-801-induced PPI deficits in rats and mice and APO-induced PPI deficits in rats. In contrast, a recent study with the novel PDE10A inhibitor TP-10 showed that TP-10 reversed *neither* baseline PPI deficits in C57/BL6 mice *nor* MK-801-induced PPI deficits in CD1 mice (Schmidt et al. 2008).

In the present study, we tested the effects of the commercially available PDE10 inhibitor PAP, in a range of DA-related PPI models. PPI studies were complemented by measures of AMPH-induced hyperlocomotion, spontaneous locomotion, and drug-induced hypothermia to evaluate bioavailability/bioactivity and drug effects on general motor behavior.

Materials and methods

Experimental animals

Adult male SD ($n=131$) and WI ($n=19$) rats (225–250 g; Harlan Laboratories, Livermore, CA, USA) were housed in groups of two to three animals per cage and maintained on a reversed light/dark schedule with water and food available ad libitum. Rats were handled within 2 days of arrival. Testing occurred during the dark phase. All experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) and were approved by the UCSD Animal Subjects Committee (protocol #S01221).

Drugs

D-Amphetamine sulfate, apomorphine hydrochloride hemihydrate, ascorbic acid, and papaverine hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). Haloperidol (HAL) solution was purchased from Novation (Irving, TX, USA). APO (0.1, 0.5 mg/kg), AMPH (1, 4 mg/kg), and HAL (0.1 mg/kg) were administered subcutaneously in a volume of 1 ml/kg. PAP (3, 10, 30 mg/kg) was administered intraperitoneally in a volume of 1.5–2 ml/kg. These doses of PAP were selected based on published in vivo assays in rodents in which PAP had significant behavioral or neurochemical effects (see, e.g., Bleickardt et al. 2007; Lu et al. 2007; Rodefer et al. 2005; Siuciak et al. 2006). Vehicle for AMPH, HAL, and PAP was saline and 0.01% ascorbate/saline for APO. All doses are based on milligram per kilogram of salts.

Apparatus

Startle chambers were housed in a sound-attenuated room and consisted of a Plexiglas cylinder 8.2 cm in diameter resting on a 12.5×25.5-cm Plexiglas frame within a ventilated enclosure. Noise bursts were presented via a speaker mounted 24 cm above the cylinder. A piezoelectric accelerometer mounted below the Plexiglas frame detected and transduced motion from within the cylinder. Stimulus delivery was controlled by the SR-LAB microcomputer and interface assembly, which also digitized (0–4095), rectified, and recorded stabilimeter readings. One-hundred-one-millisecond readings were collected beginning at stimulus onset. Startle amplitude was defined as the average of 100 readings.

Startle testing procedure—Two to 6 days before PPI drug studies were begun, rats were exposed to a short “matching” startle session. They were placed in the startle chambers for a 5-min acclimation period with a 70-dB(A) background noise, and then exposed to a total of 17 P-ALONE trials (40 ms—120 dB (A) noise bursts) that were interspersed with 3 PP12dB+P-ALONE trials (P-ALONE preceded 100 ms (onset-to-onset) by a 20-ms noise burst of 12 dB above background). Rats were assigned to drug dose groups based on average %PPI from the matching session.

Starting 2–6 days later, the effects of PAP were studied in separate groups of rats, using three different PPI protocols. All protocols used a 40-ms, 120-dB(A) noise burst as the “P-Alone”, and contained trials in which no stimulus was presented, but cage displacement was measured (NOSTIM trials). Intertrial interval was variable and averaged 15 s. NOSTIM trials were not included in the calculation of intertrial intervals.

PPI Protocol 1 was aimed to replicate key aspects of the study of Bleickardt et al. (2007) and to extend it by a strain comparison using 12 WI and 12 SD rats. A mixed-model design was used, with the APO dose (vehicle vs. 0.5 mg/kg) as the within-factor and the PAP dose (vehicle vs. 30 mg/kg) as the between-factor. Rats were treated on 2 days with either vehicle or 0.5 mg/kg APO using pseudorandom and balanced drug orders. Intertest interval was 7 days. APO injections were preceded 30 min by an injection of PAP (30 mg/kg). Immediately after the APO injection, rats were placed in the startle chambers for a 5-min acclimation period with a 70-dB(A) background noise. They were then exposed to a series of trial types which were presented in pseudorandom order: (1) P-ALONE; (2)–(4) P-ALONE preceded 100 ms (onset-to-onset) by a 20-ms noise burst of either 5 (PP5dB+P-ALONE), 10 (PP10dB+P-ALONE), or 15 dB above background (PP15dB+P-ALONE). Interspersed between these trials were NOSTIM trials. The session began with four consecutive P-ALONE trials and ended with three consecutive P-ALONE trials. Between these trials were two blocks, each consisting of eight P-ALONE trials, five PP5dB+P-ALONE trials, five PP10dB+P-ALONE trials, and five PP15dB+P-ALONE trials. Total session duration was 18.25 min.

Five days after this PAP experiment, these animals were used in a follow-up PPI experiment using the same session and experimental design. The typical antipsychotic HAL was used as a positive control to demonstrate assay sensitivity for the “detection” of antipsychotic-like compounds. Animals were “reshuffled” and assigned to dose groups matched for average % PPI values and previous PAP dose groups. A mixed-model design was used, with APO dose as the within-factor and HAL dose (vehicle vs. 0.1 mg/kg) as the between-factor. Rats were treated 2 days with either vehicle or 0.5 mg/kg APO using pseudorandom and balanced drug orders. Intertest interval was 7 days. APO injections were preceded 10 min by an injection of HAL (0.1 mg/kg). Immediately after the APO injection, rats were placed in the startle chambers and the session described above was begun (protocol 1, see above).

PPI Protocol 2 was identical to protocol 1, but used a pretreatment time for PAP of 5 min relative to the DA agonist APO (0.5 mg/kg or 0.1 mg/kg) or AMPH (4 mg/kg). The time difference between the agonist injection and the beginning of the experiment was 0 min for APO and 10 min for AMPH. These conditions were chosen to optimize bioavailability/bioactivity for PAP (see data from T° and locomotor experiments below, and Siuciak et al. 2006) and the DA agonists. Based on strong evidence for bioactivity of PAP in assays of T° and AMPH-induced hyperlocomotion in particular in SD rats (see below), only SD rats ($n=50$) from Harlan Laboratories were used for these experiments. Two APO studies were carried out, one using a standard dose of APO (0.5 mg/kg) and one using a low dose of APO (0.1 mg/kg). Intertest interval was 6–7 days.

PPI Protocol 3 also used a 5-min pretreatment time and SD ($n=24$) rats only. This session included prepulse intervals ranging from 10 to 120 ms, to enable the detection of antipsychotic-like effects at a wider range of prepulse intervals (see, e.g., Swerdlow et al. 2004). A mixed-model design was used, with the APO dose (vehicle vs. 0.5 mg/kg) as the within-factor and the PAP dose (vehicle 3, 10, 30 mg/kg) as the between factor. Rats were treated 2 days with either vehicle or 0.5 mg/kg APO using pseudorandom and balanced drug orders. Intertest interval was 7 days. Immediately after the APO injection, rats were placed in the startle chambers for a 5-min acclimation period with a 70-dB(A) background noise. They were then exposed to a series of trial types which were presented in pseudorandom order: (1) P-ALONE;

(2)–(5) P-ALONE preceded by a prepulse of 5 ms duration and a prepulse amplitude of 15 dB above background, which was presented either 10 (PP10ms+P-ALONE), 20 (PP20ms+P-ALONE), 30 (PP30ms+P-ALONE), 60 (PP60ms+P-ALONE), or 120 ms (PP120ms+P-ALONE) prior the pulse (onset-to-onset). Interspersed between these trials were NOSTIM trials. The session began with three consecutive P-ALONE trials and ended with three consecutive P-ALONE trials. Between these trials was one block consisting of six P-ALONE, six PP10ms+P-ALONE, six PP20ms+P-ALONE, six PP30ms+P-ALONE, six PP60ms+P-ALONE, or six PP120ms+P-ALONE trials. Total session duration was 15.5 min.

Locomotor protocols

D-Amphetamine-induced hyperlocomotion: AMPH-induced hyperactivity was recorded in SD ($n=8$) and WI rats ($n=7$). The testing protocol was based on Schmidt et al. (2008) and Swerdlow et al. (2006). Recordings were obtained during the dark cycle of the animals. The experimental room was illuminated, providing ~40 lx in the middle of the locomotor cages. A background of 60 dB(A) white noise was used. Rats were brought into the laboratory and placed into individual cages without water and food for 1–2 h of adaptation prior to placement into the locomotor cages. Locomotor cages were wire-mesh photocell cages (22×35×15 cm) fitted with two parallel infrared beams 1 cm above the floor, perpendicular to the long axis of the cage. The total number of beam breaks was calculated for each 10 min interval during all tests. Rats were acclimated to the locomotor cages for 180 min 1 day prior to their first drug test and these data were used to assign animals to “activity-matched” dose groups. Rats were treated 2 days with either vehicle or 30 mg/kg PAP using a within-subjects, pseudorandom and balanced drug order design. Intertest interval was 7 days. On test days, rats were placed in the activity chambers for 60 min, then removed and immediately injected with PAP (0, 30 mg/kg). Rats were then returned to their individual cages and then injected 5 min later with AMPH (1 mg/kg). Immediately after the AMPH injection, rats were returned to the activity chambers for a recording duration of 180 min.

Spontaneous locomotion: The effect of PAP on spontaneous locomotor activity was recorded in 22 SD rats. The procedure for spontaneous locomotion testing was identical to the one described for D-amphetamine-induced hyperactivity testing, except that the experiment was designed to achieve sufficient and sustained baseline locomotor activity by non-pharmacological means: (1) rats naive to the experimental environment were used, (2) recordings began at the beginning of the dark cycle, and (3) the room was dark throughout the recording. Rats were brought into the laboratory and placed into individual cages without water and food for ~45 min of adaptation prior to drug treatment. Rats were randomly assigned to dose groups and treated with either vehicle or 30 mg/kg PAP using a between-subjects design. After the injections, rats were returned to their individual cages for 5 min. After the waiting period and coinciding with the onset of the dark cycle, rats were placed in activity chambers for 180 min.

Drug-induced hypothermia— T° was recorded in 15 SD rats. The protocol was modified from Zhang et al. (2007). One day prior to the first drug testing, an “adaptation session” was carried out to familiarize the rats with the testing procedure. Rats were brought to the testing room and placed into individual cages without water and food. After approximately 2.5 h of adaptation to the experimental environment, core body temperature measurements were begun. A rat rectal probe (Model Ret-2-Iso, Physitemp Instruments, Clifton, NJ, USA) was attached to a high precision thermometer (Hi-Lo Temp Model 8200 (Malinckrodt, Argyle, NY, USA), manufactured by Sensortek (now Physitemp Instruments, Clifton, NJ, USA). The probe was coated with a lubricant (K-Y-Jelly, McNeil-PPC, Skillman, NJ, USA) and inserted approximately 3 cm into the rectum. T° was tested a total of three times with approximately 30 min between recordings. After the adaptation session, rats were returned to the vivarium.

Drug testing was begun 1 day after the adaptation session and consisted of two test days with 7 days between tests, using a mixed-model, pseudorandom balanced dose order design. PAP treatment (vehicle vs. 30 mg/kg) was used as the between-factor, and APO treatment (vehicle vs. 0.5 mg/kg) as the within-factor. A total of three T° recordings were taken on each test day. The first T° measurement was carried out to re-familiarize the animals with the procedure, but was not used for further analysis. Thirty minutes thereafter, a baseline recording was taken. After the baseline recording, rats were injected with PAP (30 mg/kg). To match the timing of drug effects on behavior with those of protocols 2 and 3 of the PPI experiment, APO (0.5 mg/kg) was administered 5 min after PAP, and T° recordings were taken 8 min after APO treatment. Thus, T° recordings were taken at a time point corresponding to the middle of the PPI session of protocol 2, relative to APO treatment.

Data analysis: PPI was defined as $100 - [(startle\ amplitude\ on\ prepulse\ trials / startle\ amplitude\ on\ P-ALONE\ trials) \times 100]$ and was analyzed by mixed design ANOVAs. Other ANOVAs were used to assess P-ALONE magnitude. PPI data were collapsed across prepulse intensities. NOSTIM levels revealed very small signal amplitudes (<1% of pulse alone amplitudes) and followed previously published patterns. D-Amphetamine-induced hyperactivity data were analyzed by ANOVAs, with strain as between-subject factor and PAP dose group, and time as the within-subject factors. An initial ANOVA was carried out to test for differences in baseline activity prior to administration of AMPH and PAP. After no significant differences between PAP dose groups were found, a second ANOVA was carried out for the activity data recorded during the first 2 h after drug administration. For the spontaneous locomotor data, the first 120 min of activity was analyzed by ANOVAs, with PAP dose group as between-subject factor and time (10 min intervals) as the within-subject factor. For T° comparisons between PAP dose groups, an ANOVA was first carried out to test for differences in baseline T° values. After no significant baseline differences between dose groups were detected, the drug-induced change in T° ($T^\circ_{post-injection} - T^\circ_{baseline}$) was calculated and submitted to an ANOVA. Post hoc comparisons of significant interactions and relevant main factor effects were conducted using Fisher's PLSD and ANOVA tests. Alpha was 0.05. When multiple post hoc comparisons were carried out for locomotor-based tests, significance levels were Bonferroni-adjusted for the number of time intervals.

Results

Prepulse inhibition

Haloperidol vs. apomorphine (PPI protocol 1)—To verify the sensitivity of the PPI protocol in detecting antipsychotic-like effects of established antipsychotics, APO (0.5 mg/kg)-induced PPI deficits were tested against the typical antipsychotic and D2 antagonist, HAL (0.1 mg/kg) in SD and WI rats.

ANOVA of %PPI revealed significant main effects of APO dose ($F=41.52$, $df\ 1, 20$, $p<0.0001$), a trend towards a main effect of HAL ($F=3.49$, $df\ 1, 20$, $p=0.076$), but no significant main effect of strain ($F=2.93$, $df\ 1, 20$, ns). There was a significant APO $\times$ HAL interaction ($F=27.00$, $df\ 1, 20$, $p<0.0001$), but no significant strain $\times$ APO interaction ($F<1$), no strain $\times$ HAL interaction ($F<1$), and no strain $\times$ APO $\times$ HAL interaction ($F=2.34$, $df\ 1, 20$, ns; Fig. 1a,b). The critical APO $\times$ HAL interaction was statistically significant in both SD ($p<0.0001$) and WI rats ($p<0.05$). Thus, HAL significantly opposed APO-induced PPI deficits in this paradigm.

ANOVA of startle magnitude on P-ALONE trials revealed no significant main effects of APO dose ($F<1$), HAL dose ($F=1.28$, $df\ 1, 20$, ns), or strain ($F<1$). There was a trend towards a strain $\times$ HAL interaction ($F=3.66$, $df\ 1, 20$, $p=0.07$), indicative of startle suppression by HAL in WI but not in SD rats, no APO $\times$ HAL interaction ($F<1$), no strain $\times$ APO interaction ($F=1.83$ $df\ 1, 20$, ns), or strain $\times$ APO $\times$ HAL interaction ($F<1$; Fig. 1, insets).

Papaverine vs. apomorphine (PPI protocol 1)

SD vs. WI rats: APO (0.5 mg/kg) was first tested against PAP (30 mg/kg) in SD and WI rats using a pretreatment time for PAP of 30 min. ANOVA of %PPI revealed significant main effects of APO dose ($F=60.85$, df 1, 20, $p<0.0001$), but no significant main effects of PAP ($F<1$), or strain ($F=2.40$, df 1, 20, ns). There was a significant strain $\times$ APO interaction ($F=9.12$, df 1, 20, $p<0.01$), but no APO $\times$ PAP interaction ($F<1$), strain $\times$ PAP interaction ($F<1$), or strain $\times$ APO $\times$ PAP interaction ($F<1$; Fig. 2a vs. b). The strain $\times$ APO interaction effect reflected greater APO PPI sensitivity of SD than WI rats, as observed previously (Swerdlow et al. 1998, 2000, 2003). Separate ANOVAs for each strain revealed significant main effects of APO in SD rats ($F=46.01$, df 1, 10, $p<0.0001$) and WI rats ($F=15.71$, df 1, 10, $p<0.01$), but no significant effect of PAP ($F<1$), or APO $\times$ PAP interaction ($F<1$) in either strain.

ANOVA of startle magnitude on P-ALONE trials revealed no significant main effects of APO dose ($F<1$), PAP dose ($F=2.55$, df 1, 20, ns), or strain ($F<1$). There were no significant interactions of strain $\times$ APO ($F=2.76$, df 1, 20, ns), strain $\times$ PAP ($F<1$), or strain $\times$ APO $\times$ PAP ($F<1$; Fig. 2 insets).

Standard vs. low doses of apomorphine (PPI protocol 2)—Another series of PPI studies tested the effects of PAP on PPI deficits induced by a standard or a low dose of APO in SD rats under conditions of optimized PAP bioavailability/bioactivity as indicated by the locomotor and hypothermia experiments, described below (pretreatment time for PAP, 5 min).

First, a standard dose of APO (0.5 mg/kg) was tested against PAP (30 mg/kg). ANOVA of %PPI revealed a significant main effect of APO dose ($F=100.98$, df 1, 10, $p<0.0001$), no effect of PAP ($F<1$), and no significant APO $\times$ PAP interaction effect ($F<1$, Fig. 3a). ANOVA of startle magnitude on P-ALONE trials revealed no significant main effects of APO dose ($F<1$), PAP dose ($F=1.40$, df 1, 10, ns), and no APO $\times$ PAP interaction ($F<1$; Fig. 3a inset).

Second, to test if PAP (30 mg/kg) can reverse more subtle APO-induced PPI deficits, a lower APO dose (0.1 mg/kg) was used. ANOVA of %PPI revealed a main effect of APO dose ($F=45.65$, df 1, 18, $p<0.0001$), no effect of PAP ($F<1$), and no significant APO $\times$ PAP interaction effect ($F<1$, Fig. 3b). ANOVA of startle magnitude on P-ALONE trials revealed a significant effect of PAP ($F=7.27$, df 1, 18, $p<0.02$), but not of APO dose ($F=1.54$, df 1, 18, ns), and no APO $\times$ PAP interaction effect ($F<1$; Fig. 3b inset).

To evaluate if PAP effects on startle magnitude could be related to the lack of PAP effects on PPI, subgroups of rats were created by consecutively eliminating “high startlers” in the vehicle group and “low startlers” in the PAP group until the startle magnitude in vehicle vs. PAP-treated rats were matched. Pulse alone amplitudes (mean $\pm$ SEM) in these subsets of rats ($n=5$ /PAP dose group) were: vehicle/vehicle treatment (122 ± 47), PAP/vehicle treatment (130 ± 10); vehicle/APO (109 ± 20), PAP/APO (127 ± 15). In these subsets of rats, %PPI followed the same pattern as in the full sample. %PPI (mean $\pm$ SEM) values were: vehicle/vehicle treatment (80 ± 8), PAP/vehicle treatment (74 ± 4); vehicle/APO (56 ± 10), PAP/APO (52 ± 11). In particular, there was no indication in favor of a reversal of APO-induced PPI deficits by PAP ($F<1$ for the PAP $\times$ APO interaction), indicating that PAP effects on startle magnitude cannot account for the lack of an antipsychotic-like profile of PAP in this study.

Multiple prepulse intervals (PPI protocol 3): Here the effects of PAP were tested against APO (0.5 mg/kg)-induced PPI deficits in SD rats at a wider range of (1) prepulse intervals (10 to 120 ms), (2) doses (3 to 30 mg/kg) and (3) under conditions of optimized bioavailability/bioactivity (pretreatment time for pap, 5 min; see data from locomotor and core body temperature experiments below).

ANOVA of %PPI revealed significant main effects of APO dose ($F=10.95$, df 1, 20, $p<0.005$), a main effect of prepulse interval ($F=18.25$, df 4, 80, $p<0.0001$), and an APO $\times$ prepulse interval interaction ($F=10.945$, df 12, 80, $p<0.005$), as observed previously (Swerdlow et al. 2004). The critical interactions of APO $\times$ PAP, and APO $\times$ PAP $\times$ prepulse interval, the interaction of PAP $\times$ prepulse interval, and the main effect of PAP, all failed to reach statistical significance ($F<1$ for all cases; Fig. 4a–d), again showing that PAP did not reverse APO-induced PPI deficits. ANOVA of startle magnitude on P-ALONE trials revealed no significant main effects of APO dose ($F<1$), or PAP dose ($F<1$), and no APO $\times$ PAP interaction ($F<1$; Fig. 4e).

Papaverine vs. D-amphetamine (PPI protocol 2)—Studies testing for PAP (30 mg/kg) effects on measures of AMPH (4 mg/kg)-induced PPI deficits in SD rats were prompted by the lack of effects of PAP on measures of APO-induced PPI deficits (Figs. 2, 3, and 4), yet significant effects of PAP in measures of AMPH-induced hyperlocomotion (Fig. 6a,b). ANOVA of %PPI revealed main effects of AMPH dose ($F=35.27$, df 1, 16, $p<0.0001$) and PAP ($F=12.65$, df 1, 16, $p<0.005$), and no significant AMPH $\times$ PAP interaction effect ($F=1.47$, df 1, 16, ns, Fig. 5). ANOVA of startle magnitude on P-ALONE trials revealed a significant effect of PAP ($F=7.62$, df 1, 16, $p<0.02$), but not of AMPH dose ($F<1$), and no AMPH $\times$ PAP interaction effect ($F<1$; Fig. 5 inset).

In these rats, PAP significantly increased PPI and reduced startle magnitude. To evaluate if the main effect of PAP on startle magnitude may have affected PAP effects on PPI, matched subgroups of rats were created as described above (Experiment 1.2.2; pulse alone amplitude (mean $\pm$ SEM): vehicle/vehicle treatment (92 ± 16), PAP/vehicle treatment (95 ± 27); vehicle/AMPH (112 ± 35), PAP/AMPH (110 ± 37)). In these subsets of rats ($n=4$ /PAP group), %PPI (mean $\pm$ SEM) was: vehicle/vehicle treatment (79 ± 1), PAP/vehicle treatment (83 ± 4); vehicle/AMPH (47 ± 2), PAP/AMPH (52 ± 6). This indicates that when PAP effects on startle were minimized, PAP effects on PPI were strongly reduced, and more importantly, there remained no evidence for an AMPH $\times$ PAP interaction effect on %PPI ($F<1$).

Locomotor behavior

D-Amphetamine-induced hyperlocomotion in SD vs. WI rats—To assess the bioavailability/bioactivity of PAP in both rat strains and to evaluate the effects of PAP in another measure with predictive validity for antipsychotics, the effects of PAP were tested in a paradigm of AMPH-induced hyperlocomotion. ANOVA of pre-drug activity revealed a significant effect of time ($p<0.0001$) and strain $\times$ time interaction ($p<0.005$). No main effect of strain was detected, and no main effect of dose group (i.e., the dose assigned, but not yet given), or any interaction with dose group was detected (all ns). After AMPH, ANOVA of locomotor activity during the first 2-h post-injection period revealed significant main effects of strain ($F=9.83$, df 1, 13, $p<0.01$) and time ($F=54.35$, df 11, 143, $p<0.0001$), a significant interaction of PAP $\times$ time ($F=9.35$, df 11, 143, $p<0.0001$), and no interaction of strain $\times$ time ($F=2.77$, df 1, 13, ns), PAP $\times$ time ($F=1.02$, df 1, 13, ns), or PAP $\times$ strain $\times$ time ($F<1$). Visual inspection of the AMPH-induced hyperlocomotion data (Fig. 6a,b) suggested that the PAP effects lasted for 20–30 min post-injection in SD rats and potentially for 20 min in WI rats. Separate post hoc tests for the two strains and these three 10-min intervals were conducted using α -levels that were Bonferroni-adjusted for the 3 time intervals analyzed ($p=0.016$). These tests revealed significant suppression of AMPH-induced hyperlocomotion by PAP when compared to vehicle for the first ($p<0.0001$), and second post-injection interval ($p<0.005$), whereas the PAP effect failed to reach significance in the third interval ($p=0.088$) post-injection in SD rats. In WI rats, a trend towards a PAP effect was detected in the first interval post-injection ($p=0.071$), but not the second and third interval post-injection (both ns). Taken together, the data suggest that PAP is able to oppose the hyperactivity induced by AMPH in

SD rats for 20–30 min after AMPH treatment, corresponding to 25–35 min after PAP treatment; these effects appear less pronounced and of shorter duration in WI rats (see Fig. 6a,b).

Spontaneous locomotion—Tests for PAP effects on measures of spontaneous locomotion were conducted in SD rats to assess if a non-specific suppression of locomotion contributes to the ability of PAP to oppose AMPH-induced hyperactivity.

ANOVA of the first 2-h post-injection period revealed significant main effects of PAP ($F=40.06$, df 1, 20, $p<0.0001$) and time ($F=2.83$, df 11, 220, $p<0.002$), and a significant interaction of PAP $\times$ time ($F=25.64$, df 11, 220, $p<0.0001$). Visual inspection of the locomotor data suggested that the locomotor-suppressing PAP effects might last for up to ~1 h post-injection. Post hoc tests were carried out for these six 10-min intervals using Bonferroni-adjusted significance levels ($\alpha=0.008$). Post hoc analysis revealed significant suppression of spontaneous locomotor activity by PAP for the first 50 min ($p<0.0001$ for intervals 1 to 4, $p<0.005$ for interval 5), whereas the interval thereafter failed to reach the Bonferroni-adjusted level of significance. Taken together, the data suggest that PAP suppresses spontaneous locomotion in SD rats for at least 50 min (see Fig. 6c).

Core body temperature: papaverine vs. apomorphine

ANOVA of T° prior to injection revealed no significant differences for APO or PAP dose groups (both main effects and the APO $\times$ PAP interaction ns) confirming similar baseline values. Subsequent ANOVA of the change in T° ($T^\circ_{\text{post-injection}} - T^\circ_{\text{baseline}}$) revealed significant main effects for PAP ($F=34.99$, df 1, 13, $p<0.0001$) and APO ($F=19.58$, df 1, 13, $p<0.001$), and a significant PAP $\times$ APO interaction ($F=20.45$, df 1, 13, $p<0.001$). Post hoc tests revealed that T° was significantly reduced in vehicle/APO-treated rats when compared to vehicle/vehicle-treated rats ($p<0.001$). This confirms the sensitivity of the T° assay to detect drug-induced reductions in T° . Importantly, T° was also reduced in PAP/vehicle-treated rats when compared to vehicle/vehicle-treated animals ($p<0.001$). This confirms the bioavailability/bioactivity of PAP in SD rats. Pretreatment with PAP did not reverse APO-induced reductions on T° ; in fact, T° tended to be further decreased after PAP/APO treatment when compared to vehicle/APO treatment ($p=0.099$; Fig. 7).

Discussion

Here we report that the preferential PDE10A inhibitor PAP does *not* reverse DA agonist-induced PPI deficits regardless of (1) PAP dose (3–30 mg/kg), (2) pretreatment time for PAP relative to APO and the beginning of the recording (5 vs. 30 min), (3) prepulse intervals (10 to 120 ms), (4) rat strain (SD vs. WI rats), and (5) whether DA receptor stimulation was direct (APO) or indirect (AMPH). The sensitivity of the APO/PPI paradigm to a comparison antipsychotic (haloperidol) was confirmed, in line with previous studies showing that haloperidol prevents DA agonist-induced PPI deficits under a wide range of experimental conditions (c.f. Geyer et al. 2001; Swerdlow et al. 2004, 2005; c.f. Swerdlow et al. 2008).

Negative findings must be viewed with caution, as they might reflect a number of factors impacting the sensitivity of detecting PAP effects, including an absence of PAP bioavailability and bioactivity. However, bioavailability and bioactivity of PAP during this time interval was confirmed in three independent behavioral measures: core body temperature, AMPH-induced hyperactivity, and locomotor activity.

Consistent with previous findings, APO led to significant decreases in T° (Varty and Higgins 1998). PAP treatment also induced marked hypothermia, consistent with a previous report (Wachtel 1982). PAP did not reverse APO-induced hypothermia; in fact, treatment with PAP and APO tended to lower T° , relative to the vehicle/APO treatment condition. Arguably, while

potential D2-like antagonistic effects of PAP in the CNS in this assay are likely to be masked by (non-dopaminergic) hypothermia-inducing effects of PAP on the peripheral vasculature, at the very least, these data provide further evidence of the bioavailability/bioactivity of PAP at a time point corresponding to the middle of PPI sessions (pretreatment time for PAP, 5 min).

Bioavailability and bioactivity of PAP was further confirmed by (1) the suppression of AMPH-induced hyperlocomotion and (2) the suppression of spontaneous locomotion by PAP treatment. Both findings are consistent with previous studies (Siuciak et al. 2006). In SD rats, inhibition of spontaneous locomotor activity was present for at least 50 min, i.e., throughout and beyond the time window in which PAP reversed AMPH-induced hyperlocomotion (20–30 min). Thus, while the suppression of AMPH-induced hyperlocomotion may—in principle—suggest an antipsychotic-like profile of PAP, it is questionable if these effects indicate a “true antipsychotic-like profile”, or merely reflect a suppression of spontaneous locomotion that is uncovered under conditions of high activity. This view is supported by comprehensive data from Siuciak et al. (2006) showing that the 95% confidence band for the ID₅₀ of PAP on the suppression of spontaneous locomotion fully included the corresponding confidence band for AMPH-induced hyperlocomotion. Data from the same study further indicate that the same case can be made for PAP effects in models of CAR and possibly NMDA-antagonist-induced hyperlocomotion (Siuciak et al. 2006). Because the main goal of this study was to assess PAP effects in the PPI model, comprehensive dose–response functions were not completed with locomotor studies. Such dose–response analyses might have enabled us to assess a “therapeutic index” for PAP, based on doses of PAP that suppressed AMPH hyperlocomotion, but not spontaneous locomotor activity. The dissociation between antipsychotic effects on generalized motor activity vs. activation associated with psychostimulants or motivational states has been a longstanding challenge that has been addressed via a number of creative experimental strategies (e.g., see, Siuciak et al. 2006).

Negative findings might also reflect the insensitivity of one particular set of PPI parameters to detecting PAP effects. Thus, we assessed the effects of PAP in measures of PPI in detail and under a wide range of experimental conditions, none of which suggested an antipsychotic-like profile of PAP in PPI measures. We cannot exclude the possibility that an antipsychotic-like profile might become evident at higher PAP doses, repeated or chronic PAP treatment, or under other experimental conditions. The conditions tested in the present study have been used in our laboratory and those of others to correctly detect antipsychotic potential for a large number of clinically effective antipsychotics that differ in both clinical properties and mechanisms of action (for reviews, see Geyer et al. 2001 and Swerdlow et al. 2008). It is also possible that antipsychotic profiles in these measures might be detected using other PDE inhibitors, particularly those active against members of the PDE4 subgroup (Kanes et al. 2007; Siuciak et al. 2008). The present studies also do not address whether inhibition of PDE10A is able to reverse NMDA-antagonist-induced PPI deficits in rats; such an effect in mice has been controversial (Lu et al. 2007; Schmidt et al. 2008).

At present, it is difficult to account for the differences between our findings and those presented in abstract form by Bleickardt et al. (2007). A full understanding of PAP effects on PPI requires analyses of—or controls for—drug-induced changes in startle magnitude, and we must await a full publication of the data from Bleickardt et al. (2007) for this type of detailed information. However, a novel study by Nishi et al. (2008) suggests that high DA D2 tone might obviate the effects of PAP in some in vivo measures. Using microdialysis, Nishi et al. (2008) reported that PAP-induced increases of cAMP/PKA levels in the striatum were only observed when D2 tone was reduced via co-treatment with a subthreshold dose of the D2 antagonist, haloperidol. Similarly, Siuciak et al. (2006) observed PAP-induced catalepsy only when PAP treatment was combined with a subthreshold dose of haloperidol. It is thus possible that PAP effects on PPI might be most easily detected under conditions of low basal D2 tone. While we have tested

PAP in several rat strains and under conditions of both strong PPI disruption mediated by high doses of DA agonists (Figs. 2, 3a, and 4) as well as small/moderate PPI disruption mediated by low doses of DA agonists (Figs. 3b and 5), we cannot exclude the possibility that PAP effects on PPI might have been detected under experimental conditions characterized by low D2 tone. Importantly, the present study was conducted under conditions most commonly used in the PPI predictive model of antipsychotic effects (Swerdlow et al. 1994).

As documented in large, prospective clinical trials such as the CATIE study (Lieberman et al. 2005), there is a clear need for the development of antipsychotics that differ fundamentally from existing first- and second-generation compounds. Major advances in our understanding of post-receptor signal transduction mechanisms have identified a number of potentially novel targets for drug action that might have the ability to regulate “downstream” effects of dopamine and/or glutamate neurotransmission. It is conceivable that, in order to detect compounds with fundamentally different mechanisms of antipsychotic action (e.g., procognitive effects), we will need fundamentally different predictive models. One such model may be attentional set-shifting in rodents, a task that mimics some fronto-cortical attentional deficits that can be observed in patients with schizophrenia. Indeed, Rodefer et al. (2005) have shown that extradimensional attentional set-shifting remains disrupted after a washout period following subchronic treatment with PCP, and this deficit was reversed by acute treatment with PAP. On the other hand, many of the challenges of developing drugs aimed at these novel targets are similar to those faced in the development of conventional antipsychotics, e.g., dose and strain sensitivity, pharmacokinetics and dynamics, behavioral specificity, target specificity, and regional distribution. To the degree that we could address these challenges in the present study, we conclude that one sensitive predictive model—the blockade of PPI-disruptive effects of DA agonists—fails to detect antipsychotic-like properties of PAP and that antipsychotic-like properties detected with locomotor-based assays may need to be treated with caution due to concurrent inhibition of spontaneous locomotion.

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Fig. 1.

Effects of haloperidol on apomorphine-induced PPI deficits and startle magnitude (*insets*) in SD (a) or WI (b) rats. ANOVA of %PPI revealed a significant main effect of APO ($p < 0.0001$, not indicated in graph) and the critical APO $\times$ HAL interaction ($p < 0.0001$), demonstrating the reversal of APO-induced PPI deficits by HAL. Asterisk symbols denote significance levels of post hoc comparisons (* $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$; $n = 6$ SD rats/HAL dose group, and six WI rats/HAL dose group)

Fig. 2.

Effects of papaverine on apomorphine-induced PPI deficits and startle magnitude (*insets*) in SD (a) or WI (b) rats. Pretreatment time for PAP relative to APO treatment and the beginning of the experiment was 30 min. ANOVA of %PPI revealed significant main effects of APO ($p < 0.0001$, not indicated in figure) and a strain $\times$ APO interaction ($p < 0.01$, not indicated in figure), indicating higher APO/PPI sensitivity of SD rats. No APO $\times$ PAP or APO $\times$ PAP $\times$ strain interaction was observed. ANOVA on P-ALONE trials did not reveal any significant main or interaction effects (all ns; $n = 6$ SD rats/PAP dose group, and six WI rats/HAL PAP group)

Fig. 3.

Effects of papaverine on PPI deficits and startle magnitude (*insets*) induced by a standard (0.5 mg/kg; **a**) and low (0.1 mg/kg; **b**) dose of APO in SD rats. Pretreatment time for PAP relative to APO treatment and the beginning of the experiment was reduced to 5 min to optimize bioavailability/bioactivity. ANOVAs revealed main effects of APO in both experiments ($p < 0.0001$; not indicated in graph). PAP did *not* reverse PPI deficits induced by either **a** 0.5 mg/kg ($n = 6$ SD rats/PAP dose group), or **b** 0.1 mg/kg ($n = 10$ SD rats/PAP dose group) APO. PAP reduced P-Alone magnitude in **b**. * $p < 0.05$

Fig. 4.

Effects of papaverine on apomorphine-induced PPI deficits (**a–d**) and startle magnitude (**e**) for prepulse intervals from 10 to 120 ms in SD rats. Pretreatment time for PAP (0, 3, 10, 30 mg/kg) relative to APO treatment and the beginning of the experiment was 5 min. **a–d** ANOVA of %PPI revealed significant effects of APO dose ($p < 0.005$), prepulse interval ($p < 0.0001$), and an APO $\times$ prepulse interval interaction effect ($p < 0.005$; all not indicated in figure). Importantly, none of the APO $\times$ PAP interaction effects reached significance at any of the prepulse intervals. **e** ANOVA of startle magnitude on P-ALONE trials revealed no significant main or interaction effects ($n=6$ SD rats/PAP dose group)

Fig. 5.

Effects of papaverine on amphetamine-induced PPI deficits and startle magnitude (*inset*) in SD rats. Pretreatment time for PAP relative to AMPH treatment was 5 min, and the experiment was begun 10 min after AMPH treatment. ANOVA revealed a significant main effect of AMPH ($p < 0.0001$, not indicated in figure) and PAP ($p < 0.005$). PAP did *not* reverse PPI deficits induced by 4 mg/kg AMPH. PAP reduced P-Alone amplitudes. * $p < 0.05$, ** $p < 0.005$; $n = 9$ SD rats/PAP dose group

Fig. 6.

Effects of papaverine on amphetamine-induced hyperlocomotion in SD (**a**) or WI (**b**) rats, and on spontaneous locomotion in SD rats (**c**). **a, b** Pretreatment time for PAP relative to AMPH treatment and the beginning of the experiment was 5 min. ANOVA of pre-drug activity revealed similar beam break activity of dose groups (i.e., the dose assigned, but not yet given). After AMPH, ANOVA of beam break activity for the 2-h post-injection period revealed main effects of strain ($p < 0.02$) and time ($p < 0.0001$), and interactions of strain $\times$ time ($p < 0.0001$) and PAP $\times$ time ($p < 0.0001$). Analyses revealed suppression of locomotion by PAP relative to vehicle for the first and second post-injection interval, and a trend towards an effect in the third interval in SD rats, but not in WI rats. Asterisk symbols denote significance levels of post hoc

comparisons between vehicle and PAP-treated rats ($n=8$ SD rats and $n=7$ WI rats). **c** Pretreatment time for PAP relative to the start of the experiment was 5 min. ANOVA of beam break activity of the first 2-h post-injection period revealed significant main effects of PAP ($p<0.0001$) and time ($p<0.002$), and a significant interaction of PAP $\times$ time ($p<0.0001$; $n=11$ SD rats/PAP dose group). Post hoc analyses revealed significant suppression of locomotion by PAP for the first 5 time intervals, whereas the later time intervals did not reach the Bonferroni-adjusted α -level. *Asterisk symbols* denote significance levels of post hoc comparisons between APO and vehicle treatment (** $p<0.005$, *** $p<0.0005$)

Fig. 7.

Effects of papaverine on apomorphine-induced hypothermia in SD rats. Pretreatment time for PAP relative to APO treatment was 5 min, and the final temperature recording was taken 8 min thereafter. ANOVA of the change in T° ($T^{\circ}_{\text{post-injection}} - T^{\circ}_{\text{baseline}}$) revealed main effects for PAP ($p < 0.0001$) and APO ($p < 0.001$), and a significant PAP $\times$ APO interaction ($p < 0.001$). PAP pretreatment did not reverse APO-induced T° reductions, but tended to further decrease T° when compared to vehicle treatment in APO-treated rats. Asterisk symbols denote significance levels of post hoc comparisons (*** $p < 0.005$, *** $p < 0.0005$; $n = 7-8$ SD rats/PAP dose group)