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ORIGINAL INVESTIGATION

Inhibition of phosphodiesterase 10A has differential effects on dopamine D1 and D2 receptor modulation of sensorimotor gating

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Abstract
Rationale Inhibitors of phosphodiesterase 10A (PDE10A), an enzyme highly expressed in medium spiny neurons of the mammalian striatum, enhance activity in direct (dopamine D1 receptor-expressing) and indirect (D2 receptor-expressing striatal output) pathways. The ability of such agents to act to potentiate D1 receptor signaling while inhibiting D2 receptor signaling suggest that PDE10A inhibitors may have a unique antipsychotic-like behavioral profile differentiated from the D2 receptor antagonist-specific antipsychotics currently used in the treatment of schizophrenia.
Objectives To evaluate the functional consequences of PDE10A inhibitor modulation of D1 and D2 receptor pathway signaling, we compared the effects of a PDE10A inhibitor (TP-10) on D1 and D2 receptor agonist-induced disruptions in prepulse inhibition (PPI), a measure of sensorimotor gating disrupted in patients with schizophrenia.
Results Our results indicate that, in rats: (1) PDE10A inhibition (TP-10, 0.32–10.0 mg/kg) has no effect on PPI disruption resulting from the mixed D1/D2 receptor agonist apomorphine (0.5 mg/kg), confirming previous report; (2) Yet, TP-10 blocked the PPI disruption induced by the D2 receptor agonist quinpirole (0.5 mg/kg); and attenuated apomorphine-induced disruptions in PPI in the presence of the D1 receptor antagonist SCH23390 (0.005 mg/kg).
Conclusions These findings indicate that TP-10 cannot block dopamine agonist-induced deficits in PPI in the presence of D1 activation and suggest that the effect of PDE10A inhibition on D1 signaling may be counterproductive in some models of antipsychotic activity. These findings, and the contribution of TP-10 effects in the direct pathway on sensorimotor gating in particular, may have implications for the potential antipsychotic efficacy of PDE10A inhibitors.

Keywords PDE10A · Phosphodiesterase inhibitor · Apomorphine · Quinpirole · SCH23390 · Prepulse inhibition · Acoustic startle · Rat

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Introduction

Approximately 95 % of all rodent striatal neurons are medium spiny neurons (MSN), the principal output neurons of the mammalian striatum (Matamales et al. 2009). Striatal MSNs are organized into two output pathways, the direct (striatonigral) and indirect (striatopallidal) pathways (Surmeier et al. 2007). The direct pathway expresses dopamine D1 receptors positively coupled to adenylyl cyclase to stimulate cAMP/PKA signaling. Conversely, the indirect pathway expresses dopamine D2 receptors which inhibit

adenylyl cyclase and cAMP/PKA signaling (Surmeier et al. 2007; Nishi et al. 2008). These pathways regulate behavioral responses after integrating cortical glutamatergic and midbrain dopaminergic inputs, both of which have been implicated in the pathophysiology of schizophrenia (O'Donnell and Grace 1998; Carlsson et al. 2001; Goff and Coyle 2001). The majority of currently approved antipsychotic medications antagonize D2 receptor neurotransmission, principally acting on the neurons of the indirect pathway (Kapur and Mamo 2003; Agid et al. 2008).

One enzyme highly expressed in the MSN is phosphodiesterase 10A (PDE10A) (Seeger et al. 2003). This enzyme is found in high levels in terminals and neuropil in the striatonigral and striatopallidal circuits while showing limited cell body expression in cortical and hippocampal regions (Seeger et al. 2003). PDE10A regulates striatal output by degrading cAMP and cGMP and downregulating cAMP/PKA signaling (Menniti et al. 2006; 2007; Nishi et al. 2008). PDE10A inhibitors activate cAMP/PKA signaling in MSNs of both striatal pathways as shown by their ability to increase substance P and enkephalin expression (markers of direct and indirect pathway, respectively) (Strick et al. 2010). These data indicate that in PDE10A expressing neurons, which are largely found in the striatum, PDE10A inhibition results in *potentiation* of dopamine D1 receptor signaling in conjunction with *inhibition* of dopamine D2 receptor signaling. Because of this combined enhancement of both the D1 receptor expressing direct pathway and the D2 receptor expressing indirect pathway, PDE10A inhibitors may have a unique clinical profile compared to currently approved D2 antagonist-specific antipsychotics (Schmidt et al. 2008; Grauer et al. 2009). PDE10A inhibitors are efficacious in several behavioral tests for antipsychotic efficacy and animal models of schizophrenia, with relatively mild potential side effects such as catalepsy (Chappie et al. 2009; Kehler and Nielsen 2011). Because PDE10A inhibition is suggested to activate indirect pathway neurons to a greater extent than direct pathway neurons (Nishi et al. 2011), the behavioral effects of PDE10A inhibitors are primarily attributed to inhibition of the downstream effects of D2 receptor signaling (Kehler and Nielsen 2011). The functional impact of PDE10A inhibitor effects on D1 signaling remains unclear, yet may have implications for their utility as therapeutic agents for schizophrenia. To evaluate the functional effect of PDE10A inhibition on direct vs. indirect pathway signaling, we compared the effects of the PDE10A inhibitor, TP-10 (Schmidt et al. 2008), on the differential contribution of D1 and D2 receptor activation to the disruption of prepulse inhibition (PPI), a measure of sensorimotor gating deficient in patients with schizophrenia.

PPI is a measure of information processing wherein the presentation of a non-startling "prepulse" inhibits the startle response to a following startling pulse. Rodent models of PPI are highly predictive of antipsychotic efficacy (Braff et al. 2001; Geyer et al. 2001; Swerdlow et al. 2008). Atypical and typical antipsychotics reverse D1/D2 agonist (e.g., apomorphine)-induced decreases in PPI. Atypical antipsychotics also reverse PPI disruptions induced by *N*-methyl-D-aspartate (NMDA)-receptor antagonists (Geyer et al. 2001). While there are reports that PDE10A inhibitors prevent decreases in PPI induced by MK-801 (NMDA receptor antagonist) in rats (Grauer et al. 2009; Bleickardt et al. 2010; but see Schmidt et al. 2008 [mouse]), these inhibitors have no effect on apomorphine-induced disruptions in PPI (Weber et al. 2009; Bleickardt et al. 2010), nor do they improve PPI in mice with low gating, unlike D2 antagonists (Schmidt et al. 2008). One possible explanation for the inconsistent effects of PDE10A inhibitors in hyperdopaminergic models of PPI is that their D1/direct pathway potentiation masks the D2-inhibitory effects. D2 receptor activation is sufficient and necessary to disrupt PPI in rats (Peng et al. 1990; Wan et al. 1996). In contrast, D1 activation alone is not sufficient to disrupt PPI in rats, although it does synergistically potentiate D2-induced disruption of PPI (Swerdlow et al. 1991; Hoffman and Donovan 1994; Wan et al. 1996; Bortolato et al. 2005). If the hypothesis that TP-10 facilitates D1 activation is correct, TP-10 treatment should block selective D2-induced disruptions in PPI, but not disruptions induced by concurrent D1/D2 activation. To test these predictions we compared the efficacy of TP-10 to prevent PPI disruptions induced by (1) a mixed D1/D2 agonist, apomorphine, (2) a D2 agonist, quinpirole, or (3) apomorphine in the presence of the D1-selective antagonist SCH23390.

Materials and methods

Animals

A total of 178 male Sprague–Dawley rats (Harlan Laboratories, San Diego, CA, USA), weighing 300–350 g at test, were used. Animals were housed two per cage in clear plastic cages inside a temperature- and humidity-controlled vivarium with a 12:12 h reverse light/dark cycle (lights off at 08:00 AM). Food and water were available ad libitum. Upon arrival, all rats were handled gently to minimize stress during testing. Testing was conducted in accordance with the principles of laboratory animal care as stated in the National Institutes of Health "Guide for the Care and Use of Laboratory Animals" and approved by the University of California San Diego Institutional Animal Care and Use Committee.

Drugs

Apomorphine hydrochloride (0.5 mg/kg, s.c.) and quinpirole hydrochloride (0.5 mg/kg, s.c.) were purchased from Sigma-

Aldrich (St. Louis, MO, USA). Haloperidol lactate solution (0.1 mg/kg, s.c.) and SCH23390 hydrochloride (0.005 mg/kg, s.c.) were purchased from Bedford Labs (Bedford, OH, USA) and Tocris (Ellisville, MO, USA), respectively. TP-10 (0.32–10.0 mg/kg, s.c.) was synthesized at Pfizer Global Research and Development. Quinpirole HCl and SCH23390 HCl were dissolved in 0.9 % saline. Saline was added to the haloperidol lactate solution to achieve the correct dose (initial concentration, 5 mg/ml). Apomorphine was dissolved in a vehicle solution of 0.1 % ascorbic acid. TP-10 was dissolved in a vehicle consisting of 5 % Emulphor, 5 % dimethyl sulfoxide and 90 % saline. Injection volume was 1 ml/kg for all drugs. Drug doses are in the salt form.

Apparatus

Testing occurred in ventilated, sound-attenuating commercial startle chambers (San Diego Instruments, San Diego, CA, USA) consisting of a nonrestrictive Plexiglas cylinder into which the animal was placed. The cylinder was attached to a Plexiglas platform. Rat movements were detected by a piezoelectric accelerometer attached below the platform. The rat's movements were then digitized and stored by an interface and computer assembly. A high-frequency loud speaker inside the chamber provided both a continuous background noise of 65 dB and various acoustic stimuli.

Behavioral testing

One week after arrival, rats were exposed to a brief baseline startle/PPI session consisting of 120 dB pulse-alone trials and prepulse+pulse trials in which a 12-dB above background noise was presented 100 ms before the onset of the 120-dB pulse. A 65-dB background was presented continuously throughout the session. The startle session began with a 5-min acclimation period. Then, 24 trials were presented in pseudorandom order: 18 presentations of the 120-dB pulse-alone trials (40 ms each) and six presentations of the prepulse+pulse trials in which a 77-dB pulse preceded the 120-dB pulse by 100 ms. Treatment groups were established by using the mean startle response to the 120 dB pulse-alone trials and the mean percent PPI calculated from the prepulse+pulse trials, so that all groups had comparable baseline startle reactivity and PPI. Drug testing began 1 week after baseline session.

The test session used in all experiments consisted of five different trial types: pulse-alone trials in which a 120-dB pulse (40 ms) was presented; three prepulse+pulse trials in which a 68-, 71-, or 77-dB prepulse (20 ms) was presented 100 ms before the onset of the 120-dB pulse; and "no stimulus" trials, which included only the background noise. All trials were presented in pseudorandom order consisting of 24 pulse-alone trials, 30 prepulse+pulse trials (ten trials at each prepulse), and eight no stimulus trials. An average of 15 s (ranging from 7 to 23 s) separated consecutive trials. Session duration totaled ~20 min.

Experimental design

Experiment 1: TP-10 in apomorphine (D1/D2 agonist)-treated rats

Apomorphine (0, 0.5 mg/kg) was administered 5 min prior to testing. TP-10 (0, 0.32, 1.0, 3.2, 10.0 mg/kg) or haloperidol (0.1 mg/kg) was administered 25 min prior to apomorphine. The haloperidol group was included as a positive control to verify the sensitivity of the PPI protocol to detect antipsychotic-like effects of known antipsychotics. Thus, in total, there were 12 groups (n = 8–10/group). An apomorphine-crossover design was utilized such that each rat was tested twice (once with 0 mg/kg and once with 0.5 mg/kg apomorphine). A 1-week washout period was provided between the crossover tests.

Experiment 2: TP-10 in quinpirole (D2 agonist)-treated rats

Quinpirole (0, 0.5 mg/kg) was administered 5 min prior to testing. TP-10 (0, 0.32, 1.0, 3.2, 10.0 mg/kg) or haloperidol (0.1 mg/kg) was administered 25 min prior to quinpirole. In total, there were 12 groups (n = 7–10/group) (quinpirole crossover design) with a 1-week washout period between each test. In a separate follow-up experiment, a single dose of TP-10 (0, 3.2 mg/kg) was administered 25 min prior to quinpirole (0, 0.5 mg/kg), for a total of four groups (n = 9–10/group) in a between subject design.

Experiment 3: TP-10 plus SCH23390 (D1 antagonist) in apomorphine-treated rats

Apomorphine (0, 0.5 mg/kg) was administered 5 min prior to testing. TP-10 (0, 3.2 mg/kg) was administered 25 min prior to apomorphine and SCH23390 was administered 35 min before apomorphine. In total there were eight groups (2 levels apomorphine × 2 levels TP-10 × 2 levels SCH23390) (n = 8–9/group). An apomorphine crossover design was utilized as described above.

Data analysis

The startle response to the 120-dB burst was recorded for each pulse-alone and prepulse+pulse trial (1 kHz sampling for 100 ms at onset of the pulse). Percentage of PPI was calculated using the formula: 100−([(average startle to the prepulse+pulse trials)/average startle to the pulse alone trials] × 100). For Experiments 1 and 2, a two-way analysis of variance (ANOVA), with TP-10 (or haloperidol) pretreatment as the

between subject factor and apomorphine (or quinpirole) treatment as the within subject factor, was used. For Experiment 3, a three-way ANOVA with TP-10 and SCH23390 as between subject factors and apomorphine as the within subject factor was used. Percent PPI or startle magnitude comprised the dependent measures. In all experiments, initial statistical models of PPI effects included prepulse intensity (68, 71 and 77 dB) as a within subject factor. These analyses showed that intensity significantly interacted with either apomorphine (Experiments 1 and 3) or quinpirole (Experiment 2) treatment ($F(2,60)$, or $F(2,70)$, or $F(3,76)>4.18$, $p<0.02$), with the most robust treatment-induced PPI disruptions consistently observed at the 77 dB prepulse intensity. This intensity effect was particularly apparent in Experiment 3 where a clear apomorphine deficit was visualized at the 77 dB prepulse trial (see Online Resource 1 for supplementary tables of drug effects across 68 and 71 dB prepulse trials). Hence, treatment effects at this intensity were most appropriate to test TP-10 efficacy to reverse dopamine agonist-induced disruption of PPI. Thus, for clarity of comparison across all studies, the startle response at 77 dB intensity was used as the dependent measure for all analyses and data visualization. Post-hoc analyses were carried out using Tukey's test (between subject) or paired t-tests (within subject) as appropriate.

Results

Experiment 1: TP-10 in apomorphine (D1/D2 agonist)-treated rats

Apomorphine (APO) significantly decreased PPI ($F(1,38)=71.85$, $p=0.0001$). TP-10 had no significant effect on PPI alone, nor did it prevent the apomorphine-induced disruption of PPI, as indicated by no significant TP-10 × APO interaction. In contrast, there was a significant main effect of haloperidol (HAL) on PPI ($F(1,16)=28.68$, $p=0.0001$) that was accompanied by a significant HAL × APO interaction ($F(1,16)=21.59$; $p=0.0003$), indicating that haloperidol blocked the effect of apomorphine on PPI. Post-hoc analysis confirmed the findings, revealing an increase in PPI produced by haloperidol in apomorphine-treated rats ($p<0.05$, HAL/APO vs. VEHICLE/APO) (Fig. 1).

There was a significant main effect of apomorphine on startle magnitude ($F(1,38)=7.70$, $p=0.009$), with apomorphine increasing startle. This effect was independent of TP-10 pretreatment. There was a trend for TP-10 to reduce startle, $F(4,38)=2.23$, $p=0.08$, although no interaction with APO. Although haloperidol tended to decrease startle ($F(1,16)=4.36$, $p=0.053$), this effect was not dependent on apomorphine treatment (Table 1).

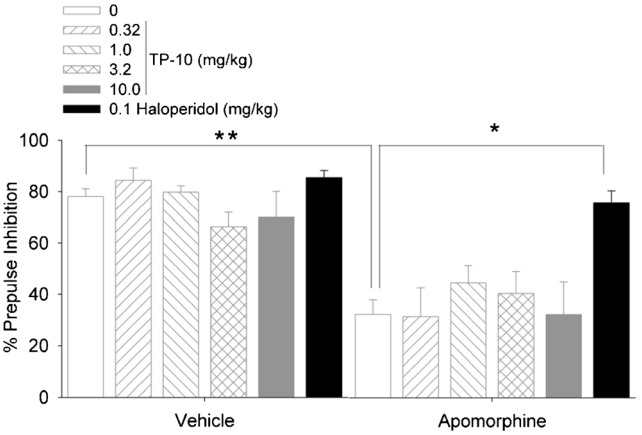

Fig. 1 Effect of TP-10 (0–10.0 mg/kg) and haloperidol (0.1 mg/kg) on apomorphine-induced decreases in % PPI. Values represent mean ± SEM. Haloperidol, but not TP-10, reversed the apomorphine-induced decrease in PPI (**$p<0.01$ paired t-test Vehicle/Vehicle vs. Apomorphine/Vehicle; *$p<0.05$, Tukey's test)

Experiment 2: TP-10 in quinpirole (D2 agonist)-treated rats

Quinpirole (QUIN) significantly decreased PPI ($F(1,38)=20.85$, $p=0.0001$), an effect blocked by pretreatment with haloperidol (HAL × QUIN: $F(1,16)=10.81$, $p=0.005$; $p<0.05$, HAL/QUIN vs. VEHICLE/QUIN, post hoc analysis), but not TP-10 (Fig. 2a). Although the TP-10 × Quinpirole interaction was not statistically significant, visual inspection of the data (Fig. 2a) indicated that a dose-dependent reversal by TP-10 of quinpirole-induced decreases in PPI may have been occluded by the inclusion of several suboptimal doses of TP-10. Indeed, when the 0.32 and 1 mg/kg doses were dropped from the analysis, the TP-10 × Quinpirole interaction was significant ($F(2,22)=6.17$, $p=0.0074$). Exploratory analysis in the quinpirole-treatment groups using TP-10 dose as the independent factor revealed a significant linear trend for PPI to be increased with increasing dose of TP-10 ($R=0.36$, $F(1,41)=6.2$, $p=0.0171$). Therefore, in a separate experiment using naïve rats, we chose to examine the effects of a single high dose of TP-10 (3.2 mg/kg) on disruptions of PPI produced by quinpirole. At this dose, a highly significant interaction between TP-10 and quinpirole was observed, suggesting that TP-10 prevented the quinpirole-induced decrease in PPI (QUIN: $F(1,35)=10.00$, $p=0.003$; TP-10 × QUIN: $F(1,35)=14.97$; $p=0.0005$). Post-hoc analysis confirmed this finding with a PPI disruption observed only in the VEHICLE/QUIN group ($p<0.05$, VEHICLE/QUIN, vs. all other groups) (Fig. 2b).

In the TP-10 dose response experiment, a significant effect on startle magnitude was found for quinpirole ($F(1,38)=12.05$, $p=0.0013$), with quinpirole decreasing startle. TP-10 also tended to decrease startle ($F(4,38)=2.42$, $p=0.065$.) Although there was a significant TP-10 × Quinpirole interaction ($F(4,38)=5.05$, $p=0.002$), post-hoc analysis indicated that no dose of TP-10 significantly reversed the quinpirole-

Table 1 Effect of TP-10 on apomorphine (Experiment 1) and quinpirole (Experiment 2)-induced changes in startle magnitude (Mean±SEM)

	TP-10 (0 mg/kg)	TP-10 (0.32 mg/kg)	TP-10 (1.0 mg/kg)	TP-10 (3.2 mg/kg)	TP-10 (10.0 mg/kg)	Haloperidol (0.1 mg/kg)
Vehicle	193±38	153±37	162±36	75±18	71±22	85±16
Apo* (0.5 mg/kg)	213±41	190±47	172±31	133±25	159±19	148±18
Vehicle	170±31	184±37	94±12	67±16	89±20	134±19
Quinpirole* (0.5 mg/kg)	76±15	86±18	112±22	63±14	77±11	106±13

*ps<0.01, Main effects of apomorphine and quinpirole

induced decrease in startle (Table 1). Decreases in startle resulting from quinpirole were independent of haloperidol pretreatment (HAL × QUIN, NS) (Table 1). In the follow-up experiment using the 3.2 mg/kg dose of TP-10, quinpirole reduced startle from 163.4±35.1 to 86.4±16.4 in the Vehicle/Vehicle and Vehicle/Quin groups, respectively. As seen previously, pretreatment with TP-10 had no effect on startle decreases resulting from quinpirole (mean startle±SEM= 94.2±23.3 in the TP-10/Quin group). TP-10 alone did not affect the startle response (mean startle±SEM=124.3±21.9 in the TP-10/Vehicle group). (QUIN: $F(1,35)=4.76$, $p=0.04$; TP-10: NS; TP-10 × QUIN: NS).

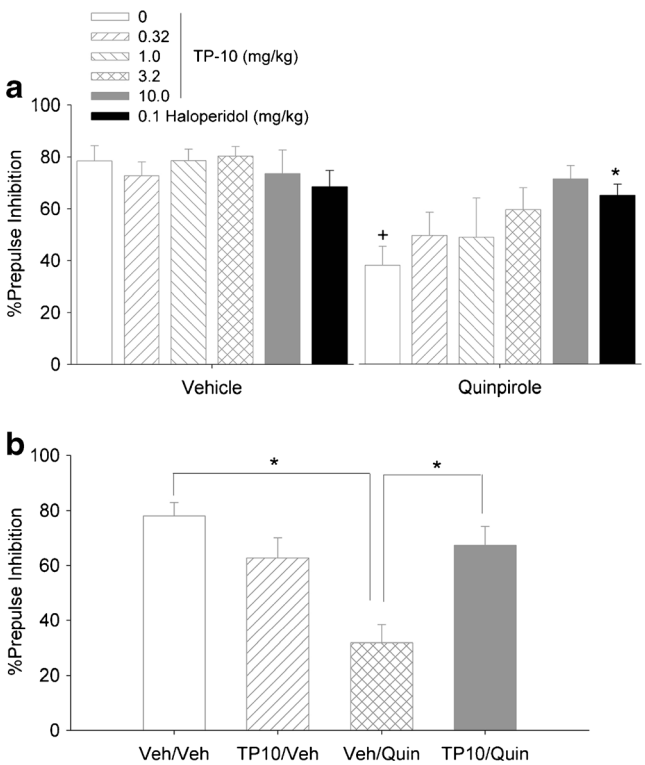

Fig. 2 Effect of TP-10 (0–10.0 mg/kg) and haloperidol (0.1 mg/kg) on quinpirole-induced decreases in % PPI. Values represent mean ± SEM. **a** Haloperidol reversed the quinpirole-induced decrease in PPI ($^{+}p<0.05$ paired t-test Vehicle/Vehicle vs. Quin/Vehicle; $^{*}p<0.05$, vs. Quin/Veh, Tukey's test). **b** TP-10 (3.2 mg/kg) reversed quinpirole-induced decreases in PPI ($^{*}p<0.05$, Veh/Quin vs. all other groups, Tukey's test)

Experiment 3: TP-10 plus SCH23390 (D1 antagonist) in apomorphine-treated rats

As in Experiment 1, apomorphine treatment produced a significant disruption in PPI (APO: $F(1,30)=20.65$, $p=0.0001$)). Apomorphine-induced decreases in % PPI also depended upon the combined pretreatment of TP-10 with SCH23390 (TP-10 × SCH23390 × APO: $F(1,30)=6.03$, $p<0.05$). To determine the source of the three-way interaction, separate two-way ANOVAs were conducted on the groups that received either apomorphine or vehicle treatment (i.e., ascorbic acid) with TP-10 and SCH23390 as factors. In the apomorphine-treated group, there was a significant TP-10 × SCH23390 interaction ($F(1,30)=8.71$, $p=0.006$). Post hoc analyses confirmed that the combination of TP-10/SCH23390 treatment significantly increased PPI compared to the apomorphine only group ($p<0.05$). This effect cannot be attributed to TP-10 alone, as the TP-10/SCH23390 combination treated group also exhibited significantly higher PPI than the TP-10 alone group ($p<0.05$, Fig. 3). Among the

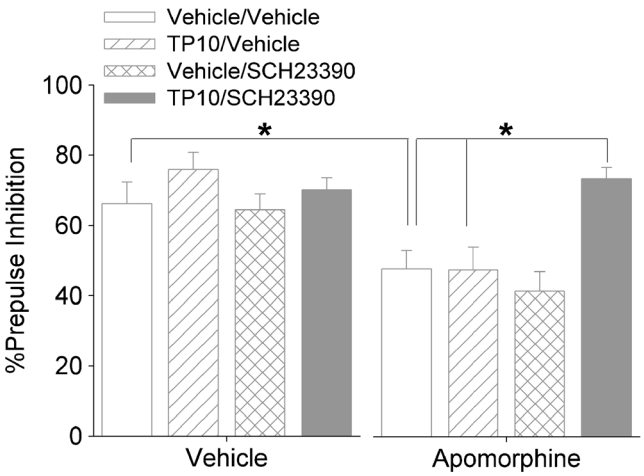

Fig. 3 Effect of TP-10 (3.2 mg/kg) plus SCH23390 (0.005 mg/kg) on apomorphine-induced decreases in % PPI. Values represent mean ± SEM. Apomorphine-induced decreases in % PPI depended upon pretreatment with TP-10 and SCH23390. Among the apomorphine-treated groups, only the combination of TP-10 plus SCH23990 reversed the decrease in PPI induced by apomorphine ($^{*}p<0.05$ via Tukey's test: Apo/TP-10/SCH vs. Apo/Veh/Veh or Apo/TP-10/Veh, or paired t-test: Apo/Veh/Veh vs. Veh/Veh/Veh)

vehicle-treated groups (i.e., ascorbic acid only), there were no significant effects of TP-10 or SCH23390 treatment, or interactions.

Apomorphine ($F(1,30)=9.83$, $p=0.004$) and TP-10 ($F(1,30)=6.57$, $p=0.02$) treatment significantly reduced startle magnitude. SCH23390 treatment significantly increased startle when given alone, but had no effect on startle in the apomorphine-treated groups (SCH23390 × APO interaction ($F(1,30)=10.92$, $p=0.003$)); Non-apomorphine treated groups: Main effect of SCH23390: $F(1,30)=9.72$, $p=0.004$; $p<0.05$, VEHICLE/SCH23390 vs. TP-10/VEHICLE, post-hoc analysis) (Table 2).

Discussion

The present studies explored the effects of a PDE10A inhibitor, TP-10, on dopamine agonist-induced disruptions of PPI. The first experiment confirmed that PDE10A inhibition had no effect on PPI disruptions induced by the mixed D1/D2 agonist apomorphine. Apomorphine-induced disruptions were blocked by the positive control, the D2 antagonist haloperidol. In contrast, the second study showed that TP-10 blocked the disruption of PPI induced by the D2 receptor agonist quinpirole. Finally, in the presence of the D1 antagonist SCH23390, TP-10 did reverse apomorphine-induced disruptions in PPI (Experiment 3). TP-10 treatment showed consistent mild reductions in startle, which did not interact with other treatments, suggesting startle effects were independent of whether TP-10 did or did not affect dopamine agonist-induced disruptions across these studies. These findings indicate that PDE10A inhibitors act on both D1 and D2 signaling pathways, activating and inhibiting D1- and D2-mediated behaviors, respectively. This is the first study, to our knowledge, showing a functional contribution of the direct pathway in the behavioral effects of TP-10.

Previous reports of PDE10A inhibitor effects in pharmacological models of PPI deficits have resulted in inconsistent results. Although PDE10A inhibitors have been reported to work in selected models of PPI in rats (e.g., NMDA antagonist-induced disruptions in Long Evans rats (Grauer et al. 2009)), the majority of studies, particularly those relying on dopaminergic disruption, have reported no effect on D1/D2 agonist (apomorphine)-induced disruptions in PPI (Weber et al. 2009; Bleickardt et al. 2010; present findings). Similarly, in naturally poor-gating C57 mice, PDE10A inhibitors failed to improve baseline PPI (Schmidt et al. 2008), an effect readily observed with D2 receptor antagonists (Ouagazzal et al. 2001). The negative findings in the PPI assay are particularly surprising given the number of preclinical assays in which PDE10A inhibitors do produce effects identical to those of D2 receptor antagonists, including several based upon reversal of the effects of direct (apomorphine) or indirect (amphetamine) agonists. For example, Grauer et al. (2009) reported that PDE10A inhibitors were effective antagonists of apomorphine-induced climbing and several groups observed antagonism of amphetamine-stimulated locomotion with PDE10A inhibitors (Siuciak et al. 2006; Sotty et al. 2009; Weber et al. 2009). Additionally, TP-10 has been reported to reverse the disruption of auditory gating by amphetamine. Based on rat studies suggesting D1 receptor stimulation contributes to the modulation of PPI in the presence of D2 activation (Geyer et al. 2001), and that D1 receptors play a role in the disruption of PPI by direct agonists in mice (Ralph-Williams et al. 2002) and rats (Ralph and Caine 2005), it is plausible that activation of the direct pathway by PDE10A inhibition interferes with the activity of these compounds in the PPI test. Such interference could in turn account for the absence of an effect of TP-10 treatment on apomorphine-induced PPI disruptions in rats and naturally poor-gating mice. Interestingly, direct D1 pathway effects of PDE10A inhibition could also account for the apparent efficacy of PDE10A inhibitors in reversing NMDA-antagonist models of PPI disruption in rats. Direct D1 agonists have been shown to weakly attenuate NMDA-induced deficits in PPI (Bubenikova-Valesova et al. 2009) and, thus, potentiation of D1 signaling via PDE10A inhibition could contribute to the efficacy of PDE10A inhibitors to reverse NMDA antagonist-induced behavioral disruption. Indeed, selective D2 inhibition alone is largely ineffective in reversing NMDA antagonist-induced deficits in PPI (Geyer et al. 2001). Taken together, these findings suggest that PDE10A inhibitors may be most effective in PPI models that induce limited D1 activation, such as selective D2/D3 agonist models (Experiment 2, present study), or when a D1 antagonist is given in conjunction with the PDE10A inhibitor following D1/D2 agonist-induced behavioral disruptions (Experiment 3, present study), or NMDA antagonist models (Grauer et al. 2009; Bleickardt et al. 2010).

The role of the D1 receptor in PPI is dependent upon the neural circuit of D1 activation, stimulus parameters, and relative activation of D2 receptors during testing. D1 agonist treatment has not been consistently shown to alter PPI (Geyer et al. 2001), thus it does not appear *sufficient* in itself to modulate PPI. However, during concomitant D2 activation, D1 may act synergistically with D2 to disrupt PPI (Wan et al.

Table 2 Effect of TP-10 and SCH23390 on apomorphine-induced decreases in startle magnitude (Mean±SEM)

	Veh/Veh	TP-10/Veh	Veh/SCH	TP-10/SCH
Vehicle	129±20	90±14	187±20^{+}	162±29
Apo* (0.5 mg/kg)	140±22	83±10	96±14	85±15

*ps<0.02, Main effects of Apo and TP-10; SCH23390 increased startle in the veh, but not apo, groups (SCH ×x Apo: $p=0.003$; $^{+}p<0.05$, Veh/SCH23390 vs. TP-10/Veh, post-hoc analysis)

1996). The data presented here support this notion because although the selective D2 inhibitor haloperidol robustly reversed the effects of D1/D2 agonist apomorphine on PPI, TP-10 alone did not. Only in the presence of a D1 antagonist (SCH23390) did TP-10 reverse apomorphine -induced PPI disruptions. The neural substrate for TP-10-induced facilitation of D1 signaling effects on PPI is most likely the striatum, which shows the highest PDE10A expression and strongly modulates markers of direct and indirect pathway signaling in this circuit (Fujishige et al. 1999). Although very limited PDE10 expression is detected in the amygdala, D1 blockade in the amygdala has been shown to increase PPI (Stevenson and Gratton 2004), thus it is possible that TP-10-induced facilitation of D1 signaling in the amygdala could also account for its lack of effect on apomorphine-induced PPI disruptions. Conversely, D1 receptor blockade in prefrontal cortex reduces PPI, thus this is an unlikely neural substrate for D1 effects of TP-10 on PPI (Ellenbroek et al. 1996; Swerdlow et al. 2005; Swerdlow et al. 2006). It is also important to note that quinpirole, used in the present study, is a D2/D3 agonist, thus TP-10 efficacy in this model could reflect modulation of D3 effects on sensorimotor gating as well as D2. D3 receptor activation in the striatum also disrupts PPI (Chang et al. 2012), thus TP-10 effects in this experiment may have been due to attenuation of either D2 or D3 signaling effects.

What does activation of the direct pathway by TP-10 and other PDE10A inhibitors mean for their potential efficacy as antipsychotics? Clearly, the efficacy profile emerging from PDE10A inhibitor studies implies significant differences between agents of this new class and clinically efficacious antipsychotics, especially with regard to models considered predictive of efficacy vs. positive symptoms. Thus, results from clinical trials with PDE10A inhibitors would not only confirm efficacy, but would also be important for preclinical assay validation. Indeed, recent results from a Phase 2a proof-of-concept trial indicate that the efficacy of TP-10 in the treatment of acute exacerbation of schizophrenia was not significantly different from placebo (DeMartinis 2012). These preliminary data taken together with the present studies might suggest that failure of TP-10 to block apomorphine-induced disruption in PPI was predictive of lack of antipsychotic efficacy, at least in blocking positive symptoms. Regardless of the activity of PDE10A inhibitors vs. positive symptoms, preclinical data point to potential efficacy against both negative and cognitive symptoms (see below). Additionally, it is possible that PDE10A inhibitors would be useful as adjunct antipsychotics in treatment-resistant patients. Further investigation in these areas will help fully characterize the potential of this novel class of agents.

Dopamine receptor agonists are currently being examined to treat cognitive disruptions in schizophrenia (McClure et al. 2010) and an amelioration of working memory deficits by D1 receptor agonists, in particular, have been observed in nonhuman primates (Roberts et al. 2010). Compounds with mixed D1 agonist/D2 antagonist profiles have also been suggested as potential antipsychotics effective in treating a broader range of symptoms of schizophrenia (Natesan et al. 2008), including negative symptoms (e.g., anhedonia, asociality, blunted affect) and/or cognitive disruptions, which are not well treated by current antipsychotic medications (Young et al. 2012). Evidence suggests that PDE10A inhibition, via pharmacological or genetic manipulations, may in fact be effective in increasing sociality in animals (Sano et al. 2008; Grauer et al. 2009). Future studies could investigate whether this efficacy extends to other behavioral paradigms modeling additional negative symptoms (e.g., sucrose preference test, a putative indicator of anhedonia). Regarding the potential for PDE10A inhibitors to treat cognitive symptoms, evidence suggests that PDE10A inhibition increases ERK and CREB phosphorylation necessary for memory formation (Siuciak et al. 2006; Nishi et al. 2008). Accordingly, PDE10A inhibitor treatment improves novel object recognition in rats (Grauer et al. 2009; Smith et al. 2013) and social odor recognition in mice (Grauer et al. 2009), and reverses attention set shift deficits in rats treated chronically with PCP (Rodefer et al. 2005). While the neural substrate for the pro-cognitive effects of these PDE10A inhibitors is not apparent, as structures critically involved in cognition (e.g., cortex and hippocampus) have relatively low PDE10A expression (compared to striatum) (Seeger et al. 2003), it nonetheless is becoming increasingly clear that many cognitive functions mediated by the cortex are modulated by basal ganglia feedback via the corticostriatal pathways (Simpson et al. 2010).

In conclusion, using a sensorimotor gating model in rats, we demonstrated that PDE10A inhibition treatment has functional effects via blockade and facilitation of D2 and D1 signaling, respectively, which under some conditions may occlude the behavior effects of each other. Although this interaction has been well-described at anatomical and functional levels, it has not been readily apparent pharmacologically with agents affecting only one pathway (as in the case of D2 antagonist) or agents affecting the two pathways in a manner consistent with differential effects on D1 and D2 receptor signaling (such as apomorphine or amphetamine). In the case of PDE10A inhibition, however, both pathways are activated in parallel, and the combined effects may play a significant role in the behavioral response. Our findings in particular indicate that the relative dopaminergic tone at D1 and D2 receptors influences the extent to which PDE10A inhibitors activate the direct pathway to modulate sensorimotor gating. If these observations are broadly applicable and if excessive dopaminergic tone plays a role in the positive symptoms of schizophrenia as is now generally accepted, the balance of activity in the direct and indirect pathway in patients may play a significant role in determining the utility of PDE10A inhibitors as effective antipsychotic agents.

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Conflict of interest The authors have nothing to disclose.

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The experiments described in this manuscript comply with the current U.S. laws on laboratory animal care.