PDE10A inhibitors stimulate or suppress motor behavior dependent on the relative activation state of the direct and indirect striatal output pathways

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Abstract
The enzyme phosphodiesterase 10A (PDE10A) regulates the activity of striatal, medium spiny neurons (MSNs), which are divided into a behaviorally stimulating, Gs-coupled D₁ receptor-expressing “direct” pathway and a behaviorally suppressant, Gi-coupled D₂ receptor-expressing “indirect” pathway. Activating both pathways, PDE10A inhibitors (PDE10AIs) combine functional characteristics of D₂ antagonists and D₁ agonists. While the effects of PDE10AIs on spontaneous and stimulated behavior have been extensively reported, the present study investigates their effects on suppressed behavior under various conditions of reduced dopaminergic neurotransmission: blockade of D₁ receptors with SCH-23390, blockade of D₂ receptors with haloperidol, or depletion of dopamine with RO-4-1284 or reserpine. In rats, PDE10AIs displayed relatively low cataleptic activity per se. After blocking D₁ receptors, however, they induced pronounced catalepsy at low doses close to those required for inhibition of apomorphine-induced behavior; slightly higher doses resulted in behavioral stimulant effects, counteracting the catalepsy. PDE10AIs also counteracted catalepsy and related behaviors induced by D₂ receptor blockade or dopamine depletion; catalepsy was replaced by behavioral stimulant effects under the latter but not the former condition. Similar interactions were observed at the level of locomotion in mice. At doses close to those inhibiting p-amphetamine-induced hyperlocomotion, PDE10AIs reversed hypolocomotion induced by D₁ receptor blockade or dopamine depletion but not hypolocomotion induced by D₂ receptor blockade. It is concluded that PDE10AIs stimulate or inhibit motor behavior dependent on the relative activation state of the direct and indirect striatal output pathways.

Abbreviations
AE, adverse event; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; CI, confidence limits; EPS, extrapyramidal symptoms; i.v., intravenous; JNJ-42314415, 3-[6-(2-methoxyethyl)pyridin-3-yl]-2-methyl-8-morpholin-4-ylimidazo[1,2-a]pyrazine; MP-10, 2-[[4-(1-methyl-4-pyridin-4-yl-1H-pyrazol-3-yl]phenoxy]methyl]quinoline; MSNs, medium spiny neurons; PDE10AI, phosphodiesterase 10A inhibitor; PQ-10, 6,7-dimethoxy-4-[3-(quinoxalin-2-yl)oxy]pyrrolidin-1-yl]quinazoline; RO-4-1284, 2-ethyl-9,10-dimethoxy-3-(2-methylpropyl)-1,3,4,6,7,11b-hexahydro-2H-pyrido[2,1-a]isoquinolin-2-ol; PDE, phosphodiesterase; PKA, protein kinase A; SCH-23390, 8-chloro-3-methyl-5-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepin-7-ol; SKF-82958, 6-chloro-1-phenyl-3-prop-2-en-1-yl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,8-diol; TP-10, 2-[[4-(pyridin-4-yl-1-yl)-2,2,2-trifluoroethyl]-1H-pyrazol-3-yl]phenoxy][methyl]quinoline; s.c., subcutaneous.
Introduction

The enzyme phosphodiesterase 10A (PDE10A) modulates signal transduction by catabolizing the intracellular second messengers cAMP and cGMP. PDE10A has higher affinity for cAMP and is more efficient with cAMP as substrate (Fujishige et al. 1999; Soderling et al. 1999). PDE10A is expressed primarily in the brain, particularly in the striatum, nucleus accumbens, and olfactory tubercle, regulating the activity of dopamine-sensitive striatal medium spiny neurons (MSNs) (Loughney et al. 1999; Seeger et al. 2003; Coskran et al. 2006; Sano et al. 2008).

MSNs are organized into a dopamine D1 receptor-expressing “direct” (striatonigral) output pathway and a dopamine D2 receptor-expressing “indirect” (striatopallidal) output pathway (Graybiel 1990, 2000). Action selection and execution takes place via the direct pathway, whereas action suppression is mediated via the indirect pathway (Delong and Wichmann 2007; Wilson 2014). Both pathways integrate behaviorally relevant information received via cortical and thalamic glutamatergic projections with input regarding the salience of this information from mid-brain dopaminergic neurons (Surmeier et al. 2007). Processed information is ultimately fed back to the frontal cortex via both pathways (Delong and Wichmann 2007; Wilson 2014), which act like the brake and accelerator in a car (Graybiel 2000).

All currently available antipsychotics block D2 receptors and, by reducing dopamine-evoked cAMP decrease via Gi-coupled D2 receptors in the indirect pathway, they activate only the indirect pathway (Kapur and Mamo 2003; Agid et al. 2008). By increasing cAMP, PDE10A inhibitors (PDE10Is) activate both the Gi-coupled D2 receptor-expressing indirect pathway and the Gs-coupled D1 receptor-expressing direct pathway (Nishi et al. 2008; Strick et al. 2010), thereby combining functional characteristics of D2 antagonists and D1 agonists. Accordingly, PDE10Is have been proposed as a novel type of antipsychotics with a broader therapeutic profile and reduced side effects (Siuciak et al. 2006; Kehler et al. 2007; Menniti et al. 2007; Siuciak 2008). However, PDE10Is may have effects beyond D1- and D2-mediated neurotransmission, also affecting striatal glutamate–NO–guanylyl cyclase signaling (Loughney et al. 1999; Seeger et al. 2003; Coskran et al. 2006; Sano et al. 2008; Grauer et al. 2009).

In order to explore their antipsychotic potential and side-effect liability, PDE10Is have been studied predominantly for effects on stimulated or spontaneous behaviors. In line with results suggesting that PDE10Is activate indirect pathway neurons to a greater extent than direct pathway neurons (Threlfell et al. 2009; Nishi et al. 2011), PDE10Is produce similar effects as D2 receptor blockers, such as inhibition of spontaneous or stimulant-induced behavior, inhibition of conditioned avoidance behavior and reversal of stimulant-induced sensory gating deficits (Menniti et al. 2007; Schmidt et al. 2008; Grauer et al. 2009; Kehler and Nielsen 2011; Gresack et al. 2013; Megens et al. 2014). Concomitant direct pathway activation explains the relatively low cataleptogenic activity (Schmidt et al. 2008; Grauer et al. 2009; Megens et al. 2014), reduced efficiency against behavioral stimulants in case of concomitant D1 receptor stimulation (Menniti et al. 2007; Sotty et al. 2009; Gresack et al. 2013; Megens et al. 2014), preferential activity against apomorphine-induced climbing versus apomorphine-induced stereotypy (Grauer et al. 2009), cognition-enhancing effects (Rodefer et al. 2005; Grauer et al. 2009), and socializing effects (Grauer et al. 2009).

The present study investigates effects of PDE10Is on suppressed behaviors (catalepsy in rats; hypolocomotion in mice) under various conditions of decreased dopaminergic neurotransmission, viz. blockade of D1 receptors with SCH-23390 (Bourne 2001), blockade of D2 receptors with haloperidol, or dopamine depletion with RO-4-1284 (Colzi et al. 1999; Filinger 1994) or reserpine. Inhibition of apomorphine-induced behavior in rats and antagonism of D-amphetamine-induced hyperlocomotion in mice are included as measures of reduced D2 receptor signaling. The following PDE10Is are tested: JNJ-42314415 (Megens et al. 2014), PQ-10 (Kehler and Kilburn 2009), TP-10 (Schmidt et al. 2008; Kehler and Kilburn 2009), and MP-10 (Grauer et al. 2009; Kehler and Kilburn 2009). TP-10 is not included in all assays because of limited availability. In some assays, the D1 agonist SKF-82958 (Wang and McGinty 1997) and the D2 antagonist haloperidol are also included for comparison. Part of the data have been presented elsewhere (Vanhoof et al. 2012; Megens et al. 2014). The present results indicate that PDE10Is stimulate or suppress motor behavior dependent on the relative activation state of the direct and indirect striatal output pathways.

Materials and Methods

Materials and sources

Company codes refer to the following chemical structures: JNJ-42314415: 3-[6-(2-methoxyethyl)pyridin-3-yl]-2-methylyl-8-morpholin-4-ylimidazo[1,2-a]pyrazine; MP-10: 2-[[4-(1-methyl-4-pyridin-4-yl-1H-pyrazol-3-yl)phenoxy]methyl] quinoline; PQ-10: 6,7-dimethoxy-4-[3-(quinoxalin-2-yl)oxy]pyrrolidin-1-yl]quinazoline); RO-4-1284: 2-ethyl-9,10-dimethoxy-3-(2-methylpropyl)-1,3,4,6,7,11b-hexahydro-2H-pyrido[2,1-a]isoquinolin-2-ol; SCH-23390: 8-chloro-3-methyl-5-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepin-7-ol; SKF-82958: 6-chloro-1-phenyl-3-prop-2-en-1-yl-2,3,4,5-tetrahydro-
1H-3-benzazepine-7,8-diol; TP-10: 2-([4-[4-pyridin-4-yl-1-(2,2,2-trifluoroethyl)-1H-pyrazol-3-yl][phenoxy]methyl] quinoline. All compounds were synthesized in our own laboratories, except apomorphine, d-amphetamine (Certa SA, Eigenbrakel, Belgium), reserpine, and SKF-82958 (Sigma-Aldrich-Fluka, Diegem, Belgium). Reserpine was dissolved in 10% hydroxypropyl-β-cyclodextrin containing ascorbic acid (4:1 ratio relative to reserpine; w:w). The other compounds were dissolved in water (apomorphine, d-amphetamine), acidified with tartaric acid if required (haloperidol, INJ-42314415, SCH-23390), or in 10% or 20% hydroxypropyl-β-cyclodextrin acidified with tartaric acid (MP-10, PQ-10, SKF-82958, TP-10). The test formulations were stored at room temperature in closed containers protected from light and dark cycle set at 12 h). According to standard procedures, all animals were fasted overnight (tap water remaining available ad libitum), allowing oral dosing on an empty stomach (not applicable to the present study). The Institutional Ethics Committee on Animal Experimentation approved the experimental protocols, in compliance with Belgian law (Royal Decree on the protection of laboratory animals 6 April 2010).

Test descriptions

Apomorphine-induced agitation in rats

Apomorphine (1.0 mg/kg, i.v., intravenous)-induced agitation was scored (0, 1, 2, or 3) every 5 min over the first hour after injection of apomorphine. Criteria for drug-induced inhibition of agitation were: less than six times score 3 (1.5% false positives), less than six times score ≥2 (0.0% false positives), or less than seven times score ≥1 (0.0% false positives).

Observation test in rats

Catalepsy was scored (0, 1, 2 or 3) by two independent observers at 1, 2, 3, and 4 h after administration of test compound or solvent. The catalepsy scores from the two observers were summed for further evaluation. Criteria for drug-induced catalepsy: score ≥2 for slight catalepsy (occurrence in 1800 control observations: 0.0%; 450 rats and four observations per rat) and score 6 for pronounced catalepsy (not observed in controls). Other abnormalities were considered drug-induced, if occurring in a dose-dependent way. The interactions with SCH-23390 (0.63 mg/kg, s.c.), haloperidol (0.63 mg/kg, s.c.), and RO-4-1284 (10 mg/kg, s.c.) were evaluated by coadministering these agents simultaneously with the test formulations.

Reserpine-induced behavior in rats

Reserpine (10 mg/kg, s.c.) induces miosis, palpebral ptosis, and sedation and blocks the tail-pinch response (the biting and grasping response when an artery clamp is put on the rat’s tail, 1 cm from the distal end). The pupil diameter (of the right eye; using a microscopic micrometer; expressed in 1/24 mm units) and the latency of the tail-pinch response (s; cutoff time: 120 sec) were measured and palpebral opening (score 0, 1, 2, 3, 4, or 5) and sedation (hypomotility; score 0, 1, 2, 3) were scored 1 h after the reserpine challenge. To assess intrinsic effects of the test compounds, the pupil diameter and the tail-pinch response were also measured just before the reserpine injection. Test compound or solvent was administered 1 h before the reserpine challenge. Based on frequency distributions obtained in a historical control population of solvent-pretreated rats (n=400), the following all-or-none criteria were selected to assess drug-induced effects: (1) before reserpine injection – induction of miosis: pupil diameter <11 units (4.3% false positive controls; n >400); induction of mydriasis: pupil diameter >25 units (5.2% false positives); inhibition and blockade of tail-pinch response: latency >45 sec and >120 sec, respectively (5.2% and 0.2% false positives, respectively); (2) after reserpine injection – reversal of the tail-pinch response blockade: latency <120 sec (4.1% false positives); reversal of miosis: pupil diameter >8 units (1.1% false positives); reversal of palpebral ptosis: score for palpebral opening >1 (3.2% false positives); and reversal of sedation: score <3 (1.1% false positives).

Locomotor activity in mice

The procedure room was only sparsely lit (3–30 lx) to provide better contrast for the video tracking. Each locomotor activity arena (gray PVC cylinder; height: 40 cm; diameter: 22.5 cm) was placed on an infrared LED (8 × 8 LEDs) lit box (white PVC squared box; 40 × 40 cm²; height 12.5 cm). An infrared-sensitive tube camera and a white light source (in arena: 4–7 lx) were mounted to the ceiling above the arena to track the animal. Distance travelled was
recorded and analyzed using the Noldus Ethovision XT Video Tracking System (Version 3.1; Noldus, Wageningen, the Netherlands). Locomotion was measured over a 30-min period by introducing the mouse into the motor activity arena 30 min after pretreatment with test compound or solvent. The arena was cleaned after each trial. In order to stimulate or suppress locomotion, following treatments were used: d-amphetamine (5.0 mg/kg, s.c.; −0.5 h), SCH-23390 (0.08 mg/kg, i.v.; 0 h), RO-4-1284 (0.63 mg/kg, s.c.; −0.5 h), or haloperidol (0.31 mg/kg, i.v.; 0 h).

**General procedure and statistics**

**General procedure**

All experiments were performed by unbiased trained technicians using coded solutions. Doses were selected from the geometrical series 0.01–0.02–0.04... 40.0–80.0–160 mg/kg. Each dose group consisted of at least five animals which were tested in separate daily experimental sessions in order to account for day-to-day variability and to minimize systematic errors. Control injections of solvent were included in each experimental session. In Figures 4–6, dose 0 mg/kg refers to results obtained in solvent-treated control animals that were tested concurrently with the test compounds.

**ED50 determination**

All-or-none criteria for drug-induced effects were defined by analyzing a frequency distribution of a series of historical control data, aiming for less than 5% responders in the control population. The fraction of animals responding to these criteria in animals pretreated with test compound was determined per dose level (n ≥ 5 in the relevant dose range; at least three doses). ED50s (the dose inducing the defined effect in 50% of the tested animals) and corresponding 95% confidence limits were determined according to the modified Spearman-Karber estimate, using theoretical probabilities instead of empirical ones (Tsutakawa 1982). This modification allows estimation of the ED50 and its confidence interval as a function of the slope of the log dose–response interval as a function of the slope of the log dose–response curve (Lewi et al. 1977).

**Time–activity relationship**

ED50s were plotted versus time after dosing using GraphPad Prism® (software version 5.00 for Windows, GraphPad Software, San Diego, CA; www.graphpad.com). The ED50 at time of peak effect and the onset and duration of action (at four times the peak-effect dose) were estimated from the curve of best fit through these data points as generated by nonlinear regression of the polynomial second order function y = a + bx + cx2. Goodness of fit was determined by calculating R² and S_y-x.

**Results**

**Apomorphine-induced agitation in rats**

Inhibition of apomorphine-induced behavior relates to the D2 receptor blocking activity of antipsychotics (Leyesen and Niemegeers 1985; Leysen 2000). Apomorphine antagonism was used in the present study as sensitive index for the functional D2 antagonism of PDE10AIs in rats, being measured at doses close to the ED50 for striatal PDE10A occupancy (Megens et al. 2014).

The ED50s of JNJ-42314415, PQ-10, TP-10, and MP-10 obtained for inhibition of apomorphine-induced behavior at various time intervals after s.c. injection are listed in Table 1 and graphically presented in Figure 1 (solid circles; the other data are discussed below). The PDE10AIs showed a fast onset of action (<0.5 h) and reached their peak effect within 0.5–1 h (nonlinear regression estimates in Table 2). JNJ-42314415, MP-10, and TP-10 were about equipotent and about three to four times more potent than PQ-10 in terms of peak-effect dose. MP-10 showed a somewhat shorter duration (2.8 h) than the other compounds (4.3–4.8 h).

**Table 1.** ED50s (and 95% confidence limits; mg/kg) of the test compounds for inhibition of apomorphine-induced agitation in rats as a function of time after s.c. injection.

| Time (h) | JNJ-42314415 (95% confidence limits; mg/kg, s.c.) | PQ-10 | TP-10 | MP-10 |
|---------|-----------------------------------------------|-------|-------|-------|
| 0.5     | 1.02 (0.75–1.38)                              | 3.6 (2.88–4.4) | 1.17 (0.86–1.58) | 1.17 (0.86–1.58) |
| 1       | 1.02 (0.75–1.38)                              | 4.1 (3.0–5.5) | 1.02 (0.75–1.38) | 0.67 (0.45–1.01) |
| 2       | 1.77 (1.44–2.18)                              | 6.2 (4.1–9.2) | 1.77 (1.18–2.65) | 2.69 (1.99–3.6) |
| 4       | 4.1 (3.0–5.5)                                 | 7.1 (4.7–10.6) | 4.1 (3.0–5.5) | 8.1 (6.0–11.0) |
| 8       | 8.2 (5.5–12.2)                                | 43 (28.7–64) | 12.3 (9.1–16.7) | |

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**Observation tests in rats**

**Interaction with the D₁ antagonist SCH-23390**

In order to study involvement of dopamine D₁ receptors, catalepsy was studied after JNJ-42314415, PQ-10, and MP-10 in rats cotreated or not cotreated with the D₁ antagonist SCH-23390 (0.63 mg/kg, s.c., 0 h). In rats not cotreated with SCH-23390 (Fig. 2: upper panel for each compound; open circles), the PDE10AIs failed to induce catalepsy in a dose-dependent and consistent manner. MP-10 tended to induce slight catalepsy (score >2) around 1.25–2.5 mg/kg (maximum 60–80% responders) but only incidentally induced pronounced catalepsy (score 5–7).

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**Figure 1.** ED₅₀ (95% confidence limits; mg/kg) of PDE10AIs for various effects in rats cotreated with the D₁ antagonist SCH-23390 (0.63 mg/kg, s.c., 0 h) in comparison with the ED₅₀ for antagonism of apomorphine-induced behavior in rats not cotreated with SCH-23390. The ED₅₀ values have been plotted as a function of time after s.c. injection of the compounds. In the rats cotreated with the D₁ antagonist, PDE10AIs induced (slight and pronounced) catalepsy at doses similar to those required for apomorphine antagonism. At 10-fold higher doses, catalepsy disappeared, being replaced by agitation. The various effects followed a parallel time course. For TP-10 only ED₅₀ for apomorphine antagonism are shown. PDE10AI, phosphodiesterase 10A inhibitor.
6). In rats cotreated with SCH-23390 (Fig. 2: bottom panel for each compound; closed circles), however, the PDE10AIs consistently induced pronounced catalepsy (score 6). The dose–response curves were bell-shaped: catalepsy decreased again at higher dose levels, being replaced by stereotyped sniffing and rearing. The ED₅₀s for the various effects in rats cotreated with SCH-23390 (Table 3) have been plotted as a function of time in Figure 1 in comparison with the ED₅₀s for apomorphine antagonism in rats not cotreated with SCH-23390. The time course for the effects in the rats cotreated with SCH-23390 was found to proceed more or less in parallel with the apomorphine antagonism in the absence of SCH-23390. In the rats cotreated with SCH-23390, the PDE10AIs already induced catalepsy at low doses comparable to those for apomorphine antagonism (Table 4), reflecting a very small safety margin under these conditions. SCH-23390 (0.63 mg/kg, s.c.) alone did not induce catalepsy (score >2 was observed only once in 35 rats tested at four time intervals).

As the functional D₁ agonism of PDE10AIs apparently counteracts the catalepsy that may be expected from their functional D₂ antagonism, it is studied below whether this activity component may also counteract catalepsy and related behaviors induced by other mechanisms, viz. D₂ receptor blockade with haloperidol or dopamine deple- tion using Ro-4-1284 or reserpine.

**Interaction with the D₂ antagonist haloperidol**

Figure 3 illustrates the dose-dependent reversal of haloperidol (0.63 mg/kg, s.c.)-induced catalepsy after cotreatment with JNJ-42314415, TP-10, MP-10, and the D₁ agonist SKF-82958. Shown are individual data and median scores per dose group obtained 2, 3, and 4 h after dosing. In solvent-cotreated rats (n = 321), haloperidol consistently induced catalepsy, which became more pronounced at later time intervals. Catalepsy scores <2 at 2 h and <5 at 3 h and 4 h occurred in only 6.2%, 4.7%, and 3.1% of these control rats, respectively, and were adopted as critical levels for reversal of haloperidol-induced catalepsy (dotted horizontal lines in Fig. 3). The three PDE10AIs dose dependently reversed the haloperidol-induced catalepsy (Fig. 3, Table 5). The lowest ED₅₀ obtained over the three time intervals was 4.7 mg/kg for JNJ-42314415, 2.69 mg/kg for TP-10, and 6.2 mg/kg for MP-10, corresponding to 4.9, 2.5, and 7.0 times, respectively, the ED₅₀ for apomorphine antagonism at time of peak effect (Table 2). SKF-82958 reversed haloperidol-induced catalepsy at 1 h with a bell-shaped dose–response curve (estimated ED₅₀: 1.02 mg/kg) but was hardly effective at the later time intervals.

**Interaction with the monoamines-depleting agent RO-4-1284**

RO-4-1284 (10 mg/kg, s.c.) consistently induced catalepsy and sedation in solvent-cotreated rats (n = 50). JNJ-42314415, PQ-10, and MP-10 dose dependently reversed the RO-4-1284-induced sedation and catalepsy, which were replaced by behavioral excitation (Table 6–8; Fig. 4). The effects occurred as doses close to the ED₅₀ for apomorphine antagonism (Table 9). The D₁ agonist SKF-82958 tended to reverse the RO-4-1284-induced catalepsy at 2.5 mg/kg without affecting sedation. At this dose, however, the combination with RO-4-1284 resulted in convulsions, spasms and body switches in two of the five rats tested (one of them died and was not included in Table 10).

**Interaction with the monoamines-depleting agent reserpine**

Reserpine (10 mg/kg, s.c.) induced palpebral ptosis, sedation, and blocked the tail-pinch response (the grasping and biting response when an artery clamp is put on the distal end of the rat’s tail; latency >120 sec). Before the reserpine challenge, the PDE10AIs inhibited the tail-pinch

| Compound     | Peak effect | Onset¹ (h) | Duration¹ (h) | R²   | Sy.x |
|--------------|-------------|------------|---------------|------|------|
| JNJ-42314415 | 0.5         | 0.95       | <0.5          | 4.3  | 0.985| 0.068|
| PQ-10        | 0.83        | 3.5        | <0.5          | 4.8  | 0.930| 0.163|
| TP-10        | 0.68        | 1.07       | <0.5          | 4.3  | 0.995| 0.044|
| MP-10        | 0.77        | 0.88       | <0.5          | 2.8  | 0.929| 0.217|

The values were interpolated from the nonlinear regression lines in Figure 1. Values for R² and Sy.x show the goodness of fit of the nonlinear regression lines.

¹Onset and duration of action were assessed at four times the peak-effect dose.
response per se (latency >45 sec) at doses only 1.2–3.2 times the ED$_{50}$ for apomorphine antagonism (Fig. 5, Table 11). The dose–response relations for the inhibition of the tail-pinch response tended to be bell-shaped, except for PQ-10 (tested in a small dose range only). While inhibiting the tail-pinch response before reserpine, the PDE10AIs in contrast dose dependently reversed the reserpine-induced blockade of the tail-pinch response 1 h after reserpine. This effect occurred at 3.3–8.5 times the ED$_{50}$ for apomorphine antagonism. The PDE10AIs even restored normal response latencies (<45 sec) at slightly higher doses (5.0–11 times the ED$_{50}$ for apomorphine antagonism). Reversal of the reserpine-induced tail-pinch response blockade was thus observed at similar doses as those inhibiting the tail-pinch response before reserpine (Table 11). The PDE10AIs also reversed the reserpine-induced sedation (at 0.54–3.2 times the ED$_{50}$ for apomorphine antagonism; Fig. 6), which was replaced by behavioral stimulation (sniffing and rearing) at 1.9–4.9 times the ED$_{50}$ for apomorphine antagonism. The PDE10AIs also tended to reverse the reserpine-induced palpebral ptosis (at 0.96–3.2 times the ED$_{50}$ for apomorphine antagonism) but did not affect pupil diameter up to the highest doses tested (data not shown).

Before the reserpine challenge, the D$_1$ agonist SKF-82958 inhibited the tail-pinch response in some rats (from 0.16 mg/kg onward; maximum 40% responders), presumably related to behavioral stimulant effects (sniffing, rearing; ED$_{50}$: 0.44 [0.36–0.55] mg/kg). After the reserpine challenge, SKF-82958 reversed the reserpine-induced sedation (ED$_{50}$: 0.097 mg/kg; Fig. 6) and the blockade of the tail-pinch response (ED$_{50}$: 0.112 mg/kg; Fig. 5, Table 11), and tended to normalize the tail-pinch response (estimated ED$_{50}$: 0.51 mg/kg) and to reverse the ptosis (from 0.31 mg/kg onward; data not shown) without affecting miosis (ED$_{50}$: >10 mg/kg; data not shown). The overt behavioral stimulation seen with SKF-82958 before the reserpine challenge was not seen after the reserpine challenge (Table 11). In contrast, the PDE10AIs showed behavioral stimulant effects after but not preceding the reserpine challenge.

Locomotor activity assays in mice

In addition to the above studies at the level of catalepsy in rats, similar studies were performed at the level of locomotion in mice. First, inhibition of D-amphetamine-induced hyperlocomotion was tested as index of functional D$_2$ antagonism. Subsequently, the ability of PDE10AIs to reverse the hypolocomotion induced by the D$_1$ antagonist SCH-23390, the monoamines-depleting agent, Ro-4-1284, or the D$_2$ antagonist haloperidol was evaluated. Total distance travelled in solvent-pretreated control mice receiving no challenge was 4167 ± 1135 cm (mean ± SD; $n = 1444$).

Inhibition of hyperlocomotion induced by the dopamine-releasing agent D-amphetamine

In solvent-pretreated control mice, D-amphetamine (5.0 mg/kg, i.c.; $−0.5$ h) increased distance travelled to 12,764 ± 4278 cm (mean ± SD; $n = 462$). Only 3.0% of these control mice travelled <5500 cm. The PDE10AIs and the D$_2$ receptor blocker haloperidol reduced the D-amphetamine-induced hyperlocomotion in a dose-dependent manner to normal values (<5500 cm (Fig. 7, first column; ED$_{50}$s in Table 12, first column).

Reversal of hypolocomotion induced by the D$_1$ antagonist SCH-23390

In solvent-pretreated control mice, SCH-23390 (0.08 mg/kg, i.v., 0 h) decreased distance travelled to 1408 ± 513 cm (mean ± SD; $n = 279$). Only 1.4% of these SCH-23390-challenged control mice travelled a distance >2500 cm. The PDE10AIs and the D$_1$ agonist SKF-82958 reversed the SCH-23390-induced hypolocomotion to normal values (>2500 cm; Fig. 7; second column). The ED$_{50}$s of the PDE10AIs for this effect were similar to those required for inhibition of D-amphetamine-induced hyperlocomotion (Table 12).

Reversal of hypolocomotion induced by the monoamines-depleting agent RO-4-1284

In solvent-pretreated control mice, dopamine depletion with RO-4-1284 (0.63 mg/kg, s.c., $−0.5$ h) induced pronounced hypolocomotion (total distance [mean ± SD]: 772 ± 411 cm; $n = 218$). Values >2500 cm were rarely measured (in only 0.4% of the control mice). The tested PDE10AIs and the D$_1$ agonist SKF-82958 reversed the RO-4-1284-induced hypolocomotion to normal values (>2500 cm; Fig. 7; third column). The dose–response curves of JNJ-42311445 and PQ-10 tended to be bell-shaped and effect size tended to be higher for MP-10 than for JNJ-42311445 and PQ-10. The PDE10AIs were somewhat (two to three times) more potent against Ro-4-1284 than against D-amphetamine or SCH-23390 while SKF-82958 was equipotent in both assays (Table 12).

Reversal of hypolocomotion induced by the D$_2$ antagonist haloperidol

In solvent-pretreated mice, the D$_2$ antagonist haloperidol (0.31 mg/kg, i.v., 0 h) induced hypolocomotion (total distance [mean ± SD]: 1257 ± 1370 cm; $n = 1833$).
Functional D<sub>1</sub> Agonism of PDE10A Inhibitors

A. A. H. P. Megens et al.

JNJ-42314415

PQ-10

MP-10
Table 3. ED_{50} (and 95% confidence limits) for induction of slight and pronounced catalepsy, the subsequent disappearance of catalepsy, and the induction of agitation in rats cotreated with SCH-23390 (0.63 mg/kg, s.c.).

| Time (h) | JNJ-42314415 | PQ-10 | MP-10 |
|---------|--------------|-------|-------|
|         | Induction of catalepsy |         |       |
|         | Slight (score ≥2) | Pronounced (score = 6) | Disappearance of slight catalepsy (score ≤2) | Induction of agitation |
| 1       | 1.54 (1.14–2.09) | 1.54 (1.14–2.09) | 10.8 (8.0–14.6) | 10.8 (8.0–14.6) |
| 2       | 1.54 (1.14–2.09) | 1.77 (1.44–2.18) | 10.8 (8.0–14.6) | 12.4 (9.1–16.7) |
| 3       | 2.03 (1.50–2.75) | 2.04 (1.51–2.76) | 16.3 (12.0–22.1) | 21.5 (13.3–35) |
| 4       | 3.5 (2.87–4.4) | 3.6 (2.88–4.4) | 16.3 (12.0–22.1) | >40 |
|         |                |                |                |       |
| 1       | 3.6 (2.37–5.3) | 4.7 (3.4–6.3) | 32 (21.7–49) | >40 |
| 2       | 5.4 (4.0–7.3) | 5.4 (4.0–7.3) | 43 (–) | >40 |
| 3       | 8.2 (6.0–11) | 10.8 (8.0–14.6) | 43 (–) | >40 |
| 4       | 18.7 (13.8–25.3) | 21.5 (15.9–29.1) | >40 | >40 |

Table 4. ED_{50} of the PDE10AIs at time of peak-effect for induction of slight and pronounced catalepsy, the subsequent disappearance of catalepsy, and the induction of agitation in rats cotreated with SCH-23390 (0.63 mg/kg, s.c.) in relation to the ED_{50} for inhibition of apomorphine-induced agitation.

| Effects | JNJ-42314415 | PQ-10 | MP-10 |
|---------|--------------|-------|-------|
| Apomorphine antagonism | 0.95 [1.0] | 3.5 [1.0] | 0.88 [1.0] |
| Induction of slight catalepsy (score ≥2) | 1.35 [1.4] | 3.6 [1.0] | 0.30 [0.34] |
| Induction of pronounced catalepsy (score 6) | 1.46 [1.5] | 4.3 [1.2] | 0.43 [0.49] |
| Disappearance of slight catalepsy (score ≤2) | 10.6 [11] | 32 [9.1] | 4.0 [4.5] |
| Induction of agitation | 9.2 [9.6] | >40 [11] | 5.2 [5.9] |

PDE10AI, phosphodiesterase 10A inhibitor.

Discussion

PDE10AIs resemble D_{2} receptor blockers by, for example, inhibiting spontaneous and stimulant-induced behavior, inhibiting conditioned behavior, and reversing stimulant-induced sensory gating deficits (see Introduction). These effects are mediated by indirect pathway activation, partially counteracted by direct pathway activation when D_{1} receptors are concomitantly stimulated (Menniti et al. 2007; Gresack et al. 2013; Megens et al. 2014). By stimulating dopamine D_{2} receptors, nonelective dopamine...
agonists such as apomorphine or endogenous dopamine (e.g., after release by D-amphetamine) deactivate the indirect pathway, thereby reducing behavioral inhibition. By concomitantly stimulating D1 receptors, such compounds also activate the direct pathway, favoring behavioral stimulation. PDE10AIs inhibit the effect of dopamine agonists on the indirect pathway but facilitate their effect on the direct pathway. Acting together, both counteracting effects provide an explanation for the relatively reduced efficacy of PDE10AIs against nonselective dopamine agonists such as apomorphine and dopamine-releasing agents such as amphetamine as compared with their pronounced effects against nondopaminergic stimulants such as NMDA antagonists and antimuscarinics or D2-selective dopamine agonists such as quinpirole (Menniti et al. 2007; Sotty et al. 2009; Gresack et al. 2013; Megens et al. 2014). Both counteracting activity components may also explain the failure of MP-10 against acute exacerbations of schizophrenia (DeMartinis et al. 2012). The present study investigated the effects of PDE10AIs on suppressed behavior induced by reducing dopaminergic neurotransmission rather than on stimulated or spontaneous behavior. The results show that PDE10AIs can reverse suppressed behavior and even stimulate behavior depending on the relative activation state of the direct and indirect pathways.

Under basal conditions, PDE10AIs have relatively low cataleptogenic activity, due to concomitant direct pathway...
activation (Kehler et al. 2007; Schmidt et al. 2008; Megens et al. 2014). After D1 receptor blockade with SCH-23390, however, PDE10AIs induced pronounced catalepsy at low doses comparable to those required for inhibition of apomorphine-induced behavior. Similar levels of catalepsy occur with D2 receptor blockers only at >10-fold higher dose increments (Langlois et al. 2012). The potentiation of PDE10AI-induced catalepsy by a D1 antagonist confirms that PDE10AIs suppress central D2 receptor-mediated neurotransmission very efficiently.

Table 5. Dose–response relations and corresponding ED50s for reversal of haloperidol (0.63 mg/kg, s.c.)-induced catalepsy in rats that received test compound simultaneously with haloperidol.

| Effect Time (h) | Criterion | Dose (mg/kg, s.c.) | 0.31 | 0.63 | 1.25 | 2.5 | 5.0 | 10 | ED50 (95% CL; mg/kg) |
|----------------|-----------|-------------------|------|------|------|-----|-----|-----|---------------------|
| JNJ-42314415   | Score <2  | 2                 | 2    | 2    | 4    | 3   | 3   | 2   | 1.02 (0.69–1.54)    |
| 3              | Score <5  | 1                 | 0    | 1    | 4    | 2   | 4   | 2   | 1.36 (0.79–2.33)    |
| 4              | Score <5  | 1                 | 0    | 0    | 3    | 4   | 4   | 2   | 1.79 (1.04–3.1)     |

Table 6. Dose–response relations and corresponding ED50s for various effects observed over a 4-h period after challenge with RO-4-1284 (10 mg/kg, s.c.) in rats that received test compound simultaneously with RO-4-1284.

| Effect Time (h) | Criterion | Dose of JNJ-42314415 (mg/kg, s.c.) | 0.63 | 1.25 | 2.5 | 5.0 | 10 | 20 | 40 | ED50 (95% CL; mg/kg) |
|----------------|-----------|-----------------------------------|------|------|-----|-----|-----|----|----|---------------------|
| Reversal of sedation/passivity | Absence (6.0%) \(^1\) | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0.59 (0.44–0.80) \(^2\) |
| 2              | Absence (4.0%) \(^1\) | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1.03 (0.69–1.54)    |
| 3              | Absence (2.0%) \(^1\) | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1.36 (0.79–2.33)    |
| 4              | Absence (16%) \(^1\) | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1.79 (1.04–3.1)     |
| Reversal of catalepsy | Score <2 (0.0%) \(^1\) | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 2.05 (1.52–2.78)    |
| 3              | Score <5 (2.0%) \(^1\) | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1.03 (0.69–1.54)    |
| 4              | Score <5 (8.0%) \(^1\) | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1.56 (1.04–2.33)    |
| Induction of excitation | Presence (0.0%) \(^1\) | 1 | 0 | 2 | 2 | 4 | 4 | 4 | 2 | 4.1 (2.38–7.0)      |
| 2              | Presence (0.0%) \(^1\) | 1 | 0 | 2 | 2 | 4 | 4 | 4 | 3 | 3.6 (2.20–5.7)      |
| 3              | Presence (0.0%) \(^1\) | 1 | 0 | 2 | 2 | 4 | 4 | 4 | 2 | 4.1 (2.53–6.6)      |
| 4              | Presence (0.0%) \(^1\) | 1 | 0 | 2 | 2 | 4 | 4 | 4 | 2 | 6.2 (3.6–10.6)      |

The number of rats responding to the listed criterion is listed per dose level (n = 5 per dose group). Values representing more than 50% responders are formatted bold.

\(^1\)Percentage of false positives (solvent-pretreated rats responding to the criterion [n = 50]).
\(^2\)ED50 estimated assuming no responders at the nontested dose of 0.31 mg/kg.
when concomitant direct pathway activation is prevented by D1 receptor blockade and implies a risk of increased EPS when combining a PDE10AI with a neuroleptic with associated D1 antagonism (e.g., clozapine or olanzapine; Schotte and Leysen 1998).

The time course for catalepsy in animals cotreated with SCH-23390 was found to proceed more or less in parallel with inhibition of apomorphine-induced behavior in the absence of SCH-23390. This confirms previously reported data on the time course of (mild) catalepsy after

Table 7. Dose–response relations and corresponding ED50s for various effects observed over a 4-h period after challenge with RO-4-1284 (10 mg/kg, s.c.) in rats that received test compound simultaneously with RO-4-1284.

| Effect                                | Criterion     | Dose of PQ-10 (mg/kg, s.c.) | 0.63 | 1.25 | 2.5 | 5.0 | 10 | 20 | 40 | ED50 (95% CL; mg/kg) |
|---------------------------------------|---------------|----------------------------|------|------|-----|-----|----|----|----|---------------------|
| Reversal of sedation/passivity        | Absence (6.0%)<sup>1</sup> | 1                           | 0    | 0    | 5   | 4   | 5  |    |    | 3.6 (2.89–4.3)      |
|                                       | Absence (4.0%)<sup>1</sup> | 2                           | 0    | 2    | 3   | 4   | 4  |    |    | 4.7 (2.38–7.0)      |
|                                       | Absence (2.0%)<sup>1</sup> | 3                           | 1    | 2    | 3   | 2   | 2  |    |    | 7.1 (–)             |
|                                       | Absence (16%)<sup>1</sup> | 4                           | 0    | 2    | 0   | 1   | 3  |    |    | 16.2 (–)            |
| Reversal of catalepsy                 | Score <2 (0.0%)<sup>1</sup> | 2                           | 0    | 0    | 0   | 1   | 3  | 5  |    | 8.2 (5.4–12.2)      |
|                                       | Score <5 (2.0%)<sup>1</sup> | 3                           | 0    | 3    | 2   | 3   | 5  |    |    | 4.7 (2.91–7.6)      |
|                                       | Score <5 (8.0%)<sup>1</sup> | 4                           | 0    | 1    | 0   | 1   | 3  | 4  |    | 10.7 (7.2–16.1)     |
| Induction of excitation               | Presence (0.0%)<sup>1</sup> | 1                           | 0    | 0    | 0   | 0   | 0  |    | 3  | 19 (–)              |
|                                       | Presence (0.0%)<sup>1</sup> | 2                           | 0    | 0    | 0   | 0   | 0  |    | 3  | 19 (–)              |
|                                       | Presence (0.0%)<sup>1</sup> | 3                           | 0    | 0    | 0   | 0   | 0  |    | 3  | 19 (–)              |
|                                       | Presence (0.0%)<sup>1</sup> | 4                           | 0    | 0    | 0   | 0   | 0  |    | >20 (–)             |

The number of rats responding to the listed criterion is listed per dose level (n = 5 per dose group). Values representing more than 50% responders are formatted bold.

<sup>1</sup>Percentage of false positives (solvent-pretreated rats responding to the criterion [n = 50]).

Table 8. Dose–response relations and corresponding ED50s for various effects observed over a 4-h period after challenge with RO-4-1284 (10 mg/kg, s.c.) in rats that received test compound simultaneously with RO-4-1284.

| Effect                                | Criterion     | Dose of MP-10 (mg/kg, s.c.) | 0.63 | 1.25 | 2.5 | 5.0 | 10 | 20 | 40 | ED50 (95% CL; mg/kg) |
|---------------------------------------|---------------|----------------------------|------|------|-----|-----|----|----|----|---------------------|
| Reversal of sedation/passivity        | Absence (6.0%)<sup>1</sup> | 1                           | 2    | 5    | 5   | 5   |    |    |    | 0.68 (0.50–0.91)    |
|                                       | Absence (4.0%)<sup>1</sup> | 2                           | 2    | 5    | 5   | 5   |    |    |    | 0.68 (0.50–0.91)    |
|                                       | Absence (2.0%)<sup>1</sup> | 3                           | 2    | 5    | 5   | 5   |    |    |    | 0.68 (0.50–0.91)    |
|                                       | Absence (16%)<sup>1</sup> | 4                           | 1    | 2    | 5   | 5   |    |    |    | 1.17 (0.78–1.76)    |
| Reversal of catalepsy                 | Score <2 (0.0%)<sup>1</sup> | 2                           | 1    | 5    | 5   | 5   |    |    |    | 0.78 (0.57–1.05)    |
|                                       | Score <5 (2.0%)<sup>1</sup> | 3                           | 1    | 4    | 5   | 5   |    |    |    | 0.89 (0.59–1.33)    |
|                                       | Score <5 (8.0%)<sup>1</sup> | 4                           | 0    | 2    | 5   | 5   |    |    |    | 1.35 (1.00–1.82)    |
| Induction of excitation               | Presence (0.0%)<sup>1</sup> | 1                           | 0    | 1    | 0   | 3   |    |    |    | 4.7 (–)             |
|                                       | Presence (0.0%)<sup>1</sup> | 2                           | 0    | 0    | 1   | 4   |    |    |    | 3.5 (2.36–5.3)      |
|                                       | Presence (0.0%)<sup>1</sup> | 3                           | 0    | 0    | 1   | 4   |    |    |    | 3.5 (2.36–5.3)      |
|                                       | Presence (0.0%)<sup>1</sup> | 4                           | 0    | 0    | 0   | 0   | 2  |    |    | >5.0                |

The number of rats responding to the listed criterion is listed per dose level (n = 5 per dose group). Values representing more than 50% responders are formatted bold.

<sup>1</sup>Percentage of false positives (solvent-pretreated rats responding to the criterion [n = 50]).
PDE10AIs alone (Kehler et al. 2007; Schmidt et al. 2008). However, catalepsy after D₂ receptor blockers intensifies over time with retesting of the animals (Stanley and Glick 1976; Hillegaart et al. 1987), this is apparently not the case after PDE10AIs. The reversal of haloperidol-induced catalepsy by PDE10AIs in the present study apparently conflicts with the reported potentiation of haloperidol-induced catalepsy after the PDE10AI papaverine (Siuciak et al. 2006). When a lower dose of haloperidol was used, however, we also showed PDE10AI-induced potentiation of catalepsy but only at low doses with a bell-shaped dose–response relation (Vanhoof et al. 2012).

The present data show that PDE10AI-induced direct pathway activation also counteracts catalepsy and related behaviors induced by reducing D₂ receptor-mediated neurotransmission via other mechanisms such as blockade of D₂ receptors with haloperidol or dopamine depletion with RO-4-1284 or reserpine. The PDE10AIs were somewhat less potent against haloperidol than against RO-4-1284 or reserpine, presumably because they had to overcome the D₂ receptor blockade by haloperidol, whereas D₂ receptors are functioning normal after dopamine depletion. Moreover, a switch in receptor-mediated signal transduction mechanism to a supersensitive form of D₁-mediated neuronal plasticity has been demonstrated in the dopamine-depleted striatum (Gerfen 2000).

The opposing effects of PDE10AIs in the reserpine test are striking. PDE10AIs inhibit the tail-pinch response

Figure 4. Reversal of Ro-4-1284 (10 mg/kg, s.c.)-induced catalepsy in rats that were cotreated (at the same time) with the PDE10AIs JNJ-42314415, PQ-10, or MP-10 or the dopamine D₁ agonist SKF-82958. Shown are individual and median catalepsy scores (symbols and stripes, respectively) per dose group measured 2, 3, and 4 h after dosing. The dotted horizontal lines represent the critical levels adopted for drug-induced reversal of catalepsy (score < 2 at 2 h; score < 5 at 3 and 4 h) that were used for the determination of the ED₅₀ values listed in Tables 6–8, 10. The PDE10AIs dose dependently reduced catalepsy scores to below the critical level. The D₁ agonist was effective at the 2-h interval and, to a lesser extent, at the 3-h time interval. PDE10AI, phosphodiesterase 10A inhibitor.

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before reserpine at doses close to the ED$_{50}$ for apomorphine antagonism, presumably via indirect pathway activation. After reserpine, however, only slightly higher doses induce the opposite effect, viz. reversal of the reserpine-induced tail-pinches response blockade, presumably via direct pathway activation. In other words, indirect or direct pathway activation predominates under conditions of normal and low dopaminergic neurotransmission, respectively.

The anticaudate properties of PDE10AIs at conditions of low dopaminergic transmission may suggest therapeutic potential in Parkinson’s disease. The anticaudate effects are mediated via direct pathway activation, an effect that is shared with D$_1$ agonists. D$_1$ agonists have therapeutic potential in Parkinson’s disease but suffer from side effects (Mailman et al. 2001; Hurley and Jenner 2006). In view of minimal expression of PDE10A in peripheral tissue (Seeger et al. 2003; Coskran et al. 2006), PDE10AIs are likely devoid of the disturbing peripheral cardiovascular side effects of direct D$_1$ receptor agonists (Westfall and Westfall 2005).

Table 9. ED$_{50}$s (95% confidence limits; mg/kg) of the PDE10AIs for interactions with RO-4-1284-induced behavior related to their ED$_{50}$ for inhibition of apomorphine-induced behavior.

| Compound     | JNJ-42314415 | PQ-10 | MP-10 |
|--------------|--------------|-------|-------|
|              | ED$_{50}$ (95% CL) | Ratio$^1$ | ED$_{50}$ (95% CL) | Ratio$^1$ | ED$_{50}$ (95% CL) | Ratio$^1$ |
| Apomorphine test |               |       |       |       |               |       |
| Inhibition of agitation | 0.95 [1.0] | 3.5 [1.0] | 0.88 [1.0] |       |       |       |
| RO-4-1284 interaction |               |       |       |       |               |       |
| Reversal of sedation | 0.59 (0.44–0.80) [0.62] |       | 3.6 (2.89–4.3) [1.0] |       | 0.68 (0.50–0.91) [0.77] |       |
| Reversal of catalepsy | 1.03 (0.69–1.54) [1.1] |       | 4.7 (2.91–7.6) [1.3] |       | 0.78 (0.57–1.05) [0.89] |       |
| Induction of excitation | 2.05 (1.37–3.1) [2.2] |       | 14.2 (8.8–22.8) [4.0] |       | 3.1 (1.91–5.0) [3.5] |       |

Listed are ED$_{50}$s obtained at time of peak effect together with the ratio over the ED$_{50}$ for apomorphine antagonism. PDE10AI, phosphodiesterase 10A inhibitor

Table 10. Dose–response relations and corresponding ED$_{50}$s for various effects observed over a 4-h period after challenge with RO-4-1284 (10 mg/kg, s.c.) in rats that received test compound simultaneously with RO-4-1284.

| Effect | Dose of SKF-82958 (mg/kg, s.c.) | ED$_{50}$ (95% CL; mg/kg) |
|--------|---------------------------------|----------------------------|
|        | Time (h) 0.04 0.08 0.16 0.31 0.63 1.25 2.5$^3$ |               |
| Reversal of sedation/passivity | 1 Absence (6.0%)$^2$ | 0 1 0 2 3 0 0/4 >2.5 |
|                                   | 2 Absence (4.0%)$^2$ | 1 0 0 2 3 0 0/4 >2.5 |
|                                   | 3 Absence (2.0%)$^2$ | 1 0 0 1 2 0 0/4 >2.5 |
|                                   | 4 Absence (16%)$^2$ | 0 0 0 0 1 0 1/4 >2.5 |
| Reversal of catalepsy | 2 Score <2 (0.0%)$^2$ | 0 1 0 1 1 1 3/4 2.1 (–) |
|                                   | 3 Score <5 (2.0%)$^2$ | 1 1 1 1 2 1 2/4 2.5 (–) |
|                                   | 4 Score <5 (8.0%)$^2$ | 0 1 0 1 1 1 1/4       |
| Induction of excitation | 1 Presence (0.0%)$^2$ | 0 0 0 0 0 0 0/4 >2.5 |
|                                   | 2 Presence (0.0%)$^2$ | 0 0 0 0 0 0 0/4 >2.5 |
|                                   | 3 Presence (0.0%)$^2$ | 0 0 0 0 0 0 0/4 >2.5 |
|                                   | 4 Presence (0.0%)$^2$ | 0 0 0 0 0 0 0/4 >2.5 |

The number of rats responding to the listed criterion is listed per dose level (n = 5 per dose group). Values representing more than 50% responders are formatted bold.

$^1$One of the five rats tested at 2.5 mg/kg died and was not included.

$^2$Percentage of false positives (solvent-pretreated rats responding to the criterion [n = 50]).
induced by the D\textsubscript{1} antagonist SCH-23390 or the monoamines-depleting agent RO-4-1284. Despite their activity against haloperidol-induced catalepsy in rats, PDE10AIs hardly affected haloperidol-induced hypolocomotion in mice. As a similar profile was obtained with the D\textsubscript{1} agonist SKF-82958, the effects of PDE10AIs against SCH-23390...
and RO-4-1284 are most likely related to direct pathway activation.

The minor effects obtained with the D₁ agonist SKF-82958 in rats do not fully support the hypothesis that common direct pathway activation mediates the effects of PDE10AI against the sedation and catalepsy induced by haloperidol, RO-4-1284, and reserpine. SKF-82958 antagonized haloperidol-induced catalepsy only at the 2-h interval but this may be related to short duration of action (Desai et al. 2005). However, the D₁ agonist also reversed the reserpine-induced effects only partially and tended to reverse RO-4-1284-induced catalepsy only at very high doses. The effect on D₁ signaling under these conditions is apparently more efficient with a PDE10AI than that with an orthosteric D₁ agonist. This may be the case because, under dopamine depletion, D₁ receptors become supersensitive resulting from a signaling switch from induction of immediate early genes (such as c-fos) toward ERK/MAP signaling (Gerfen et al. 2002), whereas PDE10AIs induce both immediate early genes and ERK/MAP signaling (Strick et al. 2010).

Behavioral stimulant effects of PDE10AIs were observed in combination with high doses of the D₁ antagonist SCH-23390 or the dopamine-depleting agents RO-4-1284 or reserpine but not in combination with the D₂ receptor blocker haloperidol. Like D₂ receptor blockers, PDE10AIs increase striatal dopamine turnover (Schmidt et al. 2008). In contrast to D₂ receptor blockers, however, PDE10AIs do not directly prevent D₂ receptor stimulation, thereby leaving dopaminergic neurotransmission largely intact.

Thus, the above behavioral stimulant effects of PDE10AIs may arise from progressively increasing activation of the direct pathway overcoming the effect of D₁ receptor blockade and resulting in behavioral stimulation as long as the indirect pathway is not disinhibited by D₂ receptor blockade. The behavioral stimulant effects are observed at doses close to those required for reversal of the cataleptic state, reflecting very efficient direct pathway activation and limited safety margin for anti-Parkinson effects. Concomitant direct pathway activation may also explain the bell-shaped dose–response for inhibition of the tail-pinch response before reserpine. Similar bell-shaped effects on glucose metabolism in the lateral habenula of mice were also attributed to increased direct pathway activation at high PDE10AI doses (Dedeurwaerdere et al. 2011).

Balance between direct and indirect pathway activation is necessary for numerous behaviors including reward processing (Lobo et al. 2010; Beutler et al. 2011). Dopamine D₁ receptors in the direct striatal pathway facilitate several dopamine-dependent functions including appetitive behaviors. Global loss of dopamine D₁ receptors demonstrates its importance for feeding and reward acquisition to the general ability to thrive (Drago et al. 1994; Xu et al. 1994; Wall et al. 2011). In this regard, direct striatal pathway activation by PDE10AIs might improve negative and depressive symptomatology in schizophrenic patients. In view of the monoamine hypothesis of depression (Hirschfeld 2000), the antidepressant potential of PDE10AIs may be supported by the reversal of the effects of monoamines depletion observed in the present study.
Together with previously reported data, the present results indicate that PDE10AIs suppress stimulant behavior via indirect pathway activation, but the effect is counteracted by D1 receptor stimulation and corresponding activation of the direct pathway. In the absence of counteracting direct pathway activation (D1 receptor stimulation), however, this mechanism is very efficient, resulting in a limited safety margin toward cataleptic side effects. Conversely, PDE10AIs can reverse behavioral suppression (catalepsy, hypolocomotion) via direct pathway activation, but the effect is counteracted by D2 receptor blockade and corresponding disinhibition of the indirect pathway. In the absence of concomitant, counteracting indirect pathway activation (D2 receptor blockade), however, this
mechanism is very efficient with a limited safety margin toward behavioral stimulant side effects. In analogy, the brake and accelerator of a car also function more efficiently when used separately rather than simultaneously, yet both are indispensable to adjust speed when out of balance. Further clinical studies have to show which patients may benefit from the dual activity profile of PDE10AIs.

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Disclosure

All authors are employees of Janssen Research & Development, a division of Janssen Pharmaceutica NV. All authors had access to the study data, provided direction and comments on the manuscript, made the final decision about where to publish these data, and approved submission to the journal.

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