Pharmacology of JNJ-42314415, a Centrally Active Phosphodiesterase 10A (PDE10A) Inhibitor: A Comparison of PDE10A Inhibitors with D₂ Receptor Blockers as Potential Antipsychotic Drugs

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ABSTRACT

The new phosphodiesterase 10A inhibitor (PDE10A) JNJ-42314415 [3-[6-(2-methoxyethyl)pyridin-3-yl]-2-methyl-8-morpholin-4-ylidazao[1,2-ajpyrazine] was compared with three reference PDE10AIs and eight dopamine 2 (D₂) receptor blockers. Despite displaying relatively low PDE10A activity in vitro, JNJ-42314415 was found to be a relatively potent and specific PDE10A inhibitor in vivo. The compound was devoid of effects on prolactin release and of receptor interactions associated with other commonly observed adverse effects of available antipsychotics. Similar to D₂ receptor blockers, the tested PDE10AIs antagonized stimulated-instantaneous behavior and inhibited conditioned avoidance behavior; these effects were observed at doses close to the ED₅₀ for striatal PDE10A occupancy. Relative to the ED₅₀ for inhibition of apomorphine-induced stereotypy, PDE10AIs blocked conditioned avoidance behavior and behaviors induced by nondopaminergic stimulants (phencyclidine, scopolamine) more efficiently than did D₂ receptor blockers; however, they blocked behaviors induced by dopaminergic stimulants (apomorphine, d-amphetamine) less efficiently. PDE10AIs also induced less pronounced catalepsy than D₂ receptor blockers. The effects of PDE10A inhibition against dopaminergic stimulants and on catalepsy were potentiated by the D₂ antagonist SCH-23390 (8-chloro-3-methyl-5-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepin-7-ol), suggesting that enhancement of D₁ receptor-mediated neurotransmission contributes to the behavioral profile of PDE10AIs. By reducing dopamine D₂ and concomitantly potentiating dopamine D₁ receptor-mediated neurotransmission, PDE10AIs may show antipsychotic activity with an improved side-effect profile relative to D₂ receptor blockers. However, the clinical implications of this dual mechanism must be further explored.

Introduction

Schizophrenia is a severe and chronic mental illness. Observations that dopamine-enhancing drugs, such as amphetamine, may induce psychosis and that all marketed antipsychotics block dopamine D₂ receptors (Niemegeers and Janssen, 1979; Leysen, 2000; Kapur and Mamo, 2003; Seeman, 2006) suggest that hyperactivity of dopaminergic neurotransmission underlies the positive symptoms of schizophrenia. In vivo brain imaging studies in patients with schizophrenia support this notion and demonstrate increased activity of dopaminergic neurons during episodes of illness exacerbation (Laruelle et al., 1999; Erritzoe et al., 2003). Blockade of central D₂ receptors is thought to be responsible for antipsychotic efficacy but also for the major adverse effects (AEs) of antipsychotics, such as extrapyramidal symptoms (EPS) and tardive dyskinesia. Blockade of peripheral D₂ receptors results in enhanced prolactin release, which can cause a number of AEs (e.g., menstrual disturbances, galactorrhea, sexual dysfunction, decreased fertility, movement disorders, and behavioral disturbances) (Dickson and Glazer, 1999).
Inhibition of the enzyme phosphodiesterase (PDE) 10A has been proposed as an alternative way to reduce central D2 receptor-mediated neurotransmission to obtain antipsychotic efficacy (Siuciak, 2008; Siuciak et al., 2006a, 2008; Kehler et al., 2007; Menitti et al., 2007). PDEs inactivate the intracellular second messengers cAMP and cGMP and thereby regulate signal transduction processes. PDE10A is a dual-substrate PDE with 20- to 60-fold higher affinity for cAMP than that for cGMP but 2- to 5-fold higher V_{max} for cGMP than that for cAMP (Fujishige et al., 1999; Soderling et al., 2003; Nishi et al., 2008), which have opposing effects on striatal dopamine-sensitive medium spiny neurons (MSNs) (Loughney et al., 1999; Seeger et al., 2003; Coskran et al., 2009). PDE10A is expressed primarily in the brain (striatum, nucleus accumbens, and olfactory tubercle) and is thought to be particularly important in regulating the activity of striatal dopamine-sensitive medium spiny neurons (MSNs) and dopamine D1 receptor-expressing indirect striatal output pathways, regulating behavioral responses on integration of cortical glutamatergic and midbrain dopaminergic input (Graybiel, 1990, 2000). Marketed antipsychotics act predominantly by blocking D2 receptors in the indirect pathway. PDE10A is expressed in both the indirect and direct pathways (Seeger et al., 2003; Nishi et al., 2008), which have opposing effects on striatal output. Although it has been emphasized that PDE10A inhibitors (PDE10AIs) combine functional characteristics of D2 antagonists and D1 agonists by reducing dopamine-evoked cAMP decrease via G_{i}-coupled D2 receptors and potentiating dopamine-evoked cAMP increase through G_{s}-coupled D1 receptors, this conceptualization is likely too narrow as it does not take into account, for example, the striatal glutamate-NO-guanylyl cyclase signaling axis (Grauer et al., 2009; Sammut et al., 2010; Kleiman et al., 2011).

The present study reports on the pharmacological profile of the novel PDE10AI JNJ-42314415 [3-6-(2-methoxyethyl) pyridin-3-yl]-2-methyl-8-morpholin-4-ylmimidazo[1,2-a]pyrazine; Fig. 1]. JNJ-42314415 is compared with three reference PDE10AIs [PQ-10 [6,7-dimethoxy-4-[3-(quinoxalin-2-yl oxy) pyrrolidin-1-yl]quinazoline] (Kehler and Kilburn, 2009); TP-10 [2-(4-[4-pyridin-4-yl-1-(2,2,2-trifluoroethyl)-1H-pyrazol-3-yl] phenoxy)methyl]quinoline] (Schmidt et al., 2008; Kehler and Kilburn, 2009); and MP-10 [PF-2545920 or 2-[4-(1-methyl-4-pyridin-4-yl)-1H-pyrazol-3-yl]phenoxy)methyl]quinoline] (Grauer et al., 2009; Kehler and Kilburn, 2009)] and eight reference D2 receptor blockers [the azapines clozapine and olanzapine, the thiapine quetiapine, the benzoxazolone risperidone, the benzothiazole ziprasidone, the butyrophenone haloperidol, the dihydroquinolinone aripiprazole, and the fast-dissociating D2 receptor blocker JNJ-37822681 [N-[1-(3,4-difluorobenzyl) piperidin-4-yl]-6-(trifluoromethyl)pyridazin-3-amine] (Langlois et al., 2012)].

JNJ-42314415 is also studied for effects on nondopaminergic systems, some of which may be responsible for AEs observed with available antipsychotic drugs, such as those mediated via (inhibition of) 5HT_{2A} receptors (weight gain), α_{1}-adrenoceptors (orthostatic hypotension, reflex tachycardia, hypnosedation), α_{2}-adrenoceptors (tachycardia), histamine H_{1} receptors (sedation, weight gain), and muscarinic receptors (blurred vision, dry mouth, constipation, cognitive impairment), or it may contribute to an improved therapeutic profile (5HT_{2A}: reduction of EPS, improvement of negative symptoms; 5HT_{2C}: anxiolytic activity). Although these nondopaminergic interactions have already been previously reported and extensively discussed for the D2 receptor blockers (Langlois et al., 2012), they are briefly presented in the present article to compare both drug classes and to characterize the D2 receptor blockers regarding nondopaminergic interactions that may interfere with the behavioral effects studied.

The present study confirms the functional D2 antagonism of PDE10AIs that may be expected from the activation of the “indirect” striatal output pathway elicited by both drug classes. However, our results also show that PDE10AIs differ from D2 receptor blockers by less efficient blockade of dopamine stimulant-induced behavior and less pronounced catalepsy. This difference may result from the associated functional D1 agonistic component of PDE10AIs via the dopamine D1 receptor-expressing “direct” striatal output pathway. To substantiate the involvement of this functional D1 agonistic component, the interaction of the D2 antagonist SCH-22390 (8-chloro-3-methyl-5-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepin-7-ol) with the effects of the MP-10 on apomorphine- and d-amphetamine-induced behavior and on catalepsy was studied.

Materials and Methods

Preparation of Test Compounds and Controls and Sources

All compounds were synthesized in our own laboratories, with the exception of quetiapine (Sequioa Research Products, Pangbourne, UK); apomorphine, clonidine, compound 48/80, histamine, norepinephrine, phystostigmine, scopolamine (Sigma-Aldrich, St. Louis, MO); d-amphetamine (Certa SA, Eigenbrakel, Belgium); phencyclidine (PCP; UK Green Scientific, London, UK); medetomidine (Domitor; Pfizer Animal Health, Ottignies-Louvain-la-Neuve, Belgium); and tryptamine (Acros Organics, Geel, Belgium). The compounds were dissolved in 0.9% NaCl (tryptamine) or in distilled water (apomorphine, clonidine, compound 48/80, d-amphetamine, histamine, medetomidine (diluted from Domitor), norepinephrine, PCP, scopolamine), if required, acidified with tartaric acid (haloperidol, clozapine, JNJ-42314415, olanzapine, R037617 [5-[(4-diphenylmethyl)piperazin-1-yl]
methyl)-1-ethyl-1H-benimidazol-2-yl)ethanone, risperidone, SCH-23390) or in 10% or 20% hydroxypropyl-β-cyclodextrin acidified with tartaric acid (aripiprazole, JNJ-37822681, MP-10, PQ-10, quetiapine, TP-10, ziprasidone). The solutions were stored at room temperature in closed containers protected from light. The preparations were subcutaneously administered in volumes of 10 μl/kg. Solvents were also tested to control for solvent-related effects.

Animals (Species, Weight, and Sex)
Sprague-Dawley rats were used for the brain tissue and plasma protein binding assays (males) and the prolactin assay (females), male Lewis rats for the compound 48/80 lethality assay, Dunkin-Hartley-Pirbright guinea pigs of both sexes for the histamine lethality assay, and male Wiga Wistar rats for all other assays. The rats ranged in body weight between 175 and 275 g, and the guinea pigs weighed between 300 and 500 g. Sprague-Dawley rat plasma was obtained from Janvier Laboratories (Saint Berthevin Cedex, France); all other animals were obtained from Charles River Breeding Laboratories (Sulzfeld, Germany) and housed under standard laboratory conditions (21 ± 2°C; 45%–65% relative humidity; light-dark cycle set at 12 hours). With the exception of the occupancy assay, the animals were fasted overnight before the start of the experiments (tap water remained available ad libitum). During the test period, they were housed in individual cages. The Institutional Ethical Committee on Animal Experimentation approved the experimental protocols, in compliance with Belgian law (Royal Decree on the Protection of Laboratory Animals, April 6, 2010).

In Vitro Assays
PDE Enzyme Assays. Human phosphodiesterase PDE11A4 was expressed in human embryonic kidney cells from a full-length human recombinant clone. Human phosphodiesterases 2A, 4D3, 5A3, 7A1, 9A1, 10A2, and rat PDE10A2 were expressed in S9f cells using a recombinant baculovirus construct. All clones consisted of the full-length sequence containing a His8 sequence following the start Met to allow metal affinity purification of the recombinant protein. Cells were harvested and the PDE protein was purified by metal chelate chromatography on nickel-Sepharose 6FF. PDE1B1, PDE3A, PDE6A8, and PDE8A were purchased as partially purified S9f cell lysates (Scottish Biomedical, Glasgow, UK). All enzymes were diluted in 50 mM Tris pH 7.8, 1.7 mM EGTA, 8.3 mM MgCl2, except for PDE9A and PDE2A, which were diluted in 50 mM Tris pH 7.8, 1.7 mM EGTA, 5 mM MnCl2, and PDE1B, which was diluted in 50 mM Tris, pH 7.8, 8.3 mM MgCl2 complemented with 624 U/ml calmodulin and 900 μM CaCl2. The effect of the compounds on PDE activity was measured by a scintillation proximity assay. PDE Ytrium Silicate SPA beads allowed PDE activity to be measured by direct binding of the primary phosphate groups of noncyclic AMP or GMP to the beads via a complex iron chelation mechanism. The amount of bound tritiated product ([3H]cAMP or [3H]GMP) was measured by liquid scintillation counting. The compounds were dissolved and diluted in 100% dimethylsulfoxide (DMSO) in polypropylene plates to a concentration of 100-fold the final concentration in the assay. Rat or human PDE10A enzyme solution (10 μl) was added to 20 μl of incubation buffer (50 mM Tris pH 7.8, 8.3 mM MgCl2, 1.7 mM EGTA), 10 μl of substrate solution consisting of a mixture of nontrinitated and tritiated substrate (240 nM cAMP, 0.032 μCi of [3H]cAMP), and 0.4 μl of compound in 100% DMSO in a 384-well plate and incubated for 60 minutes at room temperature. After incubation, the reaction was stopped with 20 μl of stop solution, consisting of PDE SPA beads (17.8 mg/ml beads in 18 mM zinc sulfate). After sedimentation of the beads for 30 minutes, the luminescence was measured in a PerkinElmer TopCount scintillation counter, and the results were expressed as counts per minute. Blank values were obtained by replacing the enzyme solution by 10 μl of incubation buffer. In control samples, compound was replaced by 0.4 μl of 100% DMSO. The same assay principle was applied for the measurement of the inhibition of other members of the PDE family, with appropriate modifications of enzyme concentration, incubation buffer, substrate solution, incubation time, and stop solution.

Brain-Tissue Binding. Diluted (1:10) rat brain tissue homogenate (from male Sprague-Dawley rats) was prepared by adding 9 ml of phosphate-buffered saline (PBS; pH = 7.4; Supelco, Bornem, Belgium) to 1 g of brain tissue. Brain tissue homogenate, containing test compound at 5 μM, was incubated at 37°C in the rapid equilibrium dialysis (RED) Device (Thermo Fisher Scientific, Geel, Belgium). The RED device consists of a Teflon 48-well base plate which contains disposable inserts. These inserts are bisection by a semipermeable (molecular mass cutoff = 8 kDa) membrane, creating two chambers. Aliquots (300 μl) of spiked (1 in 10 diluted) brain tissue homogenate were loaded in to one chamber and PBS (pH = 7.4, 500 μl) was loaded into the other. The plate was then sealed and placed in a shaking incubator at 37°C for 5 hours, after which the samples were removed and analyzed from both the buffer and brain tissue homogenate side to obtain free and bound concentrations. These concentrations were then used to calculate the percentage compound bound to brain tissue. Recovery in the experiment was checked by measuring the actual concentration of test compound remaining in the buffer and brain tissue homogenate compartments at the end of the incubation and comparing this to the actual spiked brain tissue homogenate concentration at the start of the experiment. The median of three experiments was reported.

Plasma Protein Binding. Frozen male Sprague-Dawley rat plasma (K3-EDTA; Janvier Laboratories) was thawed and warmed just before use. Plasma, containing test compound at 5 μM, was incubated at 37°C in the RED device (see preceding description). Aliquots (300 μl) of spiked plasma were loaded into one chamber, and PBS was loaded into the other chamber. The plate was then sealed and placed in a shaking incubator at 37°C for 4.5 hours. After 4.5 hours, samples were removed and analyzed from both the buffer and plasma side to obtain free and bound concentrations. These concentrations were then used to calculate the percentage of compound bound to plasma protein. Recovery in the experiment was checked by measuring the actual concentration of test compound remaining in the buffer and plasma homogenate compartments at the end of the incubation and comparing this with the actual spiked plasma homogenate concentration at the start of the experiment. The median of three experiments was reported.

Additional Assays. JNJ-42314415 was tested at a concentration of 10 μM in a battery of additional assays for inhibition of radioligand binding and enzyme activity, both in house (h5HT1A, h5HT1B, h5HT3, h5HT7, hA1A, hA2A, hA2B, hA2C, hA1b, hCaCH, hD1, hD2L, hERG (human ether-a-go-go-related gene), h1L, hLRK2, rNaCH) and by CEREP (Celle L’Evescault, France; rCaCH, rCICH, rKCH, rNaCH, rSCKaCh, CL1, h5HT1A, h5HT1B, h5HT3, h5HT7, hA1A, hA2A, hA3, hA1b, hAT1, hBCXCR2, hBK2, hCCaKa, hCCr1B, hD1, hETA, hGAL2, hH2, hM1, hMC4, hNK2, hNK3, hNPY1, hNPY2, hNT1, hOP1, hOP3, hORL1, hV1a, hVPCa1, mSST, rH1T1B, rA1a, rA2a, rBDZ, rGABA, rOP2), PLA2, cyclooxygenases-1 and -2, 12-lipoxygenase, inducible NOS, ACE, ACE-2, BACE-1, ECE-1, elastase, caspase-3, caspase-8, cathepsin D, cathepsin L, immunodeficiency virus-protase, neutral endopeptidase, matrix metalloproteinases (MMP-1, -3, -9, -9), trypstatin, TACE, adenylyl cyclase, guanylyl cyclase, PTTPB1, PP2A, phasatase CDC25A, MKP1, aceticholinesterase, MAO-A, MAO-B, CENP-E, EG5, HDAC3, HDAC4, HDAC6, HDAC11, sirtuin 1, sirtuin 2). JNJ-42314415 was tested at a concentration of 1 μM in a panel of more than 200 kinases (Upstate Profiler, Upstate, Dundee, UK).

PDE10A Enzyme Occupancy
Rats were treated subcutaneously with vehicle or test compound at five to eight dosages ranging from 0.16 to 10 mg/kg body weight (n = 3 per compound). [3H]MP-10 (10 μCi/animal) was injected
intravenously 30 minutes after drug administration. The animals were decapitated 30 minutes after the \(^{3} \text{H}\)MP-10 injection. Brains were immediately removed and rapidly frozen in dry ice-cooled 2-methylbutane (−40°C). Twenty micrometer-thick coronal sections at the level of the striatum were cut using a cryostat and collected on glass slides. Brain sections were loaded in a \(\beta\)-imager (Biopspace Laboratory, Paris, France) for 8 hours. Digital autoradiograms were quantified using the Beta Vision Program (Biopspace Laboratory). The specific binding was determined as the difference between \(^{3} \text{H}\)MP-10 binding quantified in the striatum (a brain area showing a high density of PDE10A) and in the cortex (a brain area where PDE10A is virtually absent). Occupancy was calculated as the inhibition of specific \(^{3} \text{H}\)MP-10 binding in drug-treated animals relative to vehicle-treated animals.

**In Vivo Functional Assays**

**Interactions with Non dopaminergic Receptors.** JNJ-42314415 was evaluated in various in vivo assays predicting interactions with non dopaminergic receptors in comparison with \(D_{2}\) receptor blockers. These methods and the results obtained with the \(D_{2}\) receptor blockers have been previously reported (Langlois et al., 2012). The results are included to allow comparison with PDE10AIs and to characterize the \(D_{2}\) receptor blockers in terms of non dopaminergic interactions that may interfere with the studied behavioral effects. The methods are available in Supplemental Methods.

**Apomorphine-Induced Stereotypy.** Apomorphine is a dopamine receptor stimulant that mimics the agonistic action of dopamine at the \(D_{1}\) receptor. Apomorphine (1.0 mg/kg i.v.)-induced stereotypy (compulsive sniffing, licking, chewing) was scored every 5 minutes over the first hour after injection of apomorphine. The scoring system was 3 = pronounced, 2 = moderate, 1 = slight, and (0) absent. Criterion for drug-induced inhibition of stereotypy: fewer than six scores of 3, fewer than six scores \(\geq 2\), or fewer than seven scores \(\geq 1\) (0.14% false positives in \(>5000\) solvent-pretreated control rats). Criterion for drug-induced inhibition of agitation: fewer than six scores of 3 (0.16% false positives; \(n = 2966\)), fewer than six scores \(\geq 2\) (0.0% false positives), or fewer than seven scores \(\geq 1\) (0.0% false positives). Criterion for drug-induced blockade of agitation: less than two scores of \(\geq 1\) or 0 scores of \(\geq 2\) (0.0% false positives). For evaluation of the interaction of the \(D_{1}\) antagonist SCH-23390 with the effect of MP-10 on apomorphine-induced agitation, the cumulative score for agitation over the whole 60-minute observation period was used. In this case, the following criteria for drug-induced effects were adopted: cumulative score \(<21\), \(<10\), and \(<5\) (0.9%, 0.0%, and 0.0% false positives, respectively).

**Conditioned One-Way Active Avoidance Test.** The apparatus consisted of an inner transparent box (length \(\times\) width \(\times\) height: 30 \(\times\) 30 \(\times\) 30 cm) with an open top and surrounded by an outer box. The inner box was equipped with a grid floor made of 15 pairs of iron bars (2-mm diameter; 6-mm inter-bar distance). Odd and even bars were connected with a source of alternative current (1.0 mA; Coulbourn Instruments Solid State Shocker/Distributor, Whitehall, PA), which could be interrupted by a switch. The outer box (length \(\times\) width \(\times\) height: 40 \(\times\) 40 \(\times\) 36 cm) also had an open top and kept a distance of 5 cm from the inner box at all sides. Only the front wall of the outer box was transparent to allow inspection of the animal during the test. The upper edge of the outer and inner box served as a target for the rats on which to jump with their forepaws and hindpaws, respectively.

Rats were trained to avoid an electric shock during five sessions at 15-minute intervals during a 1-hour period: the rat was placed on the non-electrified grid floor, and the grid was electrified 10 seconds later for not more than 30 seconds if the rat did not jump out of the box. Only rats that showed a correct conditioned avoidance response in all three of the last training sessions were included for further experiments and received test compound or solvent immediately after the last training session.

The rats were tested three times (i.e., 60, 90, and 120 minutes after the injection of test compound or solvent). Latency to avoidance (i.e., responding within the 10-second interval before the grid is electrified) or escape (i.e., responding after the grid has been electrified; cutoff time: 10 seconds) was recorded. The median avoidance response and the maximum escape response obtained over the three experimental sessions per rat were used. A median avoidance latency \(>8\) seconds occurred in only 1.8% of solvent-pretreated control rats ( \(n > 400\) ) and was selected as an all-or-none criterion for drug-induced inhibition of avoidance. A maximum ESC \(\geq 10\) seconds over the three trials never occurred in these control rats and was adopted as an all-or-none criterion for inhibition of escape behavior.

**Locomotor Activity Assays.** Motor activity was measured in microprocessor-based motor activity cages (length \(\times\) width \(\times\) height: 43.5 \(\times\) 43.5 \(\times\) 41.5 cm; MED Associates, St. Albans, VT) over a period of 30 minutes. The distance traveled was measured by light beam interruptions (32 infrared light beams (1.3 cm apart) were located in two arrays perpendicular to each other in a horizontal plane at 2.0 cm above the floor). Rats were pretreated with test compound or solvent (10 mg/kg s.c.) and placed in individual cages. The rats were challenged with d-amphetamine (1.25 mg/kg s.c.) 30 minutes later or with either PCP (1.25 mg/kg i.v.) or scopolamine (0.31 mg/kg i.v.) 1 hour later or were not challenged (vehicle-induced locomotion). Locomotion was measured over a period of 30 minutes in motor activity cages starting 1 hour after test compound administration (i.e., 30 minutes after d-amphetamine and immediately after PCP or scopolamine challenge).

Table 1 gives an overview on the averaged activity (mean \(\pm\) S.D.) obtained in saline-pretreated control animals in the four locomotion models and also lists the percentage of false positives obtained in saline-pretreated control animals for three distinct activity levels: 1) inhibition (reduction of hyperlocomotion below control level; only used for PCP hyperlocomotion (<11,000 cm), 2) normalization (reduction of hyperlocomotion to normal values (\(<5000\) cm), or 3) blockade (reduction of hyperlocomotion to below normal values (\(<1500\) cm). Note that the reduction of hyperlocomotion to normal values (which is called normalization) does not necessarily imply that the behavior of these rats is completely normal.

**Observation Test.** Catalsepsy, palpebral opening (before and after manipulation), and body temperature (°C; using an esophageal thermistor probe) were assessed at hourly intervals over a period of 8 hours after administration of test compound or solvent. The scoring

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<th>TABLE 1</th>
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<td><strong>Statistical data on locomotor activity obtained in saline-pretreated control rats</strong></td>
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<tr>
<td><strong>Novelty-induced locomotion</strong></td>
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<td>No. of rats tested</td>
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<tr>
<td>Total distance (mean (\pm) S.D.; cm)</td>
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<tr>
<td>Percent of rats traveling a distance (&lt;1500) cm</td>
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<td>Percent of rats traveling a distance (&gt;5000) cm</td>
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<td><strong>d-Amphetamine hyperlocomotion</strong></td>
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<td>Total distance (mean (\pm) S.D.; cm)</td>
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<td>Percent of rats traveling a distance (&lt;5000) cm</td>
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<td>Percent of rats traveling a distance (&lt;1500) cm</td>
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<td><strong>Scopolamine hyperlocomotion</strong></td>
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<td>Total distance (mean (\pm) S.D.; cm)</td>
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<td>Percent of rats traveling a distance (&lt;5000) cm</td>
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<td><strong>PCP hyperlocomotion</strong></td>
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system was as follows: for catalepsy: 3 = pronounced, 2 = moderate, 1 = slight, and 0 = absent; for palpebral opening: 5 = exophthalmos, 4 = wide open, 3 = open for three-quarters, 2 = half open, 1 = open for one-quarter, and 0 = closed. Evaluations of catalepsy and palpebral opening were based on the sum of the scores from two independent observers. Criterion for drug-induced catalepsy was score 6 (not observed in controls). Criterion for drug-induced palpebral ptosis (assessed after manipulation): score <8 for slight ptosis (in controls: 0.8%) and score <4 for pronounced ptosis (not observed in controls).

The criteria for drug-induced hypothermic effects were >1.0°C decrease of temperature for the 1-hour interval (not observed in controls) and ≥2.0°C decrease of temperature for other time intervals (0% false positives). The interaction between the D₁ antagonist SCH-23390 and MP-10 at the level of catalepsy was studied over a 4-hour period after combined subcutaneous injection compared with the corresponding data obtained over the same time interval after MP-10 alone.

**Prolactin Release.** Rats were treated with test compound or solvent and 1 hour later decapitated. Blood was collected in Vacutainer SSTO tubes and centrifuged at 3000 rpm for 10 minutes. Serum was transferred into secondary tubes and subsequently frozen. Samples were kept at <-18°C until analysis. Serum prolactin was measured with a commercially available radioimmunoassay; rat prolactin [125I]Tassay system (GE Healthcare, Chalfont St. Giles, Buckinghamshire, UK). The detection limit of the assay was 0.8 ng/ml. The interassay coefficients of variation were 9.7% at 25 ng/ml and 14% at 192 ng/ml. In solvent-pretreated control rats, the average prolactin level was 3.8 ± 5.7 ng/ml (mean ± S.D.; n = 200), ranging from 0.8 to 35 ng/ml. The following all-or-none criteria for drug-induced effects on prolactin release were adopted: prolactin concentration >20 ng/ml for a slight increase (4.0% false positives) and prolactin concentration >300 ng/ml for a pronounced increase (0.0% false positives).

**Data Analysis**

**Apparent Inhibition Constant for PDE Inhibition.** The percentage of enzyme inhibition was plotted against the log concentration of the test compound, and the sigmoidal log concentration-effect curve of best fit was calculated by nonlinear regression analysis (using GraphPad Prism version 5.01 for Windows; GraphPad Software, San Diego, CA, www.graphpad.com). From these concentration-response curves, the IC₅₀ values (concentration causing 50% inhibition of hydrolysis) with their 95% confidence limits were calculated. At least 12 concentrations and at least two points per concentration were used for curve fitting. IC₅₀ values were converted to apparent Kᵢ values assuming competitive inhibition using the Cheng-Prusoff equation.

**ED₅₀ for Occupancy (the Dose Producing 50% Occupancy).** The percentage of enzyme occupancy was plotted against dosage, and the sigmoidal log dose-effect curve of best fit was calculated by nonlinear regression analysis (using GraphPad Prism version 5.01 for Windows, GraphPad Software). From these dose-response curves (five to eight doses per compound; n = 3 per dose), the ED₅₀ values with their 95% confidence limits were calculated.

**ED₅₀ for Functional Effects (the Dose Producing 50% Responders to the Criterion Adopted for Drug-Induced Effects).** 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). ED₅₀ values and corresponding 95% confidence limits were determined according to the modified Spearman-Kaerber estimate, using theoretical probabilities instead of empirical ones (Teutakawa, 1982). This modification allows determination of the ED₅₀ and its confidence interval as a function of the slope of the log dose-response curve (Lewi et al., 1977).
Interaction Studies. One-way ANOVA with Bonferroni’s post-tests was used to evaluate the statistical significance of the interaction of the D1 antagonist SCH-23390 with MP-10 at the level of catalepsy (using GraphPad Prism version 5.01 for Windows, GraphPad Software).

Results

In Vitro Activity

JNJ-42314415 inhibited human recombinant PDE10A in vitro with an apparent $K_i$ of 35 nM and rPDE10A with a $K_i$ of 64 nM. Based on in vitro affinities, JNJ-42314415 was 10-fold less potent than PQ-10 and more than 100-fold less potent than MP-10 and TP-10 (Table 2). The compound showed relatively low in vitro activity, presumably because of low plasma protein binding and low brain tissue binding (Table 4).

In Vivo PDE10A Occupancy

The ED$_{50}$ for PDE10A occupancy in rat striatum was in the same range for JNJ-42314415 (1.09 mg/kg s.c.) as obtained for MP-10 and TP-10 (1.58 and 1.40 mg/kg s.c., respectively) and approximately 10 times lower than that obtained for PQ-10 (2.8 mg/kg s.c.) (Table 3). The order of potencies in this occupancy assay is in line with the other in vivo potencies reported in the following but differs from that in the in vitro assay. JNJ-42314415 was more potent in vivo than expected from its relatively low in vitro activity, presumably because of low plasma protein binding and low brain tissue binding (Table 4).

Antagonism of Stimulant-Induced Behavior

The effects of JNJ-42314415 and the three reference PDE10A inhibitors on stimulant-induced behavior were evaluated in several established animal models in comparison with D$_2$ receptor blockers.

Antagonism of Apomorphine-Induced Stereotypy.

The PDE10AIs inhibited the apomorphine-induced behavior at ED$_{50}$ values close to the corresponding ED$_{50}$ for PDE10A occupancy (factor $\leq$3.1; Table 3). When compared with the D$_2$ receptor blockers, JNJ-42314415, MP-10, and TP-10 were about equipotent to quetiapine, whereas PQ-10 was somewhat less potent (Fig. 2A). As inhibition of apomorphine-induced stereotypy is a common primary activity for both PDE10AIs and D$_2$ receptor blockers, all other activities discussed in the following will be expressed as ratio over the ED$_{50}$ for inhibition of apomorphine-induced behavior, which allows comparing normalized activity profiles for compounds from both drug classes.

Figure 2B compares both drug classes for the dose increments required to completely block the apomorphine-induced behavior. Complete blockade of the behavior was observed after relatively larger dose increments with the PDE10AIs (factor 48 for JNJ-42314415; 64 for MP-10) than with most D$_2$ receptor blockers (factor $\sim$10 for haloperidol, aripiprazole, risperidone, and quetiapine).

Antagonism of Stimulant-Induced Locomotion

The PDE10AIs and D$_2$ receptor blockers were compared for their effects on the locomotion induced by novelty, d-amphetamine, scopolamine, and PCP. Figure 3 shows the frequency distributions for the total distance traveled by saline-pretreated control rats in the four different models. The novelty-induced locomotion (measured in animals not saline-pretreated control rats in the four different models.

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>Striatal PDE10A Inhibition (1 hour)</th>
<th>Scopolamine Hyperlocomotion (1 hour)</th>
<th>Phencyclidine Hyperlocomotion (1 hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JNJ-42314415</td>
<td>49 (30–60)</td>
<td>37 (10–50)</td>
<td>2.03 (1.50–2.60)</td>
</tr>
<tr>
<td>PQ-10</td>
<td>41 (31–51)</td>
<td>41 (10–50)</td>
<td>2.75 (1.90–3.60)</td>
</tr>
<tr>
<td>TP-10</td>
<td>41 (30–51)</td>
<td>41 (10–50)</td>
<td>3.1 (1.92–5.0)</td>
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</table>

Table 3

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>Striatal PDE10A 1.09 (0.64–1.87)</th>
</tr>
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<tbody>
<tr>
<td>JNJ-42314415</td>
<td>12.8 (9.2–17.9)</td>
</tr>
<tr>
<td>PQ-10</td>
<td>1.40 (1.15–1.71)</td>
</tr>
<tr>
<td>TP-10</td>
<td>1.58 (1.37–1.81)</td>
</tr>
<tr>
<td>MP-10</td>
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</table>

Table 4

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>Striatal PDE10A</th>
<th>Scopolamine</th>
<th>Phencyclidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>JNJ-42314415</td>
<td>99.8 (99.1–100)</td>
<td>98.4 (99.1–100)</td>
<td>98.4 (99.1–100)</td>
</tr>
<tr>
<td>PQ-10</td>
<td>98.4 (99.1–100)</td>
<td>99.1 (99.1–100)</td>
<td>99.1 (99.1–100)</td>
</tr>
<tr>
<td>TP-10</td>
<td>99.8 (99.1–100)</td>
<td>99.8 (99.1–100)</td>
<td>99.8 (99.1–100)</td>
</tr>
</tbody>
</table>

$^a$Median of three experiments.
challenged with stimulants) is considered to reflect “normal” locomotor activity. Novelty-induced locomotion ranged from approximately 1500 to 5000 cm (Fig. 3, gray area). Challenge with scopolamine, d-amphetamine or PCP resulted consistently in hyperlocomotion (distance >5000 cm). Therefore, ED\textsubscript{50} values of the test compounds for reduction of the stimulant-induced hyperlocomotion to normal values (<5000 cm) were determined. Although this effect is called “normalization,” it does not necessarily imply that the behavior of these animals is completely normal. In all four models, also the ED\textsubscript{50}\textsubscript{SO\textsubscript{2}} of the test compounds for reducing locomotion to below “normal” values (<1500 cm) were determined, which is defined as “blockade.” In case of PCP challenge, it was possible—because of the extremely high activity level—to evaluate an additional effect level (i.e., a reduction in locomotion to below the control level of PCP-challenged rats; <11,000 cm), which was called “inhibition.”

**Antagonism of novelty-induced locomotion.** The ED\textsubscript{50} values of the PDE10AIs for antagonism of novelty-induced hyperlocomotion were comparable to those obtained for apomorphine antagonism (ED\textsubscript{50} ratio ≥1.5; Table 3; Fig. 4A). For D\textsubscript{2} receptor blockers, this margin ranged from 0.083 for clozapine to 4.0 for JNJ-37822681, although the median was close to 1.0 (0.96). Associated nondopaminergic effects of nonspecific D\textsubscript{2} receptor blockers such as clozapine (Fig. 10) and functional-selective actions of aripiprazole at different D\textsubscript{2} receptor-signaling pathways (e.g., a preferential action at presynaptic D\textsubscript{2} autoreceptors) apparently favor the suppression of locomotor activity.

**Antagonism of d-amphetamine-induced hyperlocomotion.** The ED\textsubscript{50} values of the PDE10AIs for inhibition of d-amphetamine-induced hyperlocomotion were slightly higher (factor 2.3–6.1; median 3.1) than those obtained for apomorphine antagonism (Table 3; Fig. 4B). For D\textsubscript{2} receptor blockers this factor ranged from 0.12 for clozapine and quetiapine (Fig. 10) and functional-selective actions of aripiprazole at different D\textsubscript{2} receptor-signaling pathways (e.g., a preferential action at presynaptic D\textsubscript{2} autoreceptors) apparently favor the suppression of locomotor activity. A larger differentiation between PDE10AIs and D\textsubscript{2} receptor blockers was obtained regarding blockade of the d-amphetamine-induced hyperlocomotion to values below normal (Table 3; Fig. 4C). The PDE10AIs were clearly less efficient than most D\textsubscript{2} receptor blockers in this
respect. Whereas the least specific PDE10AI PQ-10 was able to block the d-amphetamine-induced hyperlocomotion at approximately 10 times the antiapomorphine dose (i.e., the largest dose increment required among the D₂ receptor blockers), JNJ-42314415 needed a dose increment with a factor 36, whereas TP-10 and MP-10 remained ineffective up to 9.8 and 60 times, respectively, the antiapomorphine dose.

**Antagonism of scopolamine-induced hyperlocomotion.** The ED₅₀ values of the PDE10AIs for reducing scopolamine-induced locomotion to normal control values (<5000 cm; observed in animals not challenged with scopolamine) were very comparable to those obtained for apomorphine antagonism (ED₅₀ ratio ≤1.5; Table 3; Fig. 4D). D₂ receptor blockers again showed wider margins, ranging from 0.072 for clozapine to 3.0 for JNJ-37822681 (median 0.42). Blockade of the scopolamine-induced locomotion to below normal control values (<1500 cm) was obtained quite readily with the PDE10AIs (Table 3; Fig. 4E), dose increments with a factor 1.2–3.5 already being sufficient. PDE10AIs were much more efficient in this respect than D₂ receptor blockers such as aripiprazole (factor >17) or JNJ-37822681 (factor 48).

**Antagonism of PCP-induced hyperlocomotion.** The ED₅₀ values of the PDE10AIs for reducing PCP-induced hyperlocomotion to levels below the control level of PCP-challenged rats (<11,000 cm) were very comparable to those for apomorphine antagonism (ED₅₀ ratio: 0.5–1.5; Table 3; Fig. 4F). Considerably higher dose increments were required for D₂ receptor blockers such as aripiprazole (6.1) and...
JNJ-37822681 (24). Among the D2 receptor blockers, the nonspecific D2 receptor blockers clozapine and quetiapine were again most potent in inhibiting locomotion, again presumably related to their side-effect profile (Fig. 10).

Similar or even larger differences were seen for normalization (to <5000 cm) and blockade (to <1500 cm) of the PCP-induced hyperlocomotion (Fig. 4, G and H), providing a clear differentiation between PDE10AIs and specific D2 receptor blockers.

Additional Effects Related to D2 Receptor Blockade

Catalepsy, Palpebral Ptosis, and Hypothermia. Although the PDE10AIs were not completely devoid of cataleptogenic properties as indicated by the individual scores for catalepsy (maximum score over the 8-hour observation; Fig. 5), their cataleptic effects were considerably less pronounced than those obtained with D2 receptor blockers. Whereas haloperidol dose-dependently induced catalepsy and consistently achieved maximum scores of 6, the PDE10AIs hardly induced catalepsy: a maximum score >2 in >50% of the tested rats was not obtained at any dose of PQ-10, TP-10, and JNJ-42314415 and occurred with MP-10 only at 1.25 and 2.5 mg/kg (maximum score >2 occurred in only 0.51% of solvent-treated control rats). Moreover, the effects were not dose-dependent (rather they tended to decrease with an increase in dose), and maximum catalepsy (score 6) was observed only occasionally. Therefore, no ED50 values could be calculated up to the highest dose tested (Table 4; Fig. 6, A and B). The compounds were devoid of palpebral ptosis up to the highest tested dose level (Table 5; Fig. 6C). Hypothermia was observed at high doses with JNJ-42314415 (24.7 mg/kg) and MP-10 (15.2 mg/kg) but, within the limited dose ranges tested, not with PQ-10 and TP-10 (Table 5; Fig. 6D).

Inhibition of Conditioned Avoidance Response and Escape Behavior. The PDE10AIs dose-dependently inhibited the conditioned avoidance response and, at slightly higher doses, also the escape response (ESC) (Table 5; Fig. 6, E and F). The margins between inhibition of conditioned avoidance response and apomorphine antagonism ranged between a factor 2.0 and 2.6 for the PDE10AIs and from a factor 0.19 for clozapine to 28 for aripiprazole (median 5.3) for the D2 receptor blockers. The smallest margin was obtained with nonspecific D2 receptor blockers, such as clozapine and risperidone, whereas the largest margins were obtained with more specific D2 receptor blockers such as aripiprazole and JNJ-37822681. Blockade of ESC was also most readily obtained with the nonspecific D2 receptor blockers clozapine and risperidone and least readily with the specific D2 receptor blockers (aripiprazole, JNJ-37822681, and haloperidol).

![Graphs illustrating the maximum score for catalepsy as a function of dose. Individual scores for catalepsy (maximum score obtained over 1–8 hours) after subcutaneous injection of the tested PDE10AIs in comparison with haloperidol. Note that ED50 values for catalepsy in Table 4 are the lowest ED50 values obtained at time of peak effect; these tabulated ED50 values are not based on the maximum score obtained over the 8-hour period presented in this figure.](image-url)

Fig. 5.
Whereas all D<sub>2</sub> receptor blockers started to increase prolactin levels at doses below or equal to those required for apomorphine antagonism and, except clozapine, induced pronounced prolactin release after a >10-fold dose increment, the PDE10AIs were completely devoid of effect on plasma prolactin levels up to the highest doses tested (Table 5; Fig. 6, G and H).

**Prolactin Release.** Whereas all D<sub>2</sub> receptor blockers started to increase prolactin levels at doses below or equal to those required for apomorphine antagonism and, except clozapine, induced pronounced prolactin release after a >10-fold dose increment, the PDE10AIs were completely devoid of effect on plasma prolactin levels up to the highest doses tested (Table 5; Fig. 6, G and H).

**TABLE 5**
ED<sub>50</sub> values [95% (CL); mg/kg s.c.] for inhibition of conditioned avoidance and escape behavior and for induction of catalepsy, palpebral ptosis, hypothermia, and prolactin release

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>ED&lt;sub&gt;50&lt;/sub&gt; (95% CL; mg/kg s.c.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>JNJ-42314415</td>
</tr>
<tr>
<td>Observation test (1–8 h)</td>
<td></td>
</tr>
<tr>
<td>Mild catalepsy (score &gt;2)</td>
<td>&gt;80</td>
</tr>
<tr>
<td>Pronounced catalepsy (score 6)</td>
<td>&gt;80</td>
</tr>
<tr>
<td>Palpebral ptosis</td>
<td>24.7 (16.5–37)</td>
</tr>
<tr>
<td>Hypothermia</td>
<td>20.4 (12.5–37)</td>
</tr>
<tr>
<td>Conditioned avoidance test (1–2 h)</td>
<td></td>
</tr>
<tr>
<td>Inhibition of avoidance (latency &gt;8 s)</td>
<td>2.04 (1.19–3.5)</td>
</tr>
<tr>
<td>Inhibition of escape (latency &gt;9 s)</td>
<td>7.1 (4.7–10.6)</td>
</tr>
<tr>
<td>Prolactin release</td>
<td></td>
</tr>
<tr>
<td>Mild (&gt;20 ng/ml)</td>
<td>&gt;40</td>
</tr>
<tr>
<td>Pronounced (&gt;300 ng/ml)</td>
<td>&gt;40</td>
</tr>
</tbody>
</table>
Interactions with the \( \text{D}_1 \) Antagonist SCH-23390

Apomorphine Antagonism. Figure 7, A and B, illustrates the dose-dependent inhibition of agitation (cumulative score over the 1-hour observation period) to a score <21, <10, and <5 when measured 1 hour after subcutaneous injection of MP-10 and SCH-23390, respectively, alone. Figure 7, C–E, shows comparable results obtained after MP-10 in combination with coadministered SCH-23390 at doses of 0, 0.01, and 0.04 mg/kg s.c., respectively. ED\(_{50}\) values for reduction of agitation to below the indicated score levels are given in the graphs and are also listed in Tables 6 and 7. SCH-23390 alone inhibited agitation to a score <21, <10, and <5 at ED\(_{50}\) of 0.0163, 0.098, and 0.44 mg/kg; MP-10 alone inhibited agitation to a score <21 and <10 at ED\(_{50}\) of 0.67 and 18.7 mg/kg, respectively, but was (up to 80 mg/kg) not able to reduce agitation to a score <5. However, an agitation score <5 was already reached with MP-10 at an ED\(_{50}\) of 1.02 mg/kg when combined with SCH-23390 at 0.04 mg/kg, these doses being a factor >80 and 11 times, respectively, below the corresponding ED\(_{50}\) of these compounds alone. This interaction seems to be more than just additive. Cotreatment with SCH-23390 at 0.04 mg/kg also decreased the ED\(_{50}\) of MP-10 for reducing agitation to a score <10 (from 18.7 to 0.77 mg/kg; factor 24). The lower dose of SCH-23390 (0.01 mg/kg) did not potentiate the effect of MP-10.

\[ \text{d-Amphetamine Antagonism.} \] Figure 8, A and B, plots the dose-dependent inhibition of \( \text{d-} \)amphetamine-induced hyperlocomotion to a distance <5000 and <500 cm when measured 1 hour after subcutaneous injection of MP-10 and SCH-23390, respectively, alone. Fig. 8, C–E, shows similar results obtained after MP-10 in combination with coadministered SCH-23390 at doses of 0, 0.01, and 0.04 mg/kg s.c., respectively. ED\(_{50}\) values for inhibition of locomotion to a distance <5000 and <500 cm are given in the graphs, and an overview is provided in Tables 8 and 9. SCH-23390 alone inhibited the \( \text{d-} \)amphetamine-induced hyperlocomotion to a distance <5000 and <500 cm at ED\(_{50}\) of 0.0215 and 0.44 mg/kg, respectively. MP-10 alone was also able to reduce the hyperlocomotion to <5000 cm at ED\(_{50}\) of 3.1 mg/kg but was (up to 40 mg/kg) not able to reduce locomotion to a distance <500 cm. The ED\(_{50}\) of MP-10 for reducing locomotion to a distance <500 cm was reduced by a factor >60 (from >40 to >80 and 11 times, respectively, below the corresponding ED\(_{50}\) of these compounds alone. SCH-23390 at 0.04 mg/kg also decreased the ED\(_{50}\) of MP-10 for reducing agitation to a score <10 (from 18.7 to 0.77 mg/kg; factor 24). The lower dose of SCH-23390 (0.01 mg/kg) did not potentiate the effect of MP-10.

Figure 7. Inhibition of apomorphine-induced agitation 1 hour after subcutaneous injection of MP-10 and SCH-23390 alone (A and B, respectively) or MP-10 in combination with SCH-23390 (0, 0.01 or 0.04 mg/kg; C–E, respectively). Shown are cumulative agitation scores (individual data and median per dose group). ED\(_{50}\) values (and 95% confidence limits; mg/kg s.c.) for reducing agitation to a score <21, <10, and <5 are given in the graphs, close to the dotted horizontal lines representing these critical levels for drug-induced inhibition. An overview is presented in Table 5.

### TABLE 6

<table>
<thead>
<tr>
<th>Compound</th>
<th>ED(_{50}) (95% CL; mg/kg s.c.; 1 hour)</th>
<th>Score &lt;21</th>
<th>Score &lt;10</th>
<th>Score &lt;5</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCH-23390</td>
<td>0.0163 (0.0120–0.0221)</td>
<td>0.098 (0.073–0.133)</td>
<td>0.44 (0.297–0.67)</td>
<td></td>
</tr>
<tr>
<td>MP-10</td>
<td>0.67 (0.45–1.01)</td>
<td>18.7 (10.9–32)</td>
<td>&gt;80</td>
<td></td>
</tr>
</tbody>
</table>

The lower dose of SCH-23390 (0.01 mg/kg) did not potentiate the effect of MP-10.
0.67 mg/kg) when combined with SCH-23390 at 0.04 mg/kg (which is a dose 11 times below the ED$_{50}$ of SCH-23390 alone for reducing locomotion to <500 cm). As already observed in the preceding apomorphine test, the interaction between the D$_1$ antagonist and MP-10 seems to be more than just additive. Cotreatment with SCH-23390 at 0.01 mg/kg decreased the ED$_{50}$ of MP-10 for reducing locomotion to a distance <5000 cm (from 3.1 to 0.38 mg/kg; factor 8.2), but reduction of locomotion to a distance <500 cm was not achieved up to 10 mg/kg.

Catalepsy. Figure 9 plots the maximum catalepsy scores obtained in individual rats over the first 4 hours after subcutaneous injection of MP-10 in rats cotreated (closed symbols) or not cotreated (open symbols) with SCH-23390 (0.63 mg/kg s.c.). MP-10 alone induced catalepsy only occasionally and at low scores. SCH-23390 (0.63 mg/kg s.c.) alone did not induce catalepsy (score = 2 occurred only once in 25 tested rats) but significantly potentiated the effect of the PDE10AIs ($P < 0.001$; two-way ANOVA): scores for both mild catalepsy (score = 2) and pronounced catalepsy (score = 6) were consistently obtained. The ED$_{50}$ values for induction of mild and pronounced catalepsy, with and without cotreatment with SCH-23390, are listed in Table 10. The ED$_{50}$ values of the PDE10AIs for inducing pronounced catalepsy in rats cotreated with SCH-23390 were close to the corresponding ED$_{50}$s for PDE10A occupancy (ED$_{50}$ ratio 0.25–1.4) and for inhibition of apomorphine-induced agitation (ED$_{50}$ ratio: 0.50–1.5; see Table 3). Note that the dose-response relation for the cataleptic effect of MP-10 in combination with SCH-23390 was bell-shaped, catalepsy being replaced by behavioral stimulant effects (stereotyped sniffing behavior) at higher doses.

Interactions with Additional Receptors

Table 11 lists the ED$_{50}$ values (95% confidence limits; mg/kg s.c.) of JNJ-42314415 for various effects characteristic for specific receptor interactions obtained 1 hour after subcutaneous injection. Figure 10 shows the corresponding specificity profile of JNJ-42314415 relative to apomorphine antagonism and in comparison with previously reported data on the D$_2$ receptor blockers (Langlois et al., 2012). The “*” sign above a bar indicates that the ED$_{50}$ is greater than the value indicated by the height of that bar.
Serotonin 5HT_2A Receptor Antagonism. Up to 39 times the ED_50 for amphetamine antagonism, JNJ-42314415 was devoid of effects characteristic for peripheral 5-HT_2A antagonism (Fig. 10A). The blockade of tryptamine-induced bilateral convulsions is therefore most likely related to behavioral depressant effects resulting from the central D_2 antagonism, just as observed for haloperidol, JNJ-37822681, and aripiprazole. Haloperidol and JNJ-37822681 were devoid of 5-HT_2A antagonism up to >100 times the dose for central D_2 antagonism, whereas risperidone and clozapine showed 5-HT_2A antagonism already at a >10-fold lower dose than that required for central D_2 antagonism. The other compounds showed an intermediate profile.

Serotonin 5HT_2C Receptor Antagonism. JNJ-42314415 was also devoid of effects characteristic for central 5-HT_2C antagonism (Fig. 10B). Among the D_2 receptor blockers, only clozapine and olanzapine showed both 5HT_2C antagonistic effects, and only clozapine displayed these effects at doses below the ED_50 for apomorphine antagonism. Haloperidol and ziprasidone antagonized hunched back behavior only.

Histamine H_1 Receptor Antagonism. JNJ-42314415 was also devoid of effects characteristic for histamine H_1 antagonism (Fig. 10C). Among the D_2 receptor blockers, JNJ-37822681 and haloperidol were devoid of histamine H_1 antagonistic activity up to 100 times their apomorphine antagonistic dose whereas olanzapine, risperidone and, in particular, quetiapine and clozapine were potent antihistamines.

α_1-Adrenoceptor Antagonism. Up to 40 mg/kg, JNJ-42314415 was devoid of effects characteristic for blockade of α_1-adrenoceptors (Fig. 10D). Among the D_2 receptor blockers, risperidone, quetiapine, and clozapine showed antagonistic activity at α_1-adrenoceptors at doses slightly below those required for apomorphine antagonism. JNJ-37822681 was completely devoid of α_1-adrenoceptor blocking activity up to the highest dose tested.

α_2-Adrenoceptor Antagonism. Reversal of the antidiarrheal effect of clonidine could be tested only at doses devoid of intrinsic constipating effects. Likewise, the antagonism of clonidine-induced mydriasis can be evaluated for olanzapine and clozapine only up to the doses that induce mydriasis per se (both compounds have associated antimuscarinic activity; see below). JNJ-42314415 did not reverse the antidiarrheal effect of clonidine up to 10 mg/kg (higher doses showed intrinsic antidiarrheal activity) and did not reverse clonidine-induced mydriasis or antagonize or prolong metadotidine-induced loss of righting up to 40 mg/kg. Risperidone was the only compound showing all the effects characteristic of an α_2-adrenoceptor blocker. Interaction with peripheral α_2-adrenoceptor blocking activity, 10-fold higher doses were required, consistent with the poor CNS disposition of this compound. For clozapine, reversal of medotidine-induced loss of righting occurred at doses close to that required for apomorphine antagonism. The intrinsic constipating and mydriatic activity of this antimuscarinic compound hampered evaluation of the other two effects. α_2-Adrenoceptor blocking activity was not detected for any other compound (Fig. 10E).

Muscarinic Receptor Antagonism. JNJ-42314415 was devoid of antimuscarinic activity up to 40 mg/kg. Olanzapine and clozapine were the only compounds showing both mydriasis and protection against physostigmine lethality, and clozapine was the only compound doing so at doses below the ED_50 for apomorphine antagonism. Quetiapine protected against physostigmine-induced lethality but did not induce mydriasis (Fig. 10F).

### Discussion

Our results show that JNJ-42314415 is a potent and centrally active PDE10AI in models for antipsychotic efficacy. Despite having 10- to 100-fold lower PDE10A activity in vitro, JNJ-42314415 was about equipotent to MP-10 and TP-10 and 10 times more potent than PQ-10 for striatal PDE10A occupancy. The relatively low plasma protein binding and high free fraction in the brain apparently favored in vivo activity. Efficacy in models for antipsychotic activity was observed at doses close to the ED_50 for PDE10A occupancy. Efficacy was associated with a beneficial side-effect profile regarding catalepsy, palpebral ptosis, and hypothermia. JNJ-42314415 did not show activity in models that assess in vivo interactions with receptors that contribute to AEs of available antipsychotic drugs (5HT_2C: weight gain; H_1: sedation, weight gain; α_1: orthostatic hypotension; α_2: tachycardia; muscarinic: blurred vision, dry mouth, constipation, cognition disturbances) or that may contribute to an improved therapeutic profile (5HT_2A: reduction of EPS, improvement of negative symptoms; 5HT_2C: anxiolytic activity). Together with the absence of in vitro binding affinity for these receptors, this implies that PDE10A inhibition does not directly or indirectly interfere with the functional effects mediated by these receptors. In line with minimal expression of PDE10A in rat pituitary (Coskran et al., 2006), JNJ-42314415 did not increase prolactin release. Together, these results suggest that JNJ-42314415 may have antipsychotic potential with a beneficial side-effect profile.

The present results also allow for a comparison of PDE10AIs with D_2 receptor blockers. As discussed previously (Langlois et al., 2012), the investigated D_2 receptor blockers have highly variable profiles, probably due to additional interactions with
Comparison of PDE10A Inhibitors with D2 Receptor Blockers

 Differences in drug-induced effects are a well-recognized phenomenon (Fig. 10). This can include neurotransmitter effects at different D2 receptor signaling pathways (e.g., JNJ-37822681), or functional-selective actions (e.g., interactions with other receptors) (Sotty et al., 2009). However, other factors may also have contributed (e.g., interactions with other receptors), a fast rate of dissociation from the D2 receptor (in the case of JNJ-37822681), or functional-selective actions at different D2 receptor signaling pathways (in the case of aripiprazole). In contrast, the four tested PDE10AIs show a homogeneous activity profile, suggesting an absence of such interfering interactions. Therefore, a comparison of the PDE10AIs with the more specific D2 receptor blockers may be most informative and least difficult to interpret. The consistent activity profile obtained with all four PDE10AIs facilitates the comparison with the D2 receptor blockers and strengthens the reliability of the conclusions.

The present results confirm that PDE10AIs show effects that are functionally similar to those of D2 receptor blockers as indicated by inhibition of apomorphine-induced stereotypy, reversal of stimulant-induced hyperlocomotion, and antagonism of conditioned avoidance behavior at doses close to the ED50 for PDE10A occupancy. In contrast to D2 blockers and in line with previously reported data (Schmidt et al., 2008; Grauer et al., 2009), the PDE10AIs induced no or only mild catalepsy, an effect generally thought to be predictive for the EPS observed with excessive doses of D2 receptor blockers in humans. Under the conditions of the present study, PDE10AIs were also devoid of effect on palpebral opening, suggesting low sedative side-effect liability (although signs of sedative effects have been reported after MP-10 in primates (Uthayathas et al., 2014)). Furthermore, absence of effect on prolactin release would allow antipsychotic therapy in the absence of hyperprolactinemia.

The systematic analysis of the data set based on fixed criteria for drug-induced effects allows differentiating PDE10AIs from D2 receptor blockers also in models predictive of antipsychotic efficacy. The determination of ED90-8 for different effect levels especially appeared to be a powerful tool to describe differences between both drug classes that are not seen in other published data sets (Schmidt et al., 2008). Understanding such differences may help to better understand the negative phase 2a study results with MP-10 in acute schizophrenia (DeMartinis et al., 2012). In the following text, results are discussed relative to the ED50 for inhibition of apomorphine-induced behavior, as a measure for functional target engagement and antipsychotic potential that applies to both drug classes. Complete blockade of behaviors induced by nondopaminergic stimulants (PCP, scopolamine) and inhibition of conditioned avoidance and escape behavior were obtained more efficiently with PDE10AIs than with specific D2 receptor blockers, as indicated by smaller dose increments relative to the ED50 for apomorphine antagonism. However, complete blockade of behaviors induced by dopamine stimulants (apomorphine, d-amphetamine) required larger dose increments relative to the ED50 for apomorphine antagonism. Further studies also investigated PDE10AIs versus D2 receptor blockers, e.g., in the negative phase 2a study results with MP-10 in acute schizophrenia (DeMartinis et al., 2012). In the following text, results are discussed relative to the ED50 for inhibition of apomorphine-induced behavior, as a measure for functional target engagement and antipsychotic potential that applies to both drug classes. Complete blockade of behaviors induced by nondopaminergic stimulants (PCP, scopolamine) and inhibition of conditioned avoidance and escape behavior were obtained more efficiently with PDE10AIs than with specific D2 receptor blockers, as indicated by smaller dose increments relative to the ED50 for apomorphine antagonism. However, complete blockade of behaviors induced by dopamine stimulants (apomorphine, d-amphetamine) required larger dose increments relative to the ED50 for apomorphine antagonism. Further studies also investigated PDE10AIs versus D2 receptor blockers, e.g., in the negative phase 2a study results with MP-10 in acute schizophrenia (DeMartinis et al., 2012). In the following text, results are discussed relative to the ED50 for inhibition of apomorphine-induced behavior, as a measure for functional target engagement and antipsychotic potential that applies to both drug classes. Complete blockade of behaviors induced by nondopaminergic stimulants (PCP, scopolamine) and inhibition of conditioned avoidance and escape behavior were obtained more efficiently with PDE10AIs than with specific D2 receptor blockers, as indicated by smaller dose increments relative to the ED50 for apomorphine antagonism. However, complete blockade of behaviors induced by dopamine stimulants (apomorphine, d-amphetamine) required larger dose increments relative to the ED50 for apomorphine antagonism.
SCH-23390 further substantiates this countering role of the D1 agonist-like component of PDE10AIs. Likewise, the relatively more pronounced effect of PDE10AIs against non-dopaminergic stimulants and on conditioned avoidance and escape behavior most likely reflects a robust suppression of D2 receptor-mediated neurotransmission, not being counteracted by the increased D1 receptor-mediated neurotransmission that takes place when dopaminergic stimulants are used.

Blockade of D1 receptors with SCH-23390 also unmasks the ability of PDE10AIs to induce catalepsy at doses close to the ED_{50s} for PDE10A occupancy and for inhibition of apomorphine stereotypy. The neurologic safety margin obtained with SCH-23390 is not clear. Because such agitation is observed in the presence of a high dose of a D1 antagonist and at a high level of PDE10A inhibition (known to block D2 receptor-mediated neurotransmission), it is unlikely that stimulation of D1 or D2 receptors is involved.

It cannot be excluded that, besides the efficient suppression of D2 receptor-mediated neurotransmission, interference by NMDA-mediated neurotransmission contributes to the highly efficient PCP antagonism of PDE10AIs. PDE10A inhibition potentiates glutamatergic neurotransmission as indicated by increased phosphorylation of the AMPA-GluR1 and NMDA receptor NR2B subunit (Grauer et al., 2009). Interestingly, PDE10A knockout mice show a blunted locomotor response to the NMDA antagonists PCP and MK-801 compared with corresponding wild-type mice, whereas both types of mice responded similarly to the locomotor effects of the dopaminergic stimulants amphetamine and methamphetamine (Siuciak et al., 2006b). PDE10AIs show a pharmacological profile that is quite different from that of D2 receptor blockers. The unique combination of high efficacy on one hand and reduced liability for some D2 receptor–related side effects, on the other hand, suggests PDE10A inhibition to be a promising novel treatment option for application in schizophrenia and related mental diseases. However, MP-10 was ineffective in a phase 2a proof-of-concept trial against short-term exacerbations of schizophrenia (DeMartinis et al., 2012). In vivo brain imaging studies in patients with schizophrenic demonstrate increased activity of dopaminergic neurons, especially during episodes of illness exacerbation (Laruelle et al., 1999; Erritzoe et al., 2003). In light of our observation that PDE10AIs are relatively less efficacious than D2 receptor blockers against...
Fig. 10. Activity profile in tests related to interactions with various types of receptors. The ED\textsubscript{50} values in the various tests have been expressed as ratios over the ED\textsubscript{50} for inhibition of apomorphine-induced stereotypy. (A) Serotonin 5-HT\textsubscript{2A} antagonism; (B) serotonin 5-HT\textsubscript{2C} antagonism; (C) histamine H\textsubscript{1} antagonism; (D) \(\alpha\textsubscript{1}\)-adrenoceptor blockade; (E) \(\alpha\textsubscript{2}\)-adrenoceptor blockade; (F) muscarinic receptor blockade. The "\textsuperscript{a}a" sign above a bar indicates that the ED\textsubscript{50} is greater than the value indicated by the height of that bar. For explanation of the test name abbreviations, see Table 11.
nonspecific dopaminergic stimuli such as apomorphine and d-amphetamine, these increases in dopamine (acting on D1 and D2 receptor systems) associated with illness exacerbations may explain why MP-10 was not efficacious in these patients. Rather than treatment of acute schizophrenia, it may be proposed that PDE10AIs have potential for treatment-resistant schizophrenia. Such patients are not characterized by enhanced presynaptic function (Demjaha et al., 2012) and may benefit from the broad spectrum of effects of a PDE10AI as compared with D2 receptor blockers.

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**Clonidine-induced Antidiarrheal Activity.** Clonidine (0.02 mg/kg, i.v.)-induced antidiarrheal action in rats challenged simultaneously with castor oil (1 ml, p.o.) was assessed 120 min later. Criterion for drug-induced reversal: presence of diarrhea (3.2% false positive controls; n = 154). Clonidine antagonism reflects blockade of peripheral $\alpha_2$-adrenoceptors. In order to investigate whether intrinsic antidiarrheal activity might have masked the peripheral $\alpha_2$-adrenoceptor blockade, the ability of inactive compounds to block castor oil-induced diarrhea was also studied after injection of saline instead of clonidine. Criterion for antidiarrheal activity: absence of diarrhea (6.7% false positive controls; n = 194).

**Clonidine-induced Mydriasis.** The pupil diameter of the right eye was measured with a graduated microscope (Gant Type 55017; 1 unit = 1/24 mm) just before administration of the test compound or solvent, immediately before injection of clonidine (0.16 mg/kg, i.v.), and at 5, 15 and 30 min after the clonidine challenge. The median pupil diameter over the 5-30 min interval after clonidine challenge was used for further evaluation. A pupil diameter < 25 units after clonidine (occurrence in 0.8% of the control rats) was adopted as all-or-none criterion for inhibition of clonidine-induced mydriasis.

**Compound 48/80-induced Lethality.** Compound 48/80 (0.30 mg/kg, i.v.)-induced lethality was recorded up to 240 min after injection. Criterion for drug-induced protection: > 240 min survival (in controls: 1.2%; n = 750). Histamine H$_1$ antagonists protect against compound 48/80-induced lethality.

**Histamine-induced Lethality in Guinea-Pigs.** Histamine (1.25 mg/ml/kg, i.v.)-induced lethality was recorded in guinea-pigs up to 120 min after the histamine challenge. Criterion for drug-induced protection: > 120 min survival (0.6% false positive controls; n > 300). Histamine H$_1$ antagonists are active in this test.
Mast Cell Serotonin-induced Gastric Lesions. Compound 48/80 (1.0 mg/kg, i.v.)-induced gastric lesions were scored 4 h after challenge in rats (175-275 g) that were protected against lethality by injection, 1 h earlier, of the histamine H₁ antagonist (5-{{4-(diphenylmethyl)piperazin-1-yl}methyl}-1-methyl-1H-benzimidazol-2-yl)methanol (R037617; 10 mg/kg, s.c.). The scoring system was: (3) red areas covering more than half the glandular tissue, (2) large red areas covering less than half the glandular tissue, (1) at least one distinct red area, (0.5) traces of superficial erosion, (0) absent. Criteria for drug-induced effects: score ≤ 1 for inhibition (7.1% false positives in controls; n = 162); score < 1 for blockade (0.6% false positives in controls). Cyanosis of the ears was scored (0, 0.5, 1) 5 min after the injection of compound 48/80. Score < 0.5 was adopted as a criterion for antagonism of cyanosis (0.0% false positives). Protection from gastric lesions and reversal of cyanosis is obtained with peripheral serotonin 5HT₂A antagonists.

Medetomidine-induced Loss of Righting. The duration of medetomidine (0.10 mg/kg, i.v.)-induced loss of righting was recorded. Criterion for drug-induced reversal: duration = 0 min (2.4% false positive controls; n > 500). Centrally-acting α₂-adrenoceptor antagonists or behavioral stimulants antagonize the loss of righting; sedative compounds may result in prolongation.

Norepinephrine-induced Lethality. Survival time after norepinephrine (0.63 mg/kg, i.v.) was recorded up to 1 h after challenge. Survival times > 60 min were considered to reflect significant norepinephrine antagonism (0% false positives in controls; n = 175). Protection against norepinephrine lethality evaluates blockade of peripheral α₁-adrenoceptors.

Norepinephrine-induced Mydriasis. The pupil diameter of the right eye was measured (in 1/24 mm units) with a graduated microscope (Gant Type 55017) 1, 2, 3, 4 and 5 min after the
norepinephrine (0.08 mg/kg, i.v.) challenge. A pupil diameter < 25 units at 1 min after
norepinephrine challenge was used as all-or-none criterion for inhibition of the norepinephrine-
induced mydriasis (3.7% false positives in > 200 solvent-pretreated control rats).

**Physostigmine-induced lethality.** Physostigmine (1.0 mg/kg, i.v.)-induced lethality was
recorded up to 120 min after challenge. Criterion for drug-induced protection: > 120 min
survival (0.0% false positives in > 200 solvent-pretreated control rats). Immediately before the
physostigmine injection, the pupil diameter of the rats was measured with a microscopic
micrometer (1 unit = 1/24 mm). Criteria for drug-induced effects: pupil diameter > 25 units for
mydriasis (in controls: 2.4%), < 10 units for miosis (in controls: 0.5%). Protection against
physostigmine-induced lethality is observed with centrally acting antimuscarinics. Mydriasis is
an expression of peripheral antimuscarinic activity

**Tryptamine-induced Behavior.** Tryptamine (25.0 mg/kg, i.v.)-induced bilateral clonic
seizures of the forepaws and hunched back and palpebral opening were scored the first min after
injection of tryptamine. The direction of locomotion (backward, sideward, or forward) was also
noted. The scoring system was: (A) for bilateral clonic seizures, and hunched back: (3)
pronounced, (2) moderate, (1) slight, and (0) absent; (B) for palpebral opening: (5)
exophthalmos, (4) wide open, (3) open for three-quarters, (2) half open, (1) open for one-quarter,
(0) closed. Criteria for drug-induced inhibition or decrease: bilateral clonic seizures: score < 3
for inhibition (1.5% false positives; n = 300), score < 2 for blockade (0.0% false positives);
palpebral opening: score < 4 for decrease (0.0% false positives); score < 3 for hunched back
(0.0% false positives); locomotion: sideward or forward direction for reversal of backward
locomotion (0.0% false positives). Tryptamine-induced hyperemia or cyanosis of the ears, an
expression of serotonin-induced vascular congestion, was evaluated 2 min after the injection of
tryptamine. Criterion for reversal of cyanosis: hyperemia of the ears (red ears; 0.0% false positives).