Patterns of Brain Glucose Metabolism Induced by Phosphodiesterase 10A Inhibitors in the Mouse: A Potential Translational Biomarker

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ABSTRACT

Phosphodiesterase 10A (PDE10A) inhibitors have recently been proposed as a new therapy for schizophrenia. The aim of this study was to enhance our understanding of the role of PDE10A inhibitors and potentially identify a clinically useful mechanistic/functional biomarker by using 2-deoxyglucose (2-DG) autoradiography. PDE10A inhibitors papaverine (10 and 40 mg/kg), 6,7-dimethoxy-4-[(3R)-3-(2-quinoxalinyloxy)-1-pyrrolidinyl]quinazoline (PQ-10), (0.16–10 mg/kg), and 2-[{4-(1-methyl-4-pyridin-4-yl-1H-pyrazol-3-yl)phenoxy}methyl]quinoline (MP-10) (0.16–40 mg/kg) induced region-specific hypermetabolism in the globus pallidus and lateral habenula of C57BL/6 mice. Studies with MP-10 revealed a dose-dependent relative increase in globus pallidus activation, whereas a bell-shaped curve was observed for the lateral habenula. Although the relative increase in 2-DG uptake in the lateral habenula was also characteristic of the D2 antagonist haloperidol (0.01–0.63 mg/kg), relative 2-DG changes were absent in the globus pallidus. This observation probably is explained by the interaction of PDE10A inhibitors with the D1 direct pathway as suggested by experiments in combination with the D1 agonist (S)-6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide (SKF-82958) (0.16 mg/kg). The absence of an effect of MP-10 (2.5 mg/kg) on relative glucose metabolism in the globus pallidus and lateral habenula of PDE10A knockout mice confirmed the specificity of the signal induced by PDE10A inhibitors. These studies substantiate the regulatory role of PDE10A in the basal ganglia circuit and as such support the potential of PDE10A inhibitors for treating psychiatric disorders. Moreover, we could differentiate PDE10A inhibitors from haloperidol based on specific patterns of hypermetabolism probably caused by its combined action at both direct and indirect dopaminergic pathways. Finally, these specific changes in brain glucose metabolism may act as a translational biomarker for target engagement in future clinical studies.

Introduction

Phosphodiesterase 10A (PDE10A) is an enzyme that inactivates the intracellular second messengers cAMP and cGMP in striatum (Fujishige et al., 1999), as such regulating and compartmentalizing the cyclic nucleotide signaling cascades. PDE10A has high expression in the GABAergic medium spiny neurons of the striatum (Fujishige et al., 1999; Seeger et al., 2003), which provide the main inhibitory input to the basal ganglia via the indirect (D2 receptor-mediated) and direct (D1 receptor-mediated) dopaminergic pathway.

PDE10A inhibitors, which increase striatal cAMP and cGMP levels (Siuciak et al., 2006a; Schmidt et al., 2008; Torremans et al., 2010), and subsequently activate medium spiny neurons, have been suggested for the treatment of neuropsychiatric disorders (Siuciak et al., 2006a; Menniti et al., 2007; Schmidt et al., 2008; Siuciak, 2008). Indeed, the efficacy of PDE10A inhibitors has been demonstrated in preclinical models of the positive, cognitive, and negative symptoms of schizophrenia. The pharmacological inhibition of PDE10A reduced spontaneous activity and stimulant-induced increases in locomotion, attenuated...
conditioned avoidance responding in rats and mice, and blocked N-methyl-D-aspartate antagonist-induced deficits in prepulse inhibition in rats (Siuciak et al., 2006a; Schmidt et al., 2008; Grauer et al., 2009). In assays intended to address negative symptoms and cognitive deficits, improvements were observed in the social approach/social avoidance assay in mice, social odor recognition in mice, and novel object recognition in rats (Grauer et al., 2009).

We have developed and evaluated in rat a specific PET ligand for the PDE10A enzyme (Celen et al., 2010), which will help to determine the occupancy/plasma concentration relationship for early-phase clinical studies once the PET tracer is found suitable for human use. In addition to tools to determine that a clinical candidate reaches and binds the target, biomarkers that can provide evidence for target engagement (demonstration of functional activity) are of great benefit for early clinical studies (Wong et al., 2009). This can significantly aid the drug development program by preventing compounds lacking functional efficacy (at relevant doses for target occupancy) to move forward to expensive patient trials. Several studies have endeavored the use of functional imaging tools such as pharmacological magnetic resonance imaging and fluorodeoxyglucose (FDG) PET to help understand and visualize functional activation at the brain-system level after a pharmacological intervention (McKe et al., 2005; MacIntosh et al., 2008; Buchsbaum et al., 2009; Murphy and Mackay, 2010). However, direct pharmacological magnetic resonance imaging has some limitations in that nonspecific vascular effects and the influence of anesthesia used in animals could potentially bias the interpretation of the blood-oxygen level-dependent signal. FDG PET measures regional brain glucose metabolism and its high-resolution preclinical counterpart 2-deoxyglucose (2-DG) autoradiography is commonly used to examine brain function and activation in awake rodents (Sokoloff et al., 1977; Dedeurwaerder et al., 2011).

The aim of this study was to implement 2-DG autoradiography to get a better understanding of the role of PDE10A inhibitors on a brain-systems level and potentially identify a clinically useful mechanistic/functional biomarker. The dose effect of the typical antipsychotic haloperidol (0.01–0.63 mg/kg) and PDE10A inhibitors papaverine (10 and 40 mg/kg), PQ-10 (0.16–10 mg/kg), and MP-10 (0.16–40 mg/kg) was investigated on basal ganglia activation, more specifically in the globus pallidus (GP) and lateral habenula (hb) in vivo in the mouse. A study by Hosoi et al. (2001) showed decreased striatal glucose metabolism after intrastriatal infusion of dibutyryl-cAMP. Therefore, the effect of peripherally administered MP-10, which should indirectly increase striatal cAMP levels, was evaluated in the striatum. To help understand the mechanism of action of PDE10A inhibitors, an experiment was performed using the D1 agonist (+)-6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide (SKF-82958) in combination with MP-10. Finally, the effects of haloperidol and MP-10 were assessed in PDE10A wild-type (WT) and knockout (KO) mice to address the specificity of the changes in 2-DG uptake induced by the PDE10A inhibitors.

Materials and Methods

Animals. Male C57BL/6 mice (weight 24–28 g) were purchased from Charles River Laboratories (Lyon, France). PDE10A WT and KO mice (Siuciak et al., 2008) were derived in house from a breeding couple purchased from Deltagen (San Mateo, CA). Mice were genotyped by polymerase chain reaction according to protocols obtained from Deltagen. Animals were housed in individually ventilated cages and acclimated for at least 3 days under standard laboratory-controlled conditions (12-h light/dark cycle, temperature 20–24°C, and humidity 40–70%) with water and rodent pellets at libitum. Animals were fasted overnight before the start of the experiment (Fig. 1). All animals were treated in accordance with the European Council Directive (86/609/EEC), the Belgian Animal Welfare Act (Aug. 18, 1986), and the Belgian Royal Decree (April 6, 2010) concerning the protection of laboratory animals. The study protocol was approved by the local ethical committee on animal experiments at Janssen Pharmaceutica N.V. (Beersel, Belgium).

Treatment and Simplified [14C]2-DG Experimental Procedure. Compounds were sourced from Janssen Research and Development (a Division of Janssen Pharmaceutica NV). Haloperidol was dissolved in an H2O solution containing 1 Eq tartaric acid; papaverine and MP-10 (Siuciak, 2008) were dissolved in a solution of 20% 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) containing 2 Eq of tartaric acid; PQ-10 (Siuciak, 2008) was dissolved in a solution of 10% HP-β-CD containing 2 Eq of tartaric acid; and SKF-82958 was dissolved in a solution of 10% HP-β-CD. Treatment was administered subcutaneously at a volume of 10 μl/kg, and administration of solvent was used as control.

The simplified [14C]2-DG experimental procedure was performed according to our standard protocol described previously (Dedeurwaerder et al., 2011). Mice (n = 4–10 animals per treatment group) were treated at t = 0 min by subcutaneous administration of vehicle, haloperidol (0.01, 0.04, 0.16, and 0.63 mg/kg), papaverine (10 and 40 mg/kg), PQ-10 (0.16, 0.63, 2.5, and 10 mg/kg), and MP-10 (0.16, 0.63, 2.5, 10, and 40 mg/kg) in randomized order (Fig. 1). Doses were selected based on previous studies (Siuciak et al., 2006a; Grauer et al., 2009) to cover a range of target occupancies (haloperidol ED50 = 0.02 mg/kg; Natesan et al., 2005; papaverine ED50 >40 mg/kg, PQ-10 ED50 = 13 mg/kg, and MP-10 ED50 = 1.6 mg/kg in rat (in-house data)). To investigate the interaction between PDE10A inhibition and D1 agonism, a combination experiment was performed with three treatment groups (n = 7–10): vehicle of MP-10 (subcutaneously, t = −30 min) with vehicle of SKF-82958 (subcutaneously, t = 0 min), MP-10 (0.63 mg/kg s.c., dose with the highest activation of the lateral habenula, t = −30 min) with vehicle of SKF-82958 (subcutaneously, t = 0 min), and MP-10 (0.63 mg/kg s.c., t = −30 min) with a low dose of SKF-82958 (0.16 mg/kg s.c., t = 0 min) (Fig. 1). To confirm the specificity of the effect of PDE10A inhibitors on 2-DG uptake, vehicle, haloperidol (0.63 mg/kg), or MP-10 (2.5 mg/kg) were tested in male WT and KO PDE10A mice (n = 4–6; Fig. 1).

At t = 15 min, [14C]2-DG (GE Healthcare, Chalfont St. Giles, Buckinghamshire, UK; 59 mCi/mmol, 0.16 μCi/g body weight and at a volume of 10 μl/kg in saline) was administered intraperitoneally. In between procedures, animals were returned to their home cage. Animals were decapitated after a 45-min 2-DG uptake period (Fig. 1), and the brain was rapidly removed, immediately frozen in cooled 2-methylbutane (−20°C, on dry ice), and stored at −20°C until sectioned. Serial coronal sections (20 μm thick) were collected in triplicate at the level of the globus pallidus (−0.58 mm from bregma) and lateral habenula (−1.94 mm from bregma) according to Paxinos and Franklin (2001) on glass slides (SuperFrostPlus Slides; Labo-Nord, Templemars, France) using a cryostat (Leica CM 3050; van Hopplynus Instruments, Brussels, Belgium) and dried rapidly on a hotplate at 60°C. Brain sections were exposed together with a precalibrated [14C] standard on Biomax film (Kodak; PerkinElmer Life and Analytical Sciences, Waltham, MA) in light tight cassettes, which was developed after 4 days of exposure.
Semi-quantitative Densitometric Analysis of [14C]2-DG Autoradiograms. Brain sections were digitized with a light box and digital camera. Local tissue 14C concentration (nanocuries per milligram of tissue equivalent) in each region of interest was determined from the optical density of the autoradiographic brain images relative to the 14C standard, using a computer-based image analysis system (MCID Basic 7.0; InterFocus Imaging Ltd, Cambridge, UK) as described previously (Dedeurwaerdere et al., 2011) or an automated analysis software (Radiology Assay, version 6.2; DCILabs, Keerbergen, Belgium).

A preliminary evaluation was performed by visual inspection of rostral to caudal brain sections in a subset of animals. This indicated obvious activation in the globus pallidus and lateral habenula, whereas other brain regions seemed unaffected by PDE10A inhibitors; therefore, we chose to focus primarily on these two brain regions. Ratios of the globus pallidus and lateral habenula with a normalization region on the same brain section [the caudate putamen (CPu) and thalamic region (Tha), respectively] were calculated to evaluate specific changes in these brain regions relative to their normalization region. In addition, this allowed controlling for variability in raw data caused by differences in treatment conditions such as diet and consequently plasma glucose levels. When comparing data expressed as a ratio, there was no significant difference between fed and fasted animals; hence data were pooled for both dietary conditions (Fig. 1).

For one of the MP-10 dose-response experiments (study 8) plasma glucose was measured that was not significantly different across treatment groups (one-way ANOVA followed by a two-sided Dunnett’s multiple comparison test to compare treatment with vehicle) (Fig. 4). From this experiment, raw data (nanocuries per milligram of tissue equivalent) were presented for the frontoparietal cortex and caudate putamen of the striatum according to Dedeurwaerdere et al. (2011). The frontoparietal cortex, a region with relative very low tissue equivalent) were presented for the frontoparietal cortex and caudate putamen of the striatum according to Dedeurwaerdere et al. (2011).

Statistical Analysis. For analysis of relative 2-DG uptake in the globus pallidus and lateral habenula, data from several studies were grouped for statistical analysis (Fig. 1). Autoradiography data were statistically analyzed using a one-way ANOVA followed by a two-sided Dunnett’s multiple comparison test to compare treatment with vehicle or a two-sided planned Bonferroni multiple comparison test for comparison of selected pairs (Prism, version 4.02; GraphPad Software Inc., San Diego, CA). For the raw 2-DG uptake data, two-way ANOVA was used after a Bonferroni multiple comparison test with the vehicle group. Data are shown as mean ± S.E.M.

Results

Effect of PDE10A Inhibitors on 2-DG Uptake in Mice. A dose-dependent reduction in spontaneous locomotion was visually observed during treatment with the different compounds. Although only qualitative, this observation confirmed the regulatory role of PDE10A on motor function (Schmidt et al., 2008) and indicated that the compounds were tested in a relevant dose range for assessing central activity. The minimal effective dose (MED) needed for the different PDE10A inhibitors to induce specific changes in relative 2-DG uptake in the globus pallidus and lateral habenula was as follows: MP-10 (MEDGP,hb = 0.63 mg/kg) < PQ-10 (MEDGP = 10 mg/kg and MEDhb = 2.5 mg/kg) < papaverine (MEDGP,hb = 40 mg/kg) (Figs. 2 and 3). Starting at 0.63 mg/kg, MP-10 significantly increased both the globus pallidus ratio (p < 0.001) and the lateral habenula ratio (p < 0.001). The globus pallidus ratio was significantly higher with increasing doses (p < 0.001), in the lateral habenula; however, a bell-shape-like response was measured (Fig. 3). The D2 antagonist haloperidol induced a dose-dependent significant increase in relative 2-DG uptake in the lateral habenula (p < 0.001), whereas changes in the globus pallidus were absent (Figs. 2 and 3).
Fig. 2. Autoradiographic 2-DG images of brain sections across the globus pallidus (top) and lateral habenula (bottom) in mice treated with vehicle, haloperidol, papaverine, PQ-10, and MP-10. Arrows indicate the regions of interest on the brain sections, globus pallidus and lateral habenula.

Fig. 3. Dose-response effect of vehicle, haloperidol (A and B), papaverine (C and D), PQ-10 (E and F), and MP-10 (G and H) on relative 2-DG uptake in the globus pallidus and lateral habenula. Data were statistically analyzed using a one-way ANOVA followed by a two-sided Dunnett's multiple comparison test to compare treatment dose with vehicle. To statistically calculate dose-response effects for haloperidol and MP-10 a one-way ANOVA was used on the different doses, and two-sided planned Bonferroni multiple comparison test was used for comparison of selected pairs for the MP-10-induced bell-shaped curve in the lateral habenula. n.s., not significant; *, p < 0.05; **, p < 0.01; ***, p < 0.001.

Fig. 4. Dose-response effect of MP-10 on 2-DG uptake in frontoparietal cortex (FrParCx) and caudate putamen of the striatum (CPu). There were no significant effects of MP-10 on plasma glucose levels (right). To statistically calculate the dose-response effect of MP-10, a two-way ANOVA was used on the different doses, and a two-sided planned Bonferroni multiple comparison test was used for comparison of the different doses with vehicle. *, p < 0.05.

An overall significant dose-dependent reduction in 2-DG uptake was observed in the frontoparietal cortex and caudate putamen of the striatum after MP-10 treatment (p < 0.05); however, post hoc Bonferroni test did not reveal significant differences between vehicle and different doses of MP-10 (Fig. 4).

In the MP-10 and SKF-82958 combination experiment, significantly increased globus pallidus and lateral habenula ratios were confirmed at 0.63 mg/kg s.c. of MP-10 (Fig. 5). It is noteworthy that the D1 agonist SKF-82958 showed a potentiating effect on MP-10-induced relative 2-DG activation in the globus pallidus, whereas in the lateral habenula MP-10-induced brain activation was attenuated by SKF-82958 (Fig. 5).

Effect of PDE10A Inhibitors on 2-DG Uptake in PDE10A KO Mice. PDE10A KO mice, which do not express the PDE10A enzyme, have higher ratios of 2-DG uptake in the globus pallidus than WT animals (Fig. 6), similar to what is seen after administration of a PDE10A inhibitor. Relative glucose metabolism in the lateral habenula, on the other hand, is not changed in the PDE10A KO mice. Administration of haloperidol in PDE10A KO mice did not modify relative 2-DG uptake in the globus pallidus (Fig. 6) as expected. In the lateral habenula, there was a trend for...
haloperidol to induce an increase in relative 2-DG uptake signal in the PDE10A KO mice ($p = 0.06$), however, not to the same extent as in WT animals. MP-10, on the other hand, did not affect the globus pallidus or lateral habenula ratio at all in KO mice. This confirms that the hypermetabolism observed in these regions in WT animals after MP-10 administration is characteristic of the inhibition of the PDE10A enzyme.

**Discussion**

Our studies support the regulatory role of PDE10A in basal ganglia circuitry. In particular, PDE10A inhibitors increased glucose metabolism in the globus pallidus and lateral habenula, which has not been demonstrated before. Moreover, these activation patterns are specific to PDE10A inhibition, because the changes were not measured after treatment with typical antipsychotics such as haloperidol or in PDE10A KO mice. It is noteworthy that the results of this study may lead to a translational imaging biomarker that could guide dose selection in humans by using FDG PET. It is noteworthy that a dose-ascending study with MP-10 indicated a dissociation of the dose effect on the globus pallidus (dose response) compared with the lateral habenula (bell shape), which may help us understand the action of PDE10A inhibitors affecting the indirect and direct pathways of the basal ganglia. Indeed, administration of the D$_1$ agonist SKF-82958 further increased MP-10-induced 2-DG uptake in the globus pallidus (dose response), whereas reversed MP-10 induced brain activation in the lateral habenula in line with the bell-shaped curve obtained after MP-10 ascending doses. These results further substantiate that the mechanism of action of MP-10 is in part mediated by the direct pathway.

The PDE10A inhibitors papaverine, PQ-10, and MP-10 showed region-specific increases in 2-DG uptake in the globus pallidus (equivalent to the external segment of the globus pallidus in primates) and the lateral habenula in mice. The globus pallidus is part of the dopaminergic indirect pathway and involved in motor control (Kita, 2007). The lateral habenula is also connected to the basal ganglia system and involved in the processing of rewarding stimuli, motor control, avoidance learning and error monitoring, anxiety, stress, and pain (Kimura et al., 2007). Although poorly explored, the lateral habenula has been proposed to be a key nucleus in the pathophysiology of psychiatric disorders, including schizophrenia (Hikosaka, 2010). The lateral habenula processes information from limbic and striatal forebrain to regulatory midbrain nuclei and is substantially involved in the regulation of central dopaminergic transmission. The present study supports such a function.

Dose-response studies determined that the MED to induce specific changes in 2-DG uptake in the globus pallidus and lateral habenula by the different PDE10A inhibitors was as follows: MP-10 (MED = 0.63 mg/kg) < PQ-10 (MEDI$_{PQ}$ = 10 mg/kg and MED$_{hb}$ = 2.5 mg/kg) < papaverine (MED = 40 mg/kg) in accordance with their in vitro potencies, MP-10 (IC$_{so}$ = 0.48 nM) > PQ-10 (IC$_{so}$ = 4.6 nM) > papaverine (IC$_{so}$ = 36 nM) (Siuciak, 2008). Moreover, the doses required for the induction of specific 2-DG changes by the PDE10A inhibitors were also in line with doses generally needed for efficacy in behavioral assays (Siuciak et al., 2006a; Grauer et al., 2009). It is noteworthy that haloperidol, in agreement with previous studies (Pizzolato et al., 1984; Duncan et al., 1998), induced an increase in 2-DG uptake in the lateral habenula, the MED corresponding with 100% D$_2$ receptor occupancy (Natesan et al., 2005). PDE10A inhibitors, on the other hand, were already effectively inducing 2-DG changes at 10 to 40% occupancy of the PDE10A enzyme (in-house data). This could indicate that targeting this downstream cascade could be more effective than manipulation of the D$_2$ receptor itself.

The agreement between doses of 2-DG-induced changes by the PDE10A inhibitors, behavioral efficacy, and receptor occupancy adds value to the suggestion that PDE10A inhibitor-induced brain activation could act as a translational functional biomarker in FDG PET studies. Our group has demonstrated previously the translational value of preclinical 2-DG rodent studies for human FDG PET regarding the induction of brain glucose patterns by corticotrophin-releasing factor receptor type 1 antagonists (Warnock et al., 2009; Schmidt et al., 2010). Nevertheless, it remains to be evaluated in the clinic whether the inhibition of the PDE10A enzyme in humans will result in the same activation pathways and subsequent increased glucose metabolism in the globus pallidus and lateral habenula as seen here in mice. Nonhuman primate studies could act as an intermediate step before clinical FDG studies rather than rodents in view of the limited resolution of small-animal PET for visualization of the globus pallidus and lateral habenula. Results from such pharmacodynamic/pharmacokinetic imaging studies would provide information on the relationship between drug plasma concentrations and an objective measurement of the pharmacodynamic action in the brain visualized with FDG PET.
The mechanism of action of PDE10A inhibitors is thought to be mediated by increasing cAMP and cGMP levels in striatum (Siuciak et al., 2006a; Schmidt et al., 2008; Torremans et al., 2010). Direct infusion of dibutyryl-cAMP in the striatum has resulted in decreased striatal 2-DG uptake (19%), whereas Rp-adenosine-3’,5’-cyclic monophosphorothioatetriethylamine (inhibitor of cAMP-dependent protein kinase) reversed this reduction and increased striatal 2-DG uptake when administered alone (Hosoi et al., 2001). This study focused only on striatum. Our study showed a similar dose-depending decrease by MP-10 in the caudate putamen (up to 22%) and the frontoparietal cortex (up to 28%). However, these changes may be general and rather global effects of PDE10A inhibition because they were not limited to the caudate putamen. The alterations in the globus pallidus and lateral habenula, on the other hand, are very regional, and we believe they represent downstream effects of PDE10A inhibitors via the medium spiny neurons of the striatum.

During dose-escalation studies it became clear that MP-10 induced a dose-dependent hypermetabolism in the globus pallidus. Activation of the globus pallidus was not observed after administration of haloperidol and therefore was of particular interest. Comparing the 2-DG uptake of haloperidol and MP-10 allows us to better delineate the mechanism of action of PDE10A inhibitors. The well established antipsychotic haloperidol exerts its therapeutic activity by selectively blocking the dopamine D2 receptor, leading to a disinhibition of the indirect pathway. The globus pallidus is a direct projection site of the striatum. Increased brain glucose metabolism in the globus pallidus could be interpreted as a combination of increased activity in the nerve terminals of medium spiny neurons, probably because of increased cAMP levels after PDE10A inhibition, and dendrites of the globus pallidus. Increased glucose metabolism seen in the globus pallidus would then mainly reflect the activation of the caudate putamen. However, this interpretation may be too simplistic, because D2 antagonists, such as haloperidol, which result in disinhibition of the indirect pathway, do not increase 2-DG uptake in the globus pallidus. Given our working hypothesis that PDE10A activates both striatal output pathways, we also looked at the influence of D1 agonists on MP-10-induced 2-DG uptake in the globus pallidus. The observed potentiation by SKF-82958 suggested the involvement of the D1 pathway. In addition, the fact that haloperidol does not induce

![Fig. 6. Haloperidol and MP-10 studies in WT and KO PDE10A mice. A, autoradiographic 2-DG images of brain sections across globus pallidus (top) and lateral habenula (bottom) in vehicle-, haloperidol-, and MP-10-treated animals. Arrows indicate regions of interest on the brain sections: globus pallidus and lateral habenula. B to E, relative 2-DG uptake in the globus pallidus and lateral habenula after haloperidol (B and D) or MP-10 (C and E) in WT and KO PDE10A mice. 0.63 indicates 0.63 mg/kg dose, and 2.5 indicates 2.5 mg/kg dose. Data were statistically analyzed using a one-way ANOVA followed by a two-sided planned Bonferroni multiple comparison test for comparison of selected pairs. n.s., not significant; *, p < 0.05; **, p < 0.01.](https://www.aspetjournals.org/petj)
metabolic change in the globus pallidus suggests that concomitant activation of both pathways, such as occurring with a PDE10A inhibitor, is required to induce hypermetabolism in the globus pallidus. Further work combining D_1/D_2 agonist/antagonists would be necessary to better delineate this particular effect of PDE10A inhibition.

Another difference between MP-10 and haloperidol was the observation of a bell-shaped curve by ascending doses of MP-10 in the lateral habenula, whereas administration of haloperidol resulted in a dose-dependent increase. Papaverine and PQ-10, both displaying lower potencies than MP-10, were not tested at high enough doses to evaluate the bell-shaped effect in the lateral habenula. The increase of 2-DG uptake by D_2 antagonists in the lateral habenula is thought to be mediated by the GABA fibers projecting from the entopeduncular nucleus via the stria medullaris (Ellison, 1994). The entopeduncular nucleus is considered as a structure integrating signals from both the direct and indirect pathways because it receives inputs from the striatum, globus pallidus, and subthalamic nucleus (Hauber, 1998). Therefore, it is tempting to speculate that for the lateral habenula the increase of 2-DG uptake by MP-10 would be mediated through the activation of the indirect pathway, such as for the D_2 antagonists, whereas the attenuated response seen at higher doses would be related to the activation of the direct pathway. Indeed, the involvement of both pathways in the mechanism of action of the PDE10A inhibitors is likely to explain the differences demonstrated between MP-10 and the typical antipsychotic haloperidol because we showed that administration of a D_2 agonist attenuated the effect of a medium dose of MP-10 in the lateral habenula. It is noteworthy that it has been suggested that this mixed mechanism of action through both direct and indirect pathways may explain the very different catalepsy profile between PDE10A inhibitors and haloperidol (Schmidt et al., 2008; Grauer et al., 2009).

PDE10A knockout mice show a slight decrease in spontaneous locomotor activity in a novel environment and inhibition of conditioned avoidance behavior (Siciak et al., 2008). We showed that PDE10A KO mice have significantly elevated baseline levels of 2-DG uptake in the globus pallidus compared with WT animals, similar to what is seen after administration of a PDE10A inhibitor. This observation in PDE10A KO mice cannot be simply attributed to increased cGMP or cAMP levels, because the lack of PDE10A enzyme in these mice does not alter basal levels of striatal cGMP or cAMP (Siciak et al., 2008). Siciak et al. (2008) suggested the possibility of a net increase in striatal activity caused by enhanced sensitivity to glutamatergic input in the PDE10A KO mice. Conversely, glucose metabolism in the lateral habenula is not enhanced in the PDE10A KO mice. It could be hypothesized that this is caused by certain endogenous compensatory mechanisms, in an attempt to impose homeostasis in the PDE10A KO mice. However, attenuation of 2-DG uptake in the lateral habenula could at least also be partially mediated by the same mechanisms that regulate the bell-shaped curve at higher doses of MP-10.

Administration of haloperidol or MP-10 in PDE10A KO mice did not further modify normalized 2-DG uptake in the globus pallidus as expected. In the lateral habenula, haloperidol induced an increase in relative 2-DG uptake in the PDE10A KO mice, however, not to the same extent as in WT animals. Attenuation of haloperidol’s effect was also observed on cGMP measurements in PDE10A KO mice (Siciak et al., 2006b). This may relate to the fact that in PDE10A KO mice a baseline increase in lateral habenula ratio was not observed in contrast to the globus pallidus. MP-10, on the other hand, did not affect relative glucose metabolism at all in the lateral habenula of PDE10A KO mice, which confirms its action via the PDE10A enzyme.

In conclusion, our studies confirm the regulatory role of PDE10A in the dopaminergic basal ganglia circuit and suggest the potential of PDE10A inhibitors for treating psychiatric disorders. It is noteworthy that our results suggest an important role of the D_2 direct pathway in the mechanism of the action of PDE10A inhibitors. In addition, we could differentiate PDE10A inhibitors from the typical D_2 antagonist haloperidol based on the pattern of glucose hypermetabolism in the globus pallidus and lateral habenula. Finally, the specific brain activation patterns induced by PDE10A inhibitors hold promise as a translational biomarker in future studies.

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