Patterns of brain glucose metabolism induced by phosphodiesterase 10A inhibitors in the mouse: a potential translational biomarker.

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Running title page

Phosphodiesterase 10A inhibitor-induced brain activation

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Nonstandard abbreviations:

2-DG: 2-deoxyglucose
FDG: fluorodeoxyglucose
IC_{50}: 50% inhibitory concentration
KO: knockout
MED: minimal effective dose
PDE10A: phosphodiesterase 10A
phMRI: pharmacological Magnetic Resonance Imaging
WT: wild-type

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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 on and to potentially identify a clinically useful mechanistic/functional biomarker using 2-deoxyglucose (2-DG) autoradiography. PDE10A inhibitors papaverine (10 and 40 mg/kg), PQ-10 (0.16-10 mg/kg) and 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, while a bell-shape curve was observed for the lateral habenula. While the relative increase in 2-DG uptake in lateral habenula was also characteristic of D2 antagonist haloperidol (0.01-0.63 mg/kg), relative 2-DG changes were absent in the globus pallidus. This observation is likely explained by interaction of PDE10A inhibitors with the D1 direct pathway as suggested by experiments in combination with D1 agonist SKF-82958 (0.16 mg/kg). The absence of an effect of MP-10 (2.5 mg/kg) on relative glucose metabolism in 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 likely due to 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.
1. 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 signalling 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 treatment of neuropsychiatric disorders (Siuciak et al., 2006a; Menniti et al., 2007; Schmidt et al., 2008; Siuciak, 2008). Indeed, efficacy of PDE10A inhibitors has been demonstrated in preclinical models of the positive, cognitive, and negative symptoms of schizophrenia. Pharmacological inhibition of PDE10A reduced spontaneous activity and stimulant-induced increases in locomotion, attenuated conditioned avoidance responding in rats and mice and blocked NMDA 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 odour recognition in mice and novel object recognition in rats (Grauer et al., 2009).

Recently, we have developed and evaluated in rat a specific PET ligand for the PDE10A enzyme (Celen et al., 2010), which will help to determine occupancy/plasma concentration relationship for early phase clinical studies once the PET tracer is found suitable for human use. Besides 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., 2008). 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. Recently, several studies have endeavoured the use of functional imaging tools like pharmacological MRI (phMRI) and fluorodeoxyglucose (FDG) PET to help understand and to visualise functional activation at the brain-system level after a pharmacological intervention (Murphy and Mackay; McKie et al., 2005; MacIntosh et al., 2008; Buchsbaum et al., 2009). However, direct phMRI has some limitations in that non-specific vascular effects and the influence of anaesthesia used in animals, could potentially bias the interpretation of the BOLD 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; Dedeurwaerdere et al., 2011).

The aim of this study is to implement 2-DG autoradiography to get a better understanding of the role of PDE10A inhibitors on a brain systems level and to 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 globus pallidus and lateral habenula in vivo in the mouse. A study by Hosoi et al. (2002) showed decreased striatal glucose metabolism after intrastriatal infusion of db-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 D1 agonist 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.
2. Methods

2.1 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, USA). Mice were genotyped by polymerase chain reaction according to protocols obtained from Deltagen. Animals were housed in individually ventilated cages and acclimatised 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. In the more recent experiments, animals were fasted over night before the start of the experiment (Fig. 1). All animals were treated in accordance with the European Ethics Committee (decree 86/609/CEE), the Animal Welfare Act (7 USC 2131) and the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioural Research (National Research Council 2003). The study protocol was approved by the local animal experimental ethical committee at Janssen Pharmaceutica N.V. (Beerse, Belgium).

2.2 Treatment and simplified [14C]2-DG experimental procedure

Compounds were sourced from Janssen Research & Development (a Division of Janssen Pharmaceutica NV, Beerse, Belgium). Haloperidol was dissolved in a H2O solution containing 1 equivalent of tartaric acid; papaverine and MP-10 (Siuciak, 2008) were dissolved in a solution of 20% 2-hydroxylpropyl-beta-cyclodextrin (HP-β-CD) containing 2 equivalents of tartaric acid; PQ-10 (Siuciak, 2008) was dissolved in a solution of 10% HP-β-CD containing 2 equivalents of tartaric acid; SKF-82958 was dissolved in a solution of 10% HP-β-CD. Treatments were administered subcutaneously (s.c.) at a volume of 10 ml/kg and administration of solvent was used as control.
The simplified $[^{14}\text{C}]{\text{2-DG}}$ experimental procedure was performed according to our standard protocol described previously (Dedeurwaerdere et al., 2011). Mice ($n= 4-10$ animals per treatment group) were treated at $t= 0$ min by s.c. 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 $ED_{50}= 0.02$ mg/kg (Natesan et al., 2005); papaverine $ED_{50}> 40$ mg/kg, PQ-10 $ED_{50}= 13$ mg/kg and MP-10 $ED_{50}= 1.6$ mg/kg in rat (in house data)).

To investigate the interaction between PDE10A inhibition and D$_1$ agonism, a combination experiment was performed with three treatment groups ($n= 7-10$): vehicle of MP-10 (s.c., $t= -30$ min) with vehicle of SKF-82958 (s.c., $t= 0$ min), MP-10 ($0.63$ mg/kg, dose with the highest activation of the lateral habenula, s.c., $t= -30$ min) with vehicle of SKF-82958 (s.c., $t= 0$ 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, $[^{14}\text{C}]{\text{2-DG}}$ (GE Healthcare, Chalfont St Giles, UK, $59$ mCi/mmol, $0.16$ µCi/g body weight and at a volume of $10$ ml/kg in saline) was administered intraperitoneally (i.p.). In between procedures, animals were returned to their home cage. Animals were decapitated following a $45$ min 2-DG uptake period (Fig. 1), the brain rapidly removed and 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 (SuperFrost Plus Slides, LaboNord, 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 $[^{14}C]$standard on Biomax film (Kodak, Perkin Elmer, Cambridge, UK) in light tight cassettes, which was developed after four days of exposure.

2.3 Semi-quantitative densitometric analysis of $[^{14}C]2$-DG autoradiograms

Brain sections were digitised with a light box and digital camera. Local tissue $[^{14}C]$concentration (nCi/mg tissue equivalent - TE) in each region of interest was determined from the optical density of the autoradiographic brain images relative to the $[^{14}C]$standard, using a computer-based image analysis system (MCID Basic 7.0, UK) as described previously (Dedeurwaerdere et al., 2011) or an automated analysis software (Radiology assay v6.2, DCILabs, Belgium).

A preliminary evaluation was performed by visual inspection of rostral to caudal brain sections in a sub-set of animals. This indicated obvious activation in globus pallidus and lateral habenula, while other brain regions seemed unaffected by PDE10A inhibitors; therefore, we chose to focus on these two brain regions mainly. Ratios of the globus pallidus and lateral habenula with a normalisation region on the same brain section (the caudate putamen and thalamic region, respectively) were calculated to evaluate specific changes in these brain regions relative to their normalisation region. In addition, this allowed controlling for variability in raw data due to 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 and hence data was pooled for both dietary conditions (Fig. 1).

For one of the MP-10 dose-response experiments (study 8) plasma glucose was measured which was not significantly different across treatment groups (One-way ANOVA followed by a 2-sided Dunnett’s Multiple Comparison Test to compare treatment with vehicle)(Fig. 4). From this experiment, raw data (nCi/mg TE) is presented for frontoparietal
cortex and the caudate putamen of the striatum according to Dedeurwaerdere et al. (2011). The frontoparietal cortex, a region with relative very low expression of PDE10A (Seeger et al., 2003) was analysed to examine the region specific character of the effects.

2.4 Statistical analysis

For the analysis of relative 2-DG uptake in globus pallidus and lateral habenula, data from several studies was grouped for statistical analysis (Fig. 1). Autoradiography data was statistically analysed using a one-way ANOVA followed by a 2-sided Dunnett’s Multiple Comparison Test to compare treatment with vehicle or a 2-sided planned Bonferroni Multiple Comparison Test for comparison of selected pairs (GraphPad Prism, v4.02). For the raw 2-DG uptake data, two-way ANOVA was used following a Bonferroni Multiple Comparison Test with vehicle group. Data is shown as mean ± S.E.M.
3. Results

3.1 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. Whereas 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 globus pallidus (GP) and lateral habenula (hb) was as following: MP-10 (MED$_{GP,\ hb}$= 0.63 mg/kg) < PQ-10 (MED$_{GP}$= 10 mg/kg and MED$_{hb}$= 2.5 mg/kg) < papaverine (MED$_{GP,\ hb}$= 40 mg/kg) (Fig. 2 & 3). Starting at 0.63 mg/kg, MP-10 significantly increased both globus pallidus ratio (p< 0.001) and lateral habenula ratio (p< 0.001). While the globus pallidus ratio was significantly higher with increasing doses (p< 0.001), in the lateral habenula, on the contrary, a bell-shape like response was measured (Fig. 3). The D$_2$ antagonist haloperidol induced a dose-dependent significant increase in relative 2-DG uptake in lateral habenula (p< 0.001), while changes in globus pallidus were absent (Fig. 2 & 3).

An overall significant dose-dependent reduction in 2-DG uptake was observed in 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). Interestingly, D$_1$ agonist SKF-82958 showed to have a potentiating effect on the MP-10 induced relative 2-DG activation in the globus pallidus, while in the lateral habenula, on the contrary, MP-10 induced brain activation was attenuated by SKF-82958 (Fig. 5).
3.2 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.
4. Discussion

Our studies support the regulatory role of PDE10A in the basal ganglia circuitry. In particular, PDE10A inhibitors increased glucose metabolism in globus pallidus and lateral habenula, which has not been demonstrated before. Moreover, these activation patterns are specific to PDE10A inhibition, as the changes were not measured after treatment with typical antipsychotics such as haloperidol nor in PDE10A KO mice. Importantly, the results of this study may lead to a translational imaging biomarker that could guide dose-selection in humans using FDG PET. Interestingly, a dose-ascending study with MP-10 indicated a dissociation of the dose-effect on globus pallidus (dose-response) compared to 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 D1 agonist SKF-82958 further increased MP-10 induced 2-DG uptake in globus pallidus (dose-response), while reversed MP-10 induced brain activation in the lateral habenula in line with the bell-shape 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.

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 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). Whereas 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 is processing information from limbic and striatal forebrain to
regulatory midbrain nuclei and is substantially involved in the regulation of central dopaminergic transmission. The present study is supporting such function.

Dose-response studies determined that MED to induce specific changes in 2-DG uptake in globus pallidus and lateral habenula by the different PDE10A inhibitors was as following: MP-10 (MED= 0.63 mg/kg) < PQ-10 (MED_{GP}= 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_{50}= 0.48 nM) > PQ-10 (IC_{50}= 4.6 nM) > papaverine (IC_{50}= 36 nM)(Siuciak, 2008). Moreover, the doses required for induction of specific 2-DG changes by the PDE10A inhibitors were also in line with doses generally needed for efficacy in behavioural assays (Siuciak et al., 2006a; Grauer et al., 2009). Interestingly, for haloperidol, which 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 corresponds with 100% D₂ receptor occupancy (Natesan et al., 2005). PDE10A inhibitors on the other hand were already effectively inducing 2-DG changes at 10-40% occupancy of the PDE10A enzyme (in house data). This could indicate that targeting this downstream cascade could be more effective than manipulation of the D₂ receptor itself.

The agreement between doses of 2-DG induced changes by the PDE10A inhibitors, behavioural 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 previously demonstrated the translational value of preclinical 2-DG rodent studies for human FDG PET regarding the induction of brain glucose patterns by CRF₁ 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 human will result in the same activation pathways and subsequent increased glucose metabolism in globus pallidus and lateral habenula as seen here in mice. Non-human 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 visualisation of 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 visualised 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). In recent work, direct infusion of dibutyryl-cyclic adenosine monophosphate (db-cAMP) in the striatum resulted in a decreased striatal 2-DG uptake (19%), while Rp-adenosine-3',5'-cyclic monophosphorothioate triethylamine (Rp-cAMPS, inhibitor of cAMP-dependent protein kinase) reversed this reduction and increased striatal 2-DG uptake when administered alone (Hosoi et al., 2001). This study focussed on striatum only. Our study showed a similar dose-depending decrease by MP-10 in caudate putamen (up to 22%) and frontoparietal cortex (up to 28%). However, these changes may be general and rather global effects of PDE10A inhibition as they were not limited to the caudate putamen. The alterations in 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.

Performing dose-escalation studies it becomes 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 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 is exerting 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, likely due to increased cAMP levels after PDE10A inhibition, and dendrites of the globus pallidus. Increased glucose metabolism seen in the globus pallidus would then mainly reflect activation of the caudate putamen. However, this interpretation may be to simplistic, as D₂ antagonists such as haloperidol, which results 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 agonist on MP-10-induced 2-DG uptake in the globus pallidus. The observed potentiation by SKF-82958 suggested the involvement of the D₁ pathway. Also, the fact that haloperidol does not induce metabolic change in the globus pallidus suggests that concomitant activation of both pathways, like occurring with a PDE10A inhibitor, is required to induce hypermetabolism in the globus pallidus. Further work combining D₁/D₂ agonist/antagonist 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-shape curve by ascending doses of MP-10 in the lateral habenula, while administration of haloperidol on the contrary 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-shape effect in the lateral habenula. The increase of 2-DG uptake by D₂ 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 pathway since it receives inputs from the striatum, the globus pallidus and the 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, like for the D₂ 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 since we showed that administration of a D1 agonist attenuated the effect of a medium dose of MP-10 in the lateral habenula. Interestingly, 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<sup>−/−</sup> knockout mice show a slight decrease in spontaneous locomotor activity in a novel environment and inhibition of conditioned avoidance behavior (Siuciak et al., 2008). We showed that PDE10A KO mice have significantly elevated baseline levels of 2-DG uptake in the globus pallidus compared to 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, as opposingly the lack of PDE10A enzyme in these mice does not alter basal levels of striatal cGMP or cAMP (Siuciak et al., 2008). Siuciak et al (2008) suggested the possibility of a net increase in striatal activity due to 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 due to 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-shape 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. In line, attenuation of haloperidol’s effect was also
observed on cGMP measurements in PDE10A<sup>DB</sup> KO mice (Siuciak et al., 2006b). This may relate back 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 dopaminergic basal ganglia circuit, and as such support the potential of PDE10A inhibitors for treating psychiatric disorders. Interestingly, our results suggest an important role of the D<sub>1</sub> direct pathway in the mechanism of action of PDE10A inhibitors. In addition, we could differentiate PDE10A inhibitors from the typical D<sub>2</sub> antagonist haloperidol based on the pattern of glucose hypermetabolism in globus pallidus and lateral habenula. Finally, the specific brain activation patterns induced by PDE10A inhibitors hold promise to be used as a translational biomarker in future studies.
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Authorship contributions:

Participated in research design: Dedeurwaerdere, Vanhoof and Langlois

Conducted experiments: Dedeurwaerdere and Wintmolders

Contributed new reagents or analytic tools: NA

Performed data analysis: Dedeurwaerdere and Wintmolders

Wrote or contributed to the writing of the manuscript: Dedeurwaerdere, Vanhoof and Langlois
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Siuciak JA, McCarthy SA, Chapin DS, Fujiwara RA, James LC, Williams RD, Stock JL, McNeish JD, Strick CA, Menniti FS and Schmidt CJ (2006b) Genetic deletion of the


Footnotes

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Legends for figures

Fig. 1: Study design and overview of experiments.

Fig. 2: Autoradiographic 2-DG images of brain sections across globus pallidus (top panel) and lateral habenula (bottom panel) in vehicle, haloperidol, papaverine, PQ-10 and MP-10 treated (MED) mice. Arrows indicated regions of interest on the brain sections: globus pallidus and lateral habenula respectively.

Fig. 3: Dose-response effect of vehicle, haloperidol (A, B), papaverine (C, D), PQ-10 (E, F) and MP-10 (G, H) on relative 2-DG uptake in globus pallidus and lateral habenula. Abbreviations: veh, vehicle; GP, globus pallidus; CPu, caudate putamen; hb, lateral habenula; Tha, thalamic region. Data were statistically analysed using a one-way ANOVA followed by a 2-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 2-sided planned Bonferroni Multiple Comparison Test for comparison of selected pairs for the MP-10 induced bell-shape curve in the lateral habenula. *p<0.05, **p<0.01 and ***p<0.001.

Fig. 4: Dose-response effect of MP-10 on 2-DG uptake in frontoparietal cortex (FrPar Cx) and caudate putamen of the striatum (CPu). There were no significant effects of MP-10 on plasma glucose levels (right panel). Abbreviations: veh, vehicle. To statistically calculate dose-response effect of MP-10 a two-way ANOVA was used on the different doses and a 2-sided planned Bonferroni Multiple Comparison Test for comparison of the different doses with vehicle. *p<0.05.
Fig. 5: Combination experiment with MP-10 (0.63 mg/kg) and SKF-82958 (0.16 mg/kg). Relative 2-DG uptake in globus pallidus (A) and lateral habenula (B). Abbreviations: V, vehicle; J, SKF-82958; GP, globus pallidus; CPu, caudate putamen; hb, lateral habenula; Tha, thalamic region. Data were statistically analysed using a one-way ANOVA followed by a 2-sided planned Bonferroni Multiple Comparison Test for comparison of selected pairs *p<0.05 and **p<0.001.

Fig. 6: Haloperidol and MP-10 studies in wild-type (WT) and knockout (KO) PDE10A mice. A. Autoradiographic 2-DG images of brain sections across globus pallidus (top panel) and lateral habenula (bottom panel) in vehicle, haloperidol, and MP-10 treated animals. Arrows indicated regions of interest on the brain sections: globus pallidus and lateral habenula respectively. Relative 2-DG uptake in globus pallidus and lateral habenula after haloperidol (B, D) or MP-10 (C, E) in WT and KO PDE10A mice. Abbreviations: veh, vehicle; GP, globus pallidus; CPu, caudate putamen; hb, lateral habenula; Tha, thalamic region; 0.63, 0.63 mg/kg; 2.5, 2.5 mg/kg. Data was statistically analysed using a one-way ANOVA followed by a 2-sided planned Bonferroni Multiple Comparison Test for comparison of selected pairs. *p<0.05 and **p<0.01.
Fig. 1

<table>
<thead>
<tr>
<th>Study</th>
<th>Pretreatment</th>
<th>Test compound</th>
<th>Dose (mg/kg)</th>
<th>Diet</th>
<th>N per group</th>
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<tbody>
<tr>
<td>1</td>
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<td>Haloperidol</td>
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<td>0, 10 and 40</td>
<td>Fed</td>
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<td>0, 2.5 and 10</td>
<td>Fed</td>
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<td>PQ-10</td>
<td>0, 0.16, 0.63 and 2.5</td>
<td>Fed</td>
<td>4-5</td>
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<tr>
<td>6</td>
<td></td>
<td>MP-10</td>
<td>0, 2.5 and 10</td>
<td>Fed</td>
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</tr>
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<td>7</td>
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<td>MP-10</td>
<td>0, 0.16, 0.63 and 2.5</td>
<td>Fed</td>
<td>5</td>
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<tr>
<td>8</td>
<td></td>
<td>MP-10</td>
<td>0, 2.5, 10 and 40</td>
<td>Fasted</td>
<td>8-10</td>
</tr>
<tr>
<td>9</td>
<td>MP-10 or vehicle</td>
<td>SKF-82958</td>
<td>0, 0.16</td>
<td>Fasted</td>
<td>7-10</td>
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<tr>
<td>10</td>
<td>WT and KO</td>
<td>Haloperidol</td>
<td>0 and 0.63</td>
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<td>MP-10</td>
<td>0 and 2.5</td>
<td>Fasted</td>
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</table>
Fig. 2

Lateral habenula  Globus pallidus

Vehicle

Haloperidol (0.16 mg/kg)

Papaverine (40 mg/kg)

PQ-10 (10 mg/kg)

MP-10 (0.63 mg/kg)
Fig. 3

A. Haloperidol

B. Papaverine

C. PQ-10

D. MP-10

GP/Cpu ratio vs. Treatment dose (mg/kg)

hb/Tha ratio vs. Treatment dose (mg/kg)
Fig. 4

![Graph showing 2-DG uptake and plasma glucose levels in brain structures.](image-url)

- **Brain structures:** FrPar Cx, CPu
- **2-DG uptake (nCi/mg TE):**
  - FrPar Cx: veh MP-10, 2.5 mg/kg MP-10, 10 mg/kg MP-10, 40 mg/kg MP-10
  - CPu: veh MP-10, 2.5 mg/kg MP-10, 10 mg/kg MP-10, 40 mg/kg MP-10
  - Significant differences indicated by *.

- **Plasma glucose (mg/dl):**
  - Similar trends observed across different groups.
Fig. 5

A

GP/CPu ratio

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>VMP-10_VJ</th>
<th>0.63MP-10_VJ</th>
<th>0.63MP10_0.16J</th>
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<tbody>
<tr>
<td>GP/CPu ratio</td>
<td>**</td>
<td>***</td>
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B

hb/Tha

<table>
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<tr>
<th>Treatment group</th>
<th>VMP-10_VJ</th>
<th>0.63MP-10_VJ</th>
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<tbody>
<tr>
<td>hb/Tha</td>
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Fig. 6

A

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Globus pallidus</th>
<th>Lateral habenula</th>
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<tbody>
<tr>
<td>WT/Vehicle</td>
<td>![Image]</td>
<td>![Image]</td>
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<tr>
<td>WT/Haloperidol (0.63 mg/kg)</td>
<td>![Image]</td>
<td>![Image]</td>
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<tr>
<td>WT/MP-10 (2.5 mg/kg)</td>
<td>![Image]</td>
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<tr>
<td>KO/Vehicle</td>
<td>![Image]</td>
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<tr>
<td>KO/Haloperidol (0.63 mg/kg)</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>KO/MP-10 (2.5 mg/kg)</td>
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Haloperidol

B

<table>
<thead>
<tr>
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<tr>
<td>WT-veh</td>
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<td>WT-0.63</td>
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<tr>
<td>KO-veh</td>
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<tr>
<td>KO-0.63</td>
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GP/CPu ratio

C

<table>
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<tbody>
<tr>
<td>WT-veh</td>
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<tr>
<td>WT-2.5</td>
<td>0.75</td>
</tr>
<tr>
<td>KO-veh</td>
<td>1.00</td>
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<td>KO-2.5</td>
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GP/CPu ratio

D

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<tbody>
<tr>
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<tr>
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<tr>
<td>KO-veh</td>
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<td>KO-0.63</td>
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hb/Tha ratio

E

<table>
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<tr>
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<tr>
<td>KO-veh</td>
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<tr>
<td>KO-2.5</td>
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hb/Tha ratio

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