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The PDE10A inhibitor MP-10 and haloperidol produce distinct gene expression profiles in the striatum and influence cataleptic behavior in rodents

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ABSTRACT

Phosphodiesterase 10A (PDE10A) has garnered attention as a potential therapeutic target for schizophrenia due to its prominent striatal expression and ability to modulate striatal signaling. The present study used the selective PDE10A inhibitor MP-10 and the dopamine D2 antagonist haloperidol to compare effects of PDE10A inhibition and dopamine D2 blockade on striatopallidal (D2) and striatonigral (D1) pathway activation. Our studies confirmed that administration of MP-10 significantly elevates expression of the immediate early genes (IEG) *c-fos*, *egr-1*, and *arc* in rat striatum. Furthermore, we demonstrated that MP-10 induced *egr-1* expression was distributed evenly between enkephalin-containing D2-neurons and substance P-containing D1-neurons. In contrast, haloperidol (3 mg/kg) selectively activated *egr*-1 expression in enkephalin neurons. Co-administration of MP-10 and haloperidol (0.5 mg/kg) increased IEG expression to a greater extent than either compound alone. Similarly, in a rat catalepsy assay, administration of haloperidol (0.5 mg/kg) or MP-10 (3–30 mg/kg) did not produce cataleptic behavior when dosed alone, but co-administration of haloperidol with MP-10 (3 and 10 mg/kg) induced cataleptic behaviors. Interestingly, co-administration of haloperidol with a high dose of MP-10 (30 mg/kg) failed to produce cataleptic behavior. These findings are important for understanding the neural circuits involved in catalepsy and suggest that the behavioral effects produced by PDE10A inhibitors may be influenced by concomitant medication and the level of PDE10A inhibition achieved by the dose of the inhibitor.

1. Introduction

Dysfunction of the basal ganglia is a hallmark of many psychiatric disorders, including schizophrenia. Due to its complex circuitry, correcting basal ganglia dysfunction with therapeutic intervention is challenging and treatments often have limited effectiveness, or are associated with dose-limiting side effects (Tsutsumi et al., 2011). Most established antipsychotics target dopaminergic receptors in the striatum, which is consistent with the hypothesis that at least some symptoms arise from hyperactive dopaminergic signaling (Carlsson, 1978; Weinberger et al., 1988; Lewis and Lieberman, 2000; Abi-Dargham and Laruelle, 2005).

The bulk of neurons in the striatum are medium spiny neurons (MSNs) (Graveland and DiFiglia, 1985), which are categorized into two types of GABAergic neurons: striatonigral neurons that express dopamine D1 receptors (D1) and substance P (Sub P), and striatopallidal neurons that express dopamine D2 receptors (D2) and enkephalin (ENK) (Parent and Hazrati, 1995; Haber, 2003; Perez-Costas et al., 2010). Activating the striatonigral pathway is thought to facilitate movement, salience attribution, and certain patterns of cognition, whereas activation of the striatopallidal pathway inhibits those processes (Kravitz et al., 2010; Gerfen et al., 1990; Albin et al., 1989). Currently available antipsychotics are D2 antagonists, which improve positive symptoms in some patients, but also produce various mechanism-based side-effects, such as the

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extra-pyramidal symptoms (EPS) of tardive dyskinesia and parkinsonism (Green and Nuechterlein, 2004; Harvey et al., 2004; Keefe et al., 2007; Kern et al., 2004). Furthermore, D2 antagonists do not improve other symptoms of the disease, including negative symptoms (social withdrawal, anhedonia) and cognitive dysfunction (Arango et al., 2013). Therefore, there is a continuing effort to discover and develop improved therapeutics for the treatment of schizophrenia.

In recent years, cyclic nucleotide phosphodiesterases (PDEs) have been identified as novel therapeutic targets for treating schizophrenia (Chappie et al., 2012; Siuciak, 2008). PDEs function to selectively hydrolyze adenosine 3′, 5′ cyclic monophosphate (cAMP) and guanosine 3′, 5′ cyclic monophosphate (cGMP) within cells. The PDE superfamily is subdivided into 11 families (PDE1–PDE11) based on sequence homology, structure, and substrate specificity (Bender and Beavo, 2006; Conti and Beavo, 2007). The PDE10 family is encoded by a single gene, PDE10A, which is a dual substrate enzyme that can inactivate both cAMP and cGMP (Fujishige et al., 1999; Loughney et al., 1999; Soderling et al., 1999). A unique feature of PDE10A is its restricted distribution in the MSNs of the striatum, with limited expression in tissues outside of the basal ganglia (Chappie et al., 2012; Lakics et al., 2010; Coskran et al., 2006; Seeger et al., 2003; Xie et al., 2006). Due to the high level of expression in the striatum, it has been hypothesized that inhibition of PDE10A can regulate activation of MSNs and significantly influence output of the basal ganglia. Accordingly, preclinical studies have demonstrated that oral administration of selective PDE10A inhibitors elevate both cAMP and cGMP in the rodent striatum (Raheem et al., 2012; Smith et al., 2013; Grauer et al., 2009; Schmidt et al., 2008; Verhoest et al., 2009). Further investigation of signaling pathways downstream of cAMP demonstrated that PDE10A inhibitors induce significant changes in the phosphorylation of GluR1 subunit of the AMPA receptor and increase phosphorylation of extracellular-signal-regulated kinase (ERK) and cAMP-response element-binding protein (CREB). These signaling pathways impact both dopamine and glutamate systems in the striatum (Smith et al., 2013; Grauer et al., 2009; Nishi et al., 2011; Chao et al., 2002).

In these studies, we analyzed immediate early gene (IEG) and neuropeptide expression to assess the effects of the selective PDE10A inhibitor MP-10 in distinct cell populations of the striatum. The expression patterns induced by MP-10 were also compared to haloperidol to determine if PDE10A inhibition produced a unique pattern of IEG activity compared to this well characterized D2 antagonist. We also sought to determine if different levels and patterns of striatal activation induced by PDE10A inhibition produce discrete behavioral effects. To accomplish this, we examined the effects MP-10 dosed alone and in conjunction with haloperidol on rodent catalepsy, a behavior known to be influenced by pharmacological agents that regulate striatal activity.

2. Methods

2.1. Animals

Adult Wistar and Sprague Dawley rats (male, ~200–300 g) were obtained from Charles River (Wilmington, MA, USA). Upon arrival, they were separated into groups and individually housed for 7–14 days prior to studies. Animals were maintained on a 12 h light/dark cycle (lights on 07:00 h) and temperature and relative humidity were maintained at 22–24 °C and 50–55%, respectively. Principles from the Guide for the Care and Use of Laboratory Animals and National Institutes of Health were followed, and all protocols were approved by the Merck Institutional Animal Care and Use Committee. Efforts were made to minimize the numbers of animals used and minimize their suffering during experiments.

2.2. Drugs

MP-10 (2-[4-(1-Methyl-4-pyridin-4-yl-1H-pyrazol-3-yl)-phenoxymethyl]-quinoline) was synthesized in house and formulated in 10% Tween 80/90% water and administered at 5 ml/kg, p.o. via oral gavage. Haloperidol was purchased from Sigma–Aldrich (St. Louis, MO, USA) and dosed i.p. at a volume of 1 ml/kg in water. MP-10 was chosen due to its high selectivity, being >1000× more selective for PDE10A versus other PDEs, and its potency (0.3 nM Ki) (Chappie et al., 2012).

2.3. Pharmacokinetics

The concentration of compounds in plasma was sampled at necropsy for all studies. Blood was centrifuged at 1300 RCF for 10 min at 4 °C and the resultant supernatant was collected. Plasma samples were extracted using a protein precipitation method. The supernatants were injected onto a Transcend LX2 Multiplexed UPLC system coupled with a Sciex API5000 triple quadrupole mass spectrometer (Applied Biosystems, Ontario, Canada). An HTSS T3 column (50 × 2.1 mm, 1.8 μm) was used to retain the compound and the internal standard using a gradient consisting of solvent A (Water containing 0.1% formic acid) and solvent B (Acetonitrile containing 0.1% formic acid) at a flow rate of 0.750 mL/min. Peak integration and quantitative analysis was performed using Multiquant (version 2.1.1).

2.4. In situ hybridization studies

2.4.1. Animal handling and dosing

Adult Wistar rats were handled and mock dosed (either p.o. or i.p. depending on treatment group) three separate times prior to the experimental day; they were also weighed once. On the day of the experiment, two animals were dosed every five minutes in a staggered fashion with vehicle, haloperidol (3 mg/kg), or MP-10 (3 or 30 mg/kg). Each treatment condition had an n = 5.

2.4.2. Euthanization, sample collection and processing

Three hours post-dose, the animals were removed from their home room and euthanized with CO_2 inhalation. Intracardiac blood draws were performed to collect plasma for drug exposures. After decapitation, the brains were collected, frozen on aluminum foil over dry ice, and coronally sectioned using a cryostat at a 20 μm thickness throughout the striatum (from Bregma, AP 2.2 to −0.4 mm) (Paxinos and Charles, 1998). The sections were placed onto SuperFrost Plus slides and stored at −80 °C until use.

2.4.3. Riboprobe hybridization

Slides were postfixed in 4% paraformaldehyde then delipidated and dehydrated with chloroform and ethanol. Plasmids (GeneWiz Corp., South Plainfield, NJ, USA) containing *arc* (537 base-pairs, Xba1/EcoR1), *egr*-1 (540 bp, Xba1/EcoR1), *c-fos* (763 bp, Xba1/EcoR1), preproenkephalin (ENK; 935 bp, Sma1/EcoR1) and substance P (Sub P; 1067 bp, Xba1/EcoR1) were linearized and transcribed to produce antisense riboprobes labeled with ^{35}S-UTP from PerkinElmer (Waltham, MA, USA). After the slides were hybridized overnight with the riboprobes (~6 × 10^6 DPM/slide, 55 °C), a series of descending sodium citrate solutions (0.03–0.3 M NaCl, 0.003–0.03 M sodium citrate; pH 7.0, 25–67 °C) with 10 mM DTT were used to wash the slides. RNAse A treatment (20 μg/ml) removed nonspecific label. After dehydration in ethanol, the slides were opposed to BioMax (Kodak) x-ray film for 2–7 days. The films were then developed and the slides were dipped into NTB2 nuclear emulsion. The slides were incubated for 2–7 weeks in the dark at 4 °C, photographically processed, counterstained with hematoxylin and cover-slipped. A detailed description of the *in situ* hybridization method has been described previously (Ky and Shughrue, 2002; Eftekhari et al., 2013).

Other sets of tissue were used for fluorescent *in situ* hybridization (FISH) to determine the distribution of cells expressing *egr*-1 among striatal neurons that also expressed ENK or Sub P. The first half of the FISH was performed similarly to the process above, using the same cDNA probes. Instead of ^{35}S-UTP, the *egr*-1 riboprobes were labeled with digoxigenin (DIG)-UTP and the ENK and SubP riboprobes labeled with fluorescein (FITC)-UTP. After hybridization, they were blocked with 2% hydrogen peroxide and 0.5% of PerkinElmer blocking reagent, and then incubated overnight at 4 °C in 1 ug/ml of anti-FITC-POD (Roche, Indianapolis, IN, USA). The following day, fluorescein tyramide amplification reagent was applied to the slides at a 1:50 concentration (TSA-Plus Cyanine 3/Fluorescein System, PerkinElmer, Waltham, MA, USA). This procedure was repeated, the second time incubating with the anti-DIG-POD (Roche, Indianapolis, IN, USA) and amplifying with the cyanine-3 reagent. The slides were coverslipped with ProLong Gold containing DAPI.

2.4.4. Analysis of in situ hybridizations

Autoradiographs were digitized with a computer-based image analysis system (MCID Basic Version 7, InterFocus Imaging Research Inc., Linton, England). Optical density (OD) measurements of the autoradiographs for the anterior and middle striatal levels were determined with the MCID system. Representative images were processed for brightness/contrast enhancement and arranged into plates using Photoshop (Adobe, San Jose, CA). For slides that had been processed with the NTB2 nuclear emulsion, resultant images of the silver grains that demonstrated gene expression were digitized using the Aperio XT slide scanner (Leica Biosystems, Buffalo Grove, IL, USA). Cells expressing IEGs, as indicated by clusters of >30 silver grains per nucleus, were counted from the dorsolateral, dorsomedial and ventral (nucleus accumbens) regions of the middle striatal sections using Halo software (Indica Labs, Corrales, NM, USA). ANOVA and Dunnett t-tests (respective to vehicle)

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