

Assessment of Lumateperone Partial Agonist Activity at Presynaptic Dopamine D₂ Autoreceptors: Reversal of Haloperidol-Induced Tyrosine Hydroxylase Phosphorylation in Mouse Striatum

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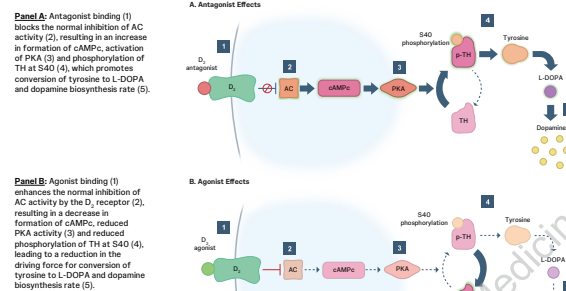
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Background

- Lumateperone is FDA approved for the treatment of schizophrenia in adults, for the treatment of depressive episodes associated with bipolar I or II disorder (bipolar depression) in adults as monotherapy and as adjunctive therapy with lithium or valproate, and as an adjunctive treatment for major depressive disorder¹
- Lumateperone is a mechanistically novel, multimodal antipsychotic that modulates serotonergic and dopaminergic neurotransmission, and indirectly modulates glutamatergic neurotransmission²
 - Lumateperone has high binding affinity for serotonin 5-HT_{2A} receptors (K_d=0.54 nM) and moderate binding affinity for dopamine D₂ receptors (K_d=32 nM); D₂ receptor occupancy of lumateperone (39%) is lower than that of other antipsychotics (65%-80%)³
- Most available antipsychotics act in part as D₂ antagonists (eg, haloperidol, quetiapine, lurasidone)^{4,5} or as partial D₂ agonists (eg, aripiprazole, brexpiprazole, cariprazine)⁶ (Figure 1)
 - While D₂ receptor blockade is essential for antipsychotic activity, extrapyramidal symptoms (EPS) are observed with high occupancy from D₂ receptor antagonists^{3,4}
- The novel mechanism of lumateperone, including presynaptic partial agonism while maintaining postsynaptic D₂ blockade with low D₂ receptor occupancy, balances out the dopaminergic tone and may contribute to the low risk of EPS and akathisia with lumateperone⁷
- Tyrosine hydroxylase (TH) is the rate-limiting enzyme in dopamine synthesis, and phosphorylation at serine 40 (S40) is essential for its catalytic activity to drive dopamine production^{8,9}
 - In vivo, treatment with the D₂ receptor antagonist haloperidol increases the levels of phosphorylated TH (p-TH) at residue S40 in the mouse striatum^{4,8}

- This analysis provides evidence for the presynaptic partial agonism of lumateperone at D₂ receptors, which is a key contributor to the favourable safety profile observed with lumateperone in clinical trials¹⁰
- The hypothesis investigated here was that lumateperone acting as a presynaptic partial agonist would reduce the levels of haloperidol-induced p-TH in mouse striatum

Figure 1. Effects of Dopamine D₂ Receptor Antagonist (A) and Agonist (B) on Dopamine Biosynthesis Through Modulation of TH Phosphorylation



AC, adenylyl cyclase; cAMP, cyclic adenosine monophosphate (cAMP); PKA, protein kinase A; S40, serine 40; TH, tyrosine hydroxylase.

Methods

Animals and Treatments	Striatal Tissue Collection	Western Blotting
- Adult C57BL/6 male mice were co-administered an intraperitoneal injection of the D₂ antagonist drug haloperidol (1 mg/kg) or its vehicle with one of the following: - Full dopamine D₂ receptor agonist → Quinpirole hydrochloride (1 or 2 mg/kg) - D₂ partial agonists → Aripiprazole (3 mg/kg)¹¹, Cariprazine (3 mg/kg)¹², Brexpiprazole (10 mg/kg)¹³, Lumateperone (6 mg/kg)¹⁴ - D₂ receptor antagonists → Lurasidone hydrochloride (1 or 3 mg/kg)¹⁵, Quetiapine fumarate (5 or 10 mg/kg)¹⁶	- One hour after drug treatment, mice were humanely euthanized using focused microwave irradiation of the head (Muromachi Kika) - Striatal tissue was bilaterally dissected and snap frozen in liquid nitrogen	- BCA assay (Pierce, Thermo Fisher) was used to quantify the protein concentration of each sample - Normalized striatal samples (20 µg/µL) were separated on 20%-14% Bis-Tris gradient protein gels, running at 140 volts for 75 min - Proteins were then transferred to nitrocellulose membranes for analysis
Data Analysis		
- Data were analyzed by 1-way analysis of variance (ANOVA) using GraphPad Prism 9 software, and results are presented as mean ± SEM - Each treatment group was normalized to the control group - Analysis was followed by a post hoc pairwise multiple comparison procedure using the Bonferroni method if the interaction was significant (P<0.05) - Outliers were removed using the median absolute deviation (MAD) equation (median ±2.5× the MAD method for outlier detection), which is a more robust measure of dispersion than the mean ±2 or 3 standard deviation¹⁷		

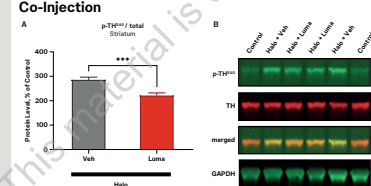
Table 1. Drugs and Antibodies

Drugs	Manufacturer
- Lumateperone (ITI-007) - Haloperidol (H1512) - Lurasidone hydrochloride (#SML2069) (-) Quinpirole hydrochloride (#1061) - Aripiprazole (#5584) - Quetiapine hemifumarate (#4735) - Brexipiprazole - Cariprazine hydrochloride	- As a tosylate salt, provided by the Medicinal Chemistry group at Intra-Cellular Therapies, a Johnson & Johnson company (lot #13337075) - Obtained from Sigma-Aldrich - Obtained from BioTecnique (Tocris) - Obtained from Advanced Chemblocks (#I-9700) - Obtained from MedChemExpress (#HY-14763A)
Antibodies	Manufacturer
- Phospho-tyrosine hydroxylase (Ser40) - Total anti-tyrosine hydroxylase antibody - Loading control: GAPDH, vinculin, or β-actin	- Obtained from Cell Signaling (#2791S); dilution 1:750 - Obtained from Abcam (ab76442); dilution 1:2,000 - Obtained from Cell Signaling: GAPDH (#2118; 1:15,000), vinculin (#13901; 1:2,000), β-actin (#4967; 1:15,000)

Results

- In mouse striatum, the D₂ antagonist haloperidol (which blocks D₂ receptors on striatal dopamine terminals) significantly increased dopamine response, inducing an ~3-fold increase in p-TH at the regulatory S40 residue (P<0.01) (Figure 2A)
 - Co-injection of haloperidol with lumateperone 3 mg/kg (P<0.01) or 6 mg/kg (P<0.01) significantly reduced p-TH levels compared with haloperidol alone
 - A representative Western blot of striatal samples from mice injected with vehicle, haloperidol, or haloperidol + lumateperone is shown in Figure 2B

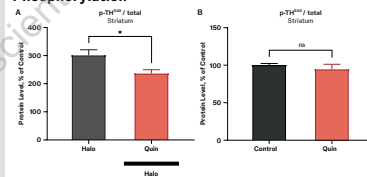
Figure 2. Reduction in Haloperidol-Induced Phosphorylation of TH With Lumateperone Co-Injection



P<0.01, *P<0.001. Control group significantly differed from all other groups (P<0.01). Concentrations: Halo, 1 mg/kg; Luma, 6 mg/kg. Halo, haloperidol; Luma, lumateperone; Veh, vehicle.

- The effect of lumateperone in reducing haloperidol-induced p-TH levels in the striatum was replicated by the full D₂ agonist quinpirole, with significant reduction with haloperidol + quinpirole co-injection versus haloperidol alone (P<0.05) (Figure 3A)
 - Injection of quinpirole alone did not affect basal levels of p-TH (Figure 3B)

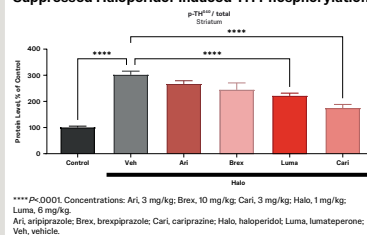
Figure 3. Co-Administration of a Full D₂ Agonist (Quinpirole) Suppressed Haloperidol-Induced TH Phosphorylation



*P<0.05. Concentrations: Halo, 1 mg/kg; Quin, 2 mg/kg (Panel A) or 1 mg/kg (Panel B). Halo, haloperidol; ns, not significant; Quin, quinpirole.

- In this in vivo model, the partial D₂ agonists aripiprazole, brexpiprazole, and cariprazine mimicked the effect of lumateperone and the full agonist quinpirole in reducing haloperidol-induced TH phosphorylation, with cariprazine reaching statistically significant reduction (P<0.001) (Figure 4)

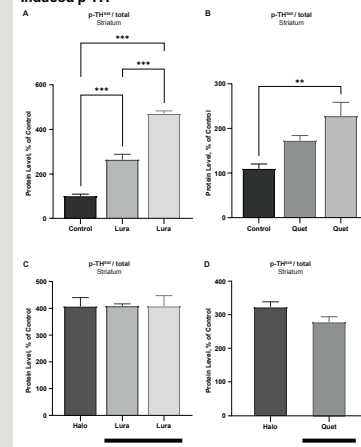
Figure 4. Co-Administration of Partial D₂ Agonists Suppressed Haloperidol-Induced TH Phosphorylation



****P<0.0001. Concentrations: Ari, 3 mg/kg; Brex, 10 mg/kg; Carl, 3 mg/kg; Halo, 1 mg/kg; Luma, 6 mg/kg. Ari, aripiprazole; Brex, brexpiprazole; Carl, cariprazine; Halo, haloperidol; Luma, lumateperone; Veh, vehicle.

- Lurasidone and quetiapine, which are antipsychotics with D₂ antagonist (but not agonist) activity, alone elevated basal phosphorylated TH (Figure 5A,B) without suppressing haloperidol-induced phosphorylated TH (Figure 5C,D)

Figure 5. Co-Administration of D₂ Antagonists (Lurasidone and Quetiapine) Increased Basal p-TH Levels But Did Not Suppress Haloperidol-Induced p-TH



Concentrations: Halo, 1 mg/kg; Lura, 1 mg/kg (darker grey) or 3 mg/kg (lighter grey); Quet, 5 mg/kg (darker grey) or 10 mg/kg (lighter grey). Halo, haloperidol; Lura, lurasidone; Quet, quetiapine; Veh, vehicle.

Conclusions

Lumateperone demonstrated in vivo suppression of haloperidol-induced increases in the phosphorylation of TH at S40, as expressed locally in striatal terminals

Lumateperone's suppression of haloperidol-induced p-TH occurred in a manner consistent with the actions of full/partial dopamine D₂ receptor agonists (cariprazine, quinpirole, aripiprazole, and brexpiprazole) at presynaptic dopamine D₂ receptors

In contrast, D₂ antagonists increased p-TH (haloperidol, lurasidone, quetiapine) and did not suppress haloperidol effects (lurasidone, quetiapine)

The results suggest that lumateperone acts as a dopamine D₂ receptor presynaptic partial agonist and does not act as a dopamine D₂ receptor presynaptic antagonist when measured in vivo

The unique mechanism of action of lumateperone on the dopaminergic system may contribute to the low risk of EPS and akathisia, and overall favourable safety profile, observed with lumateperone in clinical trials¹⁰

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Disclosures

GL Snyder, S Dutheil and M Chen are full-time employees of Johnson & Johnson.

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