The presynaptic D₂ partial agonist lumateperone acts as a postsynaptic D₂ antagonist

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Abstract
Lumateperone is a compound in clinical development as a treatment for schizophrenia, bipolar depression, agitation associated with dementia and other neuropsychiatric and neurodegenerative diseases. This novel compound exhibits high affinity in vitro binding to dopamine D₂ receptors and occupies striatal dopamine D₂ receptors in human brain at therapeutic doses. At dopamine D₂ receptors lumateperone displays dual properties, acting both as a functional post-synaptic antagonist and pre-synaptic partial agonist. To further characterize these unique receptor interactions, we compared the activity of lumateperone relative to three known D₂ partial agonists of differing intrinsic efficacy, aripiprazole, brexpiprazole, and bifeprunox, in cell-based functional assays that permit characterization of compounds of differing intrinsic efficacy (e.g. full agonism to neutral antagonism). Lumateperone had no demonstrable agonist activity in CHO cells expressing recombinant human D₂L (or D₂S) receptors, in assays where other known partial agonists displayed different degrees of agonist activity.

Introduction
Available drugs for treatment of schizophrenia are largely based on antagonism of dopamine D₂ receptors. Unalloyed D₂ antagonism, however, is associated with significant limiting side effects including acute motoric disturbances and chronic irreversible dyskinesia (i.e. tardive dyskinesia). These side effects are ameliorated in second-generation antipsychotics, of which most combine antagonism of serotonin (5-HT) ₂A receptors with D₂ antagonism. The two current antipsychotic drugs, aripiprazole and brexpiprazole, are D₂ receptor partial agonists [1] [2] [3] [4]. These compounds have high affinity for D₂ receptors but low intrinsic efficacy, meaning that they have a reduced ability to stimulate functional activity of the receptor relative to the endogenous ligand or full agonist drugs [5]. Recently, Li et al. have described lumateperone, a candidate antipsychotic that has potent 5-HT₂A antagonism and potent activity at D₂ receptors [6]. Although lumateperone occupies striatal dopamine D₂ receptors in human brain at therapeutic doses [7], and in vivo in mouse brain it demonstrates postsynaptic antagonism, at the same doses it demonstrates presynaptic partial agonism [8]. To study this unique dual behavior better, we compared the intrinsic efficacy of lumateperone to the known partial agonists, aripiprazole, brexpiprazole, and bifeprunox, in assays of dopamine D₂ receptor function, using two naturally occurring variants of the human D₂ receptor in recombinant cells.

Objective
To characterize the G-protein-mediated functional activity of lumateperone at the D₂L and D₂S dopamine receptors under conditions sensitive to D₂ partial agonists.
The presynaptic D2 partial agonist lumateperone acts as a postsynaptic D2 antagonist

Figure Legend

Figure 1. Functional activity of lumateperone and atypical antipsychotics at recombinant D2L dopamine receptor.

(A) Antagonist activity at the D2L receptor. CHO-K1 cells expressing human recombinant (hD2L) receptor (FAST-0101C) were obtained from OGEDA and used according to supplier’s recommendations. For antagonist test cells were mixed with test compound at increasing concentrations and incubated 10 min at room temperature. Thereafter a mix of quinpirole (final assay concentration 30 nM, corresponding to its measured EC80) and forskolin (10 µM final assay concentration) was added and the plates were incubated for 30 min at room temperature. After addition of lysis buffer and 1 h incubation, cAMP concentrations were measured according to manufacturer’s specification, with the Cisbio “cAMP Dynamic2 Assay Kit” (Cisbio, 62AM4PEB). All assay points were determined in triplicate and data is presented as average values. Curve fitting was performed using XLfit software (IDBS), and affinity constants were determined using a 4-parameter logistic fit.

(B) Agonist activity at the D2L receptor. For agonist test cells were mixed with forskolin (10 µM final assay concentration) and test compound at increasing concentrations and incubated 30 min at room temperature. After addition of lysis buffer and 1 h incubation, cAMP concentrations were measured according to manufacturer’s specification, with the Cisbio “cAMP Dynamic2 Assay Kit” (Cisbio, 62AM4PEB). All assay points were determined in triplicate and data is presented as average values. Curve fitting was performed using XLfit software (IDBS), and affinity constants were determined using a 4-parameter logistic fit.

(C) Antagonist and D. Agonist activity at the D2S receptor. CHO-K1 cells expressing human recombinant (hD2S) receptor (FAST-0102L) were obtained from OGEDA and assayed essentially as described for hD2L.
Results & Discussion

To evaluate the agonist or antagonist activity of compounds, we measured the ability of these compounds to either inhibit forskolin-stimulated cAMP accumulation or reverse the inhibition produced by 30 nM of quinpirole in a Chinese Hamster Ovary (CHO) cell line expressing recombinant human dopamine D2L or D2S receptors. D2 receptors in this cell line couple with Goα/i/o protein subunits to suppress adenylate cyclase activity.

In antagonist assays using the D2-L receptor (Fig. 1A), lumateperone potently (IC50 = 32 nM) and completely antagonized the activity of quinpirole, with an IC50 in the same range as its published affinity for D2 receptors in a ligand-displacement assay (32 nM; Li et al., 2014). We compared this to haloperidol (IC50 = 0.33 nM), brexpiprazole (1.68 nM) and aripiprazole (3.32 nM). In keeping with their partial agonist character, brexpiprazole and aripiprazole only partially antagonized the activity of quinpirole, reaching ~70% and ~45% maximum blockade, respectively.

This assay can also be run in agonist mode. Agents such as aripiprazole, bifeprunox, and brexpiprazole act as partial agonists, partially suppressing adenylate cyclase activity [9]. Depending on receptor abundance and efficiency of receptor coupling to second messengers in the recombinant system, the intrinsic efficacy for aripiprazole can range from 20 to 90% that of dopamine [2]. Figure 1B summarizes the result of a direct comparison of lumateperone with dopamine (full agonist), bifeprunox (partial agonist with high intrinsic efficacy), [10] aripiprazole (partial agonist with medium intrinsic activity) [11], and brexpiprazole (partial agonist with low intrinsic activity) [3] in the D2-L receptor-induced suppression of forskolin-stimulated adenylate cyclase activity. Bifeprunox reached 77% activity relative to quinpirole, with an EC50 of 4.26 nM (Fig. 1B and C). Aripiprazole reached 37% activity, with an EC50 of 4.43 nM. Brexpiprazole has less intrinsic activity than aripiprazole at D2 receptors, with a maximum effect of 11% and an EC50 of 1.32 nM. Lumateperone (up to 3 µM) had no demonstrable agonist activity in this assay.

In vivo, D2L receptors are abundantly found in postsynaptic dopamine-responsive cells. D2S, a splice variant of this receptor lacking exon 6 encoding the third intracellular loop, is most abundant on the presynaptic cells, and has been hypothesized to be the usual form functioning as the presynaptic autoreceptor (reviewed in [12]). Therefore, we asked whether the partial agonist activity of lumateperone would be revealed in assays using this receptor. In CHO cells expressing recombinant human D2S receptors, however, this was not the case (Fig. 1C and D). Once again, while aripiprazole and brexpiprazole acted as functional partial agonists, lumateperone showed only antagonist activity. This may reflect the fact that the G-protein repertoire of CHO cells does not replicate the presynaptic receptor cellular milieu. Prior work with many cell lines expressing recombinant D2 receptors has demonstrated that the cellular milieu, rather than the splice variant, is the major influence determining functional readout from D2 receptors [2].

Conclusions

We used whole cell based assays to detect D2-receptor-dependent suppression of adenylate cyclase, a functional mimic of postsynaptic D2 receptors, to compare the activity of lumateperone to older, partial agonist, atypical antipsychotics. In multiple assays that revealed partial agonist activity of existing drugs, lumateperone acted as an antagonist. Other approaches may be needed to reveal the presynaptic factors that underlie the functional properties in vivo of this compound.

Limitations

It has been documented that differences in receptor coupling to second messenger partners in test systems with recombinant receptors can influence the ability to detect partial agonism. Full understanding of the activity of this compound may require selective assay of presynaptic receptors in their authentic cellular milieu, which is beyond the scope of this report.

Our working hypothesis is that presynaptic D2 autoreceptors exist in a unique receptor complex that we cannot replicate in CHO cells. Further work could include experiments
assaying functional activity of a selective population of brain-derived presynaptic neurons, or genetic and biochemical approaches designed to reveal the nearest-neighbors of the D2 receptor in these cells.

Additional Information

Methods and Supplementary Material
Please see https://sciencematters.io/articles/201712000006.

Funding Statement
This study was funded by Intra-Cellular Therapies, Inc.

Ethics Statement
Not Applicable.

Citations


https://doi.org/10.1073/pnas.0730708100.