The modulation of cyclic nucleotide dependent pathways in the rat striatum by CPL-500-036-02 - a phosphodiesterase 10A inhibitor.

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Introduction

Phosphodiesterase 10A (PDE10A) is a dual-substrate (cAMP and cGMP) phosphodiesterase which highly expressed in striatal medium spiny neurons (MSNs). MSNs mediate corticostriatal and nigrostriatal transmissions. Disruption of these pathways leads to aberrant neuronal activity in the cerebral cortex. PDE10A regulates striatal outputs by its effect on both the cAMP and cGMP pathways, that results in changes in phosphorylation of a variety of intracellular proteins. Therefore, it is believed that inhibition of PDE10A in MSNs may alleviate both positive and negative symptoms of schizophrenia – the feature of which present antipsychotics are practically deprived of.

In the present study, activity, selectivity and ex vivo pharmacodynamics of CPL-500-036-02, a novel PDE10A inhibitor, were assessed. We examined time- and dose-dependent effect of CPL 500-036-02 on phosphorylation of proteins involved in striatal function: GluR1 (Ser845; one of the AMPA receptors for glutamate subunits), CREB (Ser133; cAMP-response element binding protein), DARRP-32 (Thr34; dopamine and cAMP-regulated phosphorylase-32), ERK\(^1\)\(^2\) (Thr202/Tyr204; extracellular signal-regulated kinase 1 and 2), MSK1 (Ser376; mitogen- and stress-activated kinase 1), H3 (Ser10; histone H3).

Activity & Selectivity

IC\(50\) determination

IC\(50\) = 1.03 nM

Selectivity at 100 nM

<table>
<thead>
<tr>
<th>PDE</th>
<th>Average IC(50) (nM)</th>
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<tbody>
<tr>
<td>PDE10A</td>
<td>5%</td>
</tr>
<tr>
<td>PDE11A</td>
<td>8%</td>
</tr>
<tr>
<td>PDE12A</td>
<td>6%</td>
</tr>
<tr>
<td>PDE15A</td>
<td>3%</td>
</tr>
<tr>
<td>PDE16A</td>
<td>15%</td>
</tr>
<tr>
<td>PDE17A</td>
<td>100%</td>
</tr>
</tbody>
</table>

Dose-response study

Pharmacodynamic, dose-response study was performed on striata of rats treated with 0.3, 1 or 3 mg/kg of CPL-500-036-02 or vehicle administered p.o.

Phosphorylation changes of proteins which function depends on cAMP and cGMP concentration were analysed by Western blot method in lysates collected 2h after compound or vehicle administration.

In this study we demonstrate significant and dose-dependent increase in phosphorylation of multiple proteins in rat striatum, compared to the vehicle. The control analysis for lysates from hippocampus or prefrontal cortex showed no changes in the phosphorylation of mentioned targets’ set (data not shown).

Representative Western blot results and data quantification from the study are presented (*p<0.05; **p<0.01; ***p<0.001).

Time course study

Pharmacodynamic, time course study was performed on striata of rats treated with 3 mg/kg of CPL-500-036-02 or vehicle administered p.o. Phosphorylation changes of proteins which function depends on cAMP and cGMP concentration were analysed by Western blot method in lysates collected 2h, 4h and 8h after compound or vehicle administration.

In this study we demonstrate significant and time dependent increase in phosphorylation of multiple proteins in rat striatum, compared to the vehicle, at given time point.

Representative Western blot results and data quantification from the study are presented (*p<0.05; **p<0.01; ***p<0.001).

Conclusions

- CPL-500-036-02 is a potent and selective PDE10A inhibitor.
- CPL-500-036-02 increased phosphorylation levels of GluR1, CREB, DARRP-32, MSK1 and histone H3 in rat striata in a dose-dependent manner, 2h after compound administration, compared to the vehicle.
- Time course study revealed that phosphorylation levels of GluR1, DARRP-32, MSK1, ERK\(^1\)\(^2\), and histone H3 change over time with maximum increase at 1 and 2h after administration of CPL-500-036-02, compared to the vehicle.
- CPL-500-036-02 is a new potent PDE10A inhibitor which can modulate cAMP and cGMP dependent pathways.

Methods

- Activity, selectivity, and selectivity against representative members of all other PDE families were determined by Enzyme Kinase Activity Assay (EKA; Stratagene).
- Pharmacodynamic study: Male Sprague Dawley (n = 4), were administered 0.3, 1 or 3 mg/kg of CPL-500-036-02 or vehicle (i.p., 0.9% saline) and sacrificed for tissue processing at 10 min (PDE10A), 15 min (PDE12A), 30 min (PDE16A), 2h (PDE17A) and 3h (PDE15A) after administration, for dose-dependent and time-course study respectively. The study was performed on 6 rats in each group.
- Phosphorylation analysis: Striate, hippocampal and prefrontal cortex from all rats were dissected and homogenized in 4% SDS. Subsequently, they were transferred to 80% EtOH. Tissue homogenates were centrifugated and supernatants were analysed. Lysates were used in Western blot analysis of total and phosphorylated levels using commercially available antibodies directed against phospho-GluR1 (Cell Signaling Technology), CREB (Cell Signaling Technology), DARRP-32 (Cell Signaling Technology), ERK\(^1\)\(^2\) (Cell Signaling Technology) and histone H3 (Cell Signaling Technology). Blots were probed with secondary antibodies labeled with horseradish peroxidase (from Cell Signaling Technology), all transferred to nitrocellulose (Whatman Millipore) filters. All antibodies were used according to manufacturer’s protocol.
- Statistics: Data and statistical analysis were done using GraphPad Prism v.6.0. Statistical significances were determined by one way ANOVA with Tukey post test for dose-response study and two way ANOVA with Bonferroni post test for time-course study.

References