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

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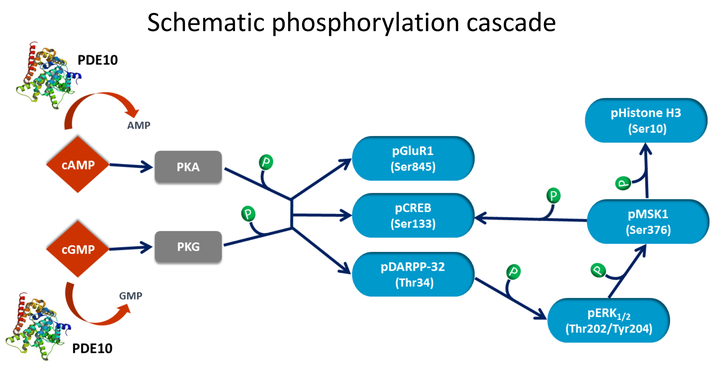
INTRODUCTION

Phosphodiesterase 10 (PDE10) is a dual-substrate (cAMP and cGMP) phosphodiesterase which highly expressed in striatal medium spiny neurons (MSNs). MSNs modulate both corticostriatal and nigrostriatal transmissions. Disruption of these pathways leads to aberrant neuronal activity in the cerebral cortex. PDE10 regulates striatal outputs by its effect on both the cAMP and cGMP pathways, that results in changes in phopshorylation of a variety of intracellular proteins. Therefore, it is believed that inhibition of PDE10 in MSNs may alleviate both positive and negative symptoms of schizophrenia – the feature of which present antipsychotic drugs are practically deprived of.

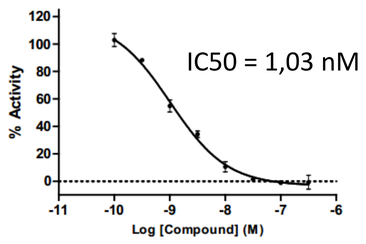
In the present study, activity, selectivity and ex vivo pharmacodynamics of CPL-500-036-02, a novel PDE10A inhibitor, were assessed. We examined time- and dose-dependent effect of CPL 500-036-02 on phosphorylation of proteins involved in striatal function: GluR1 (Ser845; one of the AMPA receptors for glutamate subunits), CREB (Ser133; cAMP-response element binding protein), DARPP-32 (Thr34; dopamine and cAMP-regulated phophoprotein-32), $ERK_{1/2}$ (Thr202/204; extracellular signal-regulated kinase 1 and 2), MSK1 (Ser376; mitogen- and stress- activated kinase 1), H3 (Ser10; histone H3).

Schematic phosphorylation cascade



ACTIVITY & SELECTIVITY

IC50 determination



Selectivity at 100 nM

PDE	Average Inh.
PDE1C	5%
PDE2A	9%
PDE3B	7%
PDE4B1	4%
PDE5A1	6%
PDE6C	18%
PDE7A	26%
PDE8A1	-1%
PDE9A2	-3%
PDE10A1	98%
PDE11A	9%

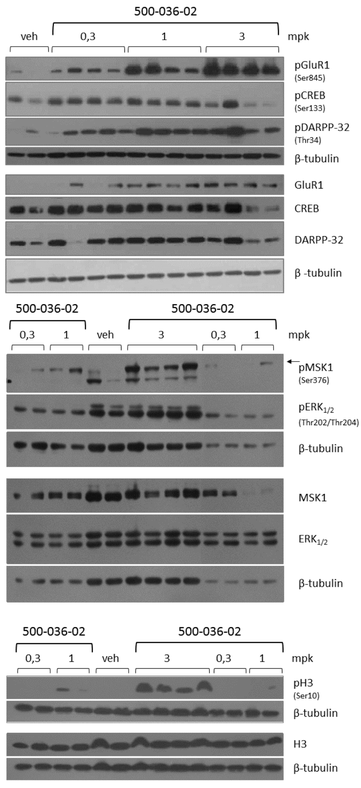
DOSE-RESPONSE STUDY

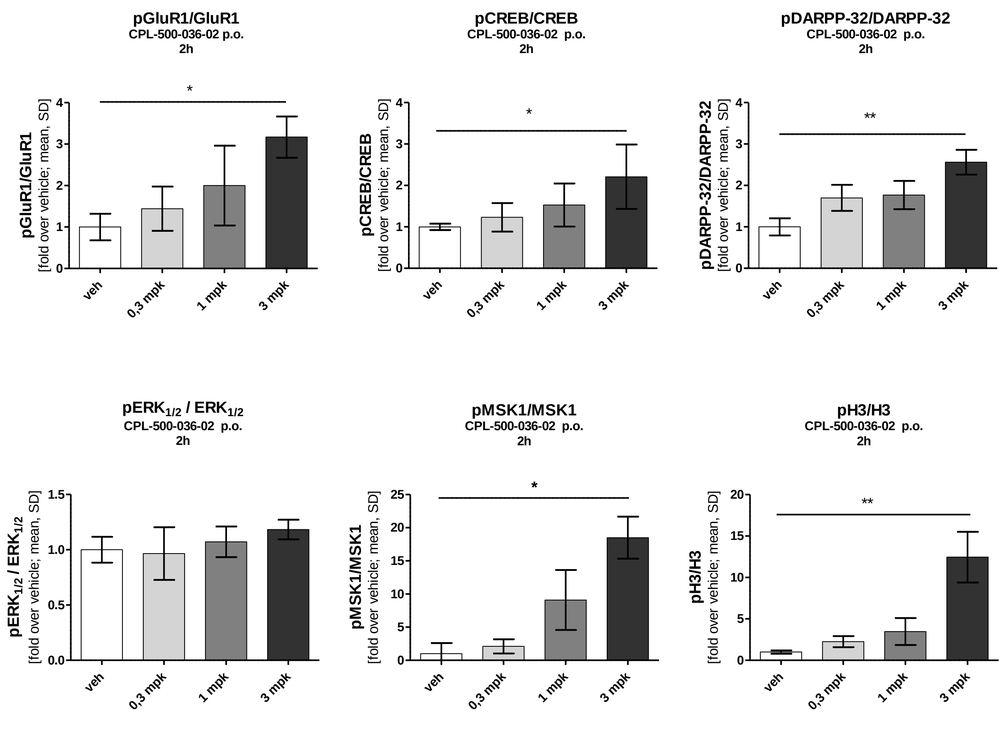
Pharmacodynamic, dose-response study was performed on striata of rats treated with 0,3, 1 or 3 mpk of CPL-500-036-02 or vehicle administered p.o.

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).



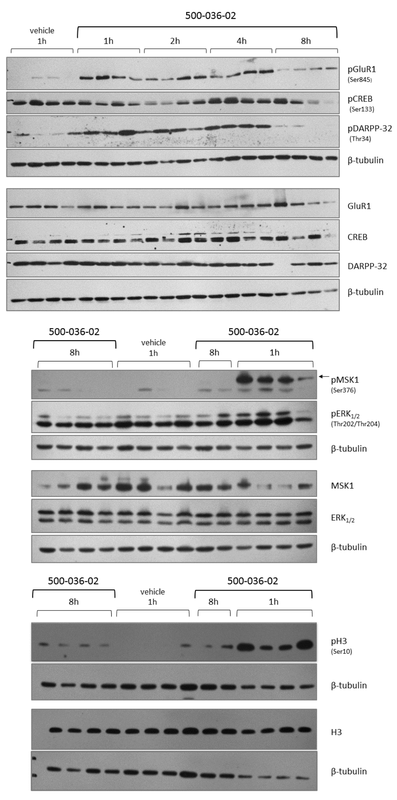


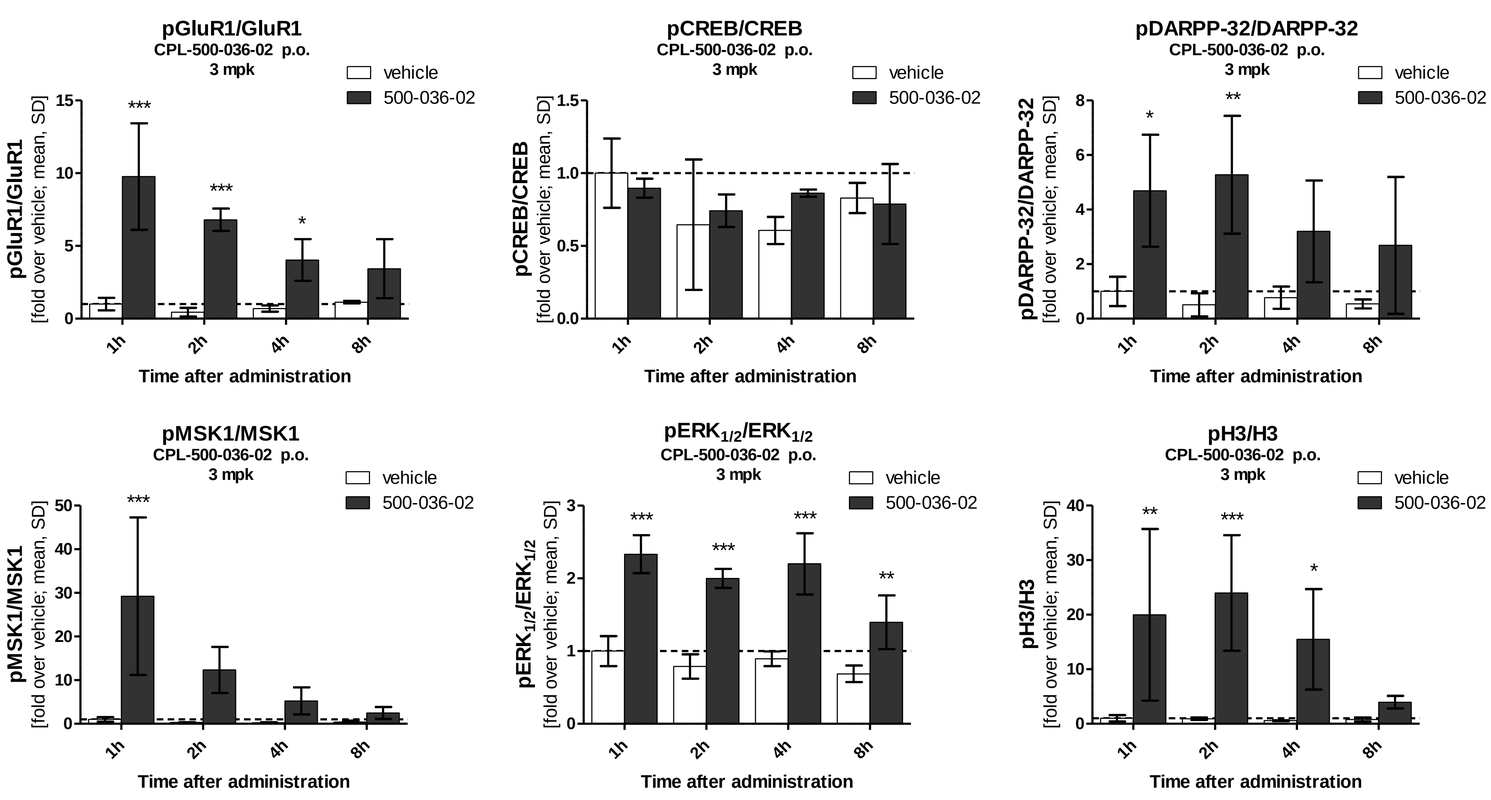
TIME COURSE STUDY

Pharmacodynamic, time course study was performed on striata of rats treated with 3 mpk 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 1h, 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).





METHODS

Activity, selectivity: IC50 and selectivity against representative members of all other PDE families was determined by PerkinElmer Discovery Services (selectivity at 100 nM).

Pharmacodynamics study: Male Sprague-Dawley rats (n = 4/group) were administered 0,3, 1 or 3 mpk of CPL-500-036-02 or vehicle (0,5%MC + 2%Tween80) and sacrificed by focused microwave irradiation 2 h after administration or administered 3 mpk of the compund or vehicle and sacrificed at 1h, 2h, 4h or 8h after administration, for dose-dependent or time-course study respectively. The studies received the approval of the respective Local Bioethics Committees.

Phosphorylation analysis: Striata, hippocampus and prefrontal cortex from all rats were dissected and frozen at -80 °C. Subsequently, they were homogenized in RIPA buffer or trichloroacetic acid and sonicated. Lysates were used to Western blot analysis of total and phosphoprotein levels using antibodies against: GluR1, GluR1 pSer845 (both from Merck Millipore), DARPP-32, DARPP-32 pThr34, CREB, CREB pSer133, MSK1, MSK1 pSer376, $ERK_{1/2}$, $ERK_{1/2}$ pThr202/Tyr204, histone H3, histone H3 pSer10 (all from Cell Signaling Technology), all normalized to β-tubulin (Merck Millipore) levels. All antibodies were used according to manufacturer's protocol.

Statitistics: Data and statistical analysis were done using GraphPad Prism v.5.0. Statistical significance were determined by one way ANOVA Kruskal-Wallis test with Dunns post-test for dose-response study and two way ANOVA with Bonferroni post-test for time course study.

CONCLUSIONS

- CPL-500-036-02 is a potent and selective PDE10A inhibitor.
- CPL-500-036-02 increased phosphorylation levels of GluR1, CREB, DARPP-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, DARPP-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.

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