Esmethadone (REL-1017) Reduces Glutamate-Induced Currents in NMDA Receptors with the GluN2D Subunit

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INTRODUCTION

- Esmethadone (REL-1017; dextromethadone; DXT) is a novel NMDA receptor (NMDAR) antagonist currently in Phase 3 trials for the treatment for major depressive disorder (MDD).

OBJECTIVES

- To characterized esmethadone ability to block heterodimeric NMDARs, in the presence of physiological concentration of extracellular magnesium and at different membrane potentials.

METHODS

- CHO cells stably expressing recombinant heterodimeric human NMDARs were used in automated patch clamp experiments (QPatch HTX).
- Cells were clamped at -80 mV holding potential.
- Voltage protocol included a depolarizing 2 seconds step pulse to +60 mV followed by a 2 seconds ramp back to holding potential.
- Currents were induced by 10 µM or 1 µM L-glutamate with 1 mM extracellular $MgCl_2$ and with or without 10 µM esmethadone.

RESULTS

Figure 1 - Voltage protocol diagram

A special protocol was designed to discard cells not perfectly clamped.
10 µM or 1 µM L-glutamate was added, in presence of 10 µM glycine and 1 mM $MgCl_2$, and in the absence or presence of 10 µM esmethadone, 500 ms after depolarizing step pulse to +60 mV. Cells not perfectly clamped showed leak current during the first 500 ms of depolarization, even in absence of L-glutamate, and were discarded.

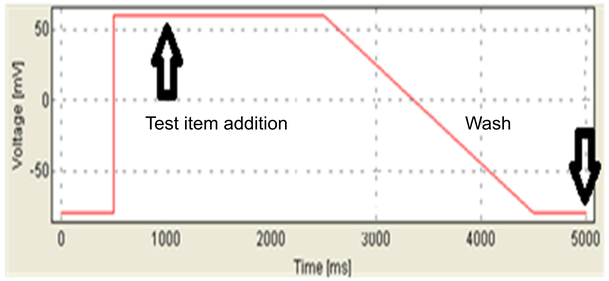


Figure 2 - L-glutamate CRC in presence of 1 mM magnesium

All used NMDAR cell lines showed desired response to glutamate CRC.
Sample superimposed current traces obtained with 0.2, 1, 10 and 100 µM consecutive L-glutamate additions in the presence of 1 mM magnesium and 10 µM glycine in sample cells expressing different NMDAR subunits.

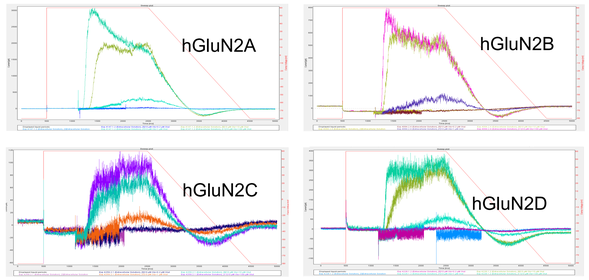


Figure 3 - Voltage dependence of 1 mM magnesium block

NMDAR currents decreased at negative voltages in presence of magnesium, since the ion was blocking NMDAR pore at negative voltages. We verified that magnesium block at negative voltages was less pronounced for hGluN1/hGluN2C and hGluN1/hGluN2D compared to hGluN1/hGluN2A, hGluN1/hGluN2B, by normalizing currents recorded at various negative voltages to current recorded at -30 mV. All currents were elicited by 10 µM L-glutamate and 10 µM glycine in presence of 1 mM $MgCl_2$ (n = 4 for each cell line). % current at -60 mV resulted 24 ± 2.4 %, 26 ± 1.8 %, 64 ± 5.6 %, 55 ± 1.8 % (mean ± SEM, n = 4) %, for hGluN1/hGluN2A, hGluN1/hGluN2B, hGluN1/hGluN2C and hGluN1/hGluN2D, respectively.

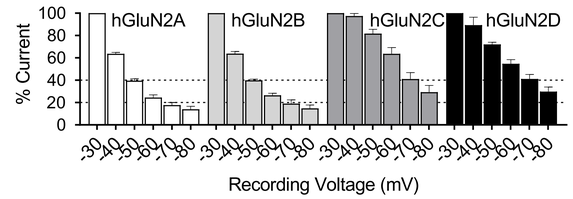
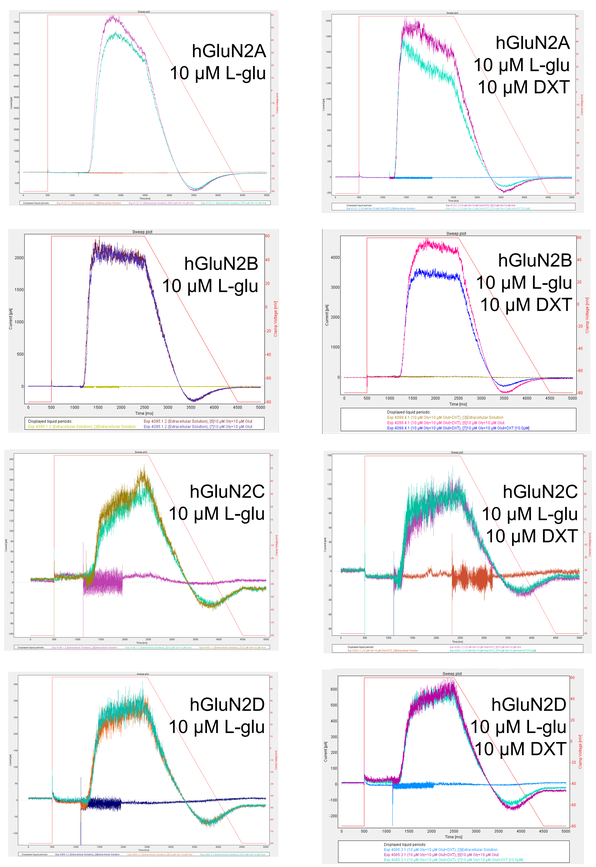


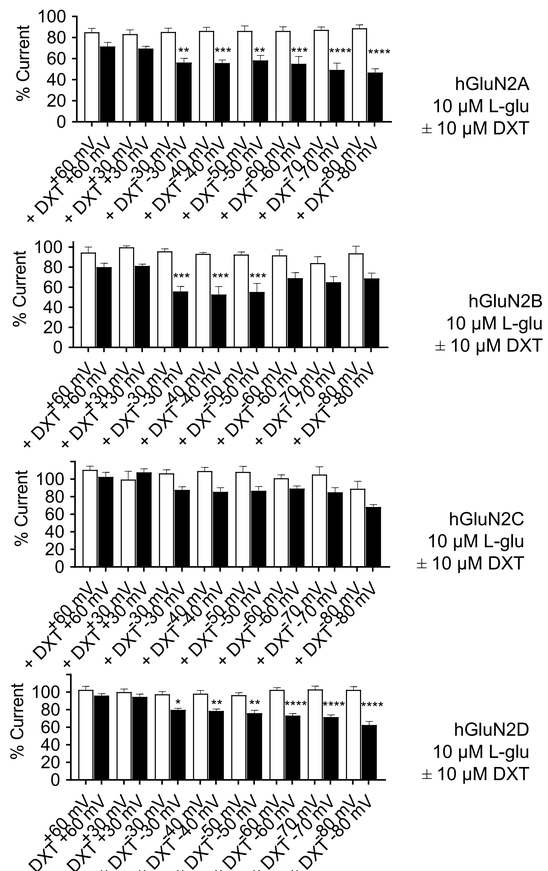
Figure 4 (Panels A, B, C, D) – Esmethadone effect on different heterodimeric NMDAR in presence of 1 mM magnesium

10 µM esmethadone blocked different heterodimeric NMDAR similarly, when activated by 10 µM L-glutamate (panels A and B). Instead 10 µM esmethadone preferentially blocked NMDAR containing hGluN2D, compared to other heterodimeric NMDAR, when activated by 1 µM L-glutamate (panels C and D).
Current traces (panels A and C) represent single cell recordings for the indicated cell line, obtained in continuous presence of 1 mM $MgCl_2$. Intracellular solution was (in mM): 80 CsF, 50 CsCl, 0.5 $CaCl_2$, 10 HEPES, 11 EGTA, adjusted to pH 7.25 with CsOH. Extracellular solution (assay buffer) was (in mM): 155 NaCl, 3 KCl, 1.5 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 10 D-glucose adjusted to pH 7.4 with NaOH. Each cell recording shows three superimposed current traces, obtained after consecutive addition of: assay buffer as first addition (baseline trace); then 10 µM (panels A) or 1 µM (panels C) L-glutamate plus 10 µM glycine as second addition (highest trace); finally, again 10 µM (panels A) or 1 µM (panels C) L-glutamate plus 10 µM glycine, in presence or absence of 10 µM esmethadone (DXT), as third addition. Third addition recordings were normalized to second addition recordings for every cell, then averaged and plotted in graphs reported in panels B and D, for traces obtained in presence of 10 µM and 1 µM L-glutamate, respectively. Column data represent mean ± SEM (n=4) of current recordings at different voltages, measured during hyperpolarizing ramp, in absence (□) or presence (■) of 10 µM esmethadone (DXT). Statistical results of one-way ANOVA followed by Tukey's multiple comparisons test is also reported: P < 0.05 (*), P < 0.01 (**), P < 0.001 (***), P < 0.0001 (****).

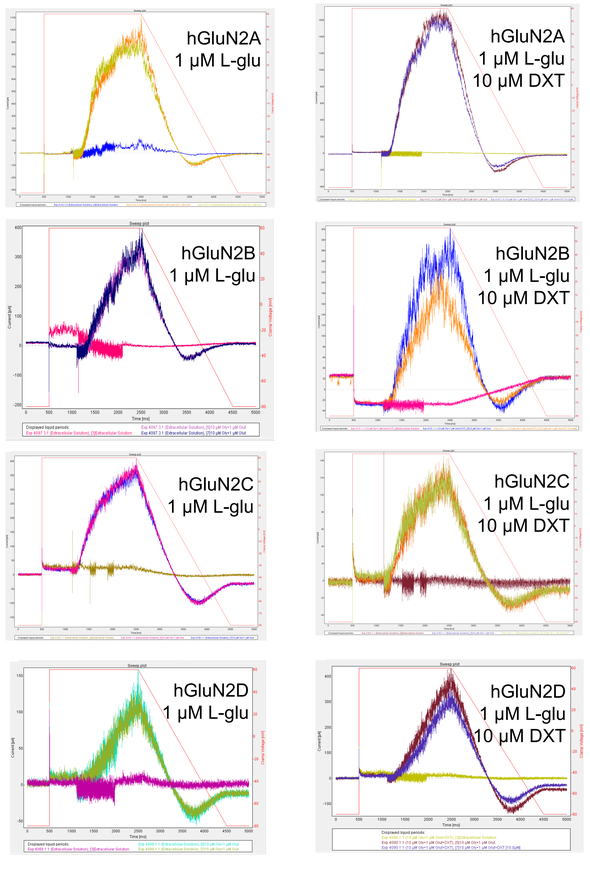
PANEL A



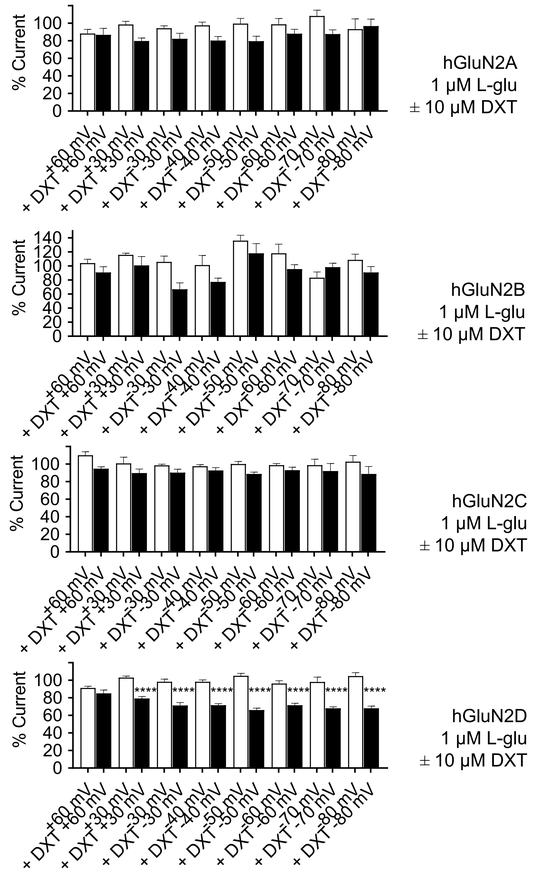
PANEL B



PANEL C



PANEL D



CONCLUSIONS

- Esmethadone preferentially reduced 1 µM L-glutamate induced currents at NMDAR including GluN2D subunit, in the presence of 1 mM $MgCl_2$..
- Since GluN2D subunit is expressed in inhibitory interneurons, esmethadone might affect interneuron activity in presence of low ambient L-glutamate concentrations.

REFERENCES

1. Perszyk RE, et al. (2016). Mol Pharmacol 90: 689–702.

DISCLOSURES

- This research was sponsored by Relmada Therapeutics, Inc. Drs. Inturrisi, Stahl, Pappagallo, and Manfredi are paid consultants for Relmada Therapeutics. Drs. Inturrisi and Manfredi are inventors on esmethadone patents and other patents and patent applications

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