Esmethadone (REL-1017) Reduces NMDA Receptor Currents in a Concentration Dependent Manner

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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 examine esmethadone role as an NMDA channel blocker in continuous presence of low L-glutamate concentration, as it might occur in pathological conditions, we characterized esmethadone ability to reduce NMDAR mediated current, after a 120 s perfusion period in the presence of 1 μM L glutamate and extracellular 1 mM magnesium, at -60 mV membrane potential.

METHODS

- CHO cells stably expressing recombinant diheteromeric human NMDARs, co-expressing hGluN1 with hGluN2A, hGluN2B, hGluN2C, or hGluN2D subunit, were used in manual whole cell patch clamp experiments.
- Cells were clamped at -60 mV holding potential, in the presence of 1 mM extracellular $MgCl_2$.
- Voltage protocol included depolarizing 2 seconds step pulses to +40 mV, followed by a 2 second ramps back to holding potential.
- The voltage stimulation was repeated 5 times at 15 s intervals
- Intracellular solution was composed of (in mM): 80 CsF, 50 CsCl, 0.5 $CaCl_2$, 10 HEPES, 11 EGTA, adjusted to pH 7.25 with CsOH.
- Extracellular solution was composed of (in mM): 155 NaCl, 3 KCl, 1.0 $MgCl_2$, 1.5 $CaCl_2$, 10 HEPES, 10 D-glucose; pH 7.4 with NaOH
- NMDAR mediated currents were measured -60 mV after 120 s perfusion with 1 μM L-glutamate, first in the absence, then in the presence of 1, 3, 10, 30, or 100 μM esmethadone.
- Current values in presence of esmethadone were percentualized to current values previously recorded in absence of esmethadone and expressed as mean ± standard error mean (SEM).
- Concentration response curve data were fitted by GraphPad Prism to four parameters logistic equation:

$$I(\%) = 100 / (1+10^{((\log IC_{50} - \log[esmethadone])*HillSlope)})$$

CONCLUSIONS

- Esmethadone reduced 1 μM L-glutamate-induced current in all NMDAR isoforms, in the presence of 1 mM extracellular magnesium and 10 μM glycine.
- Esmethadone showed preference for NMDAR containing GluN2D subunit
- Esmethadone preference for GluN2D subunit, in the presence of relatively low glutamate concentration, may play a role in its therapeutic antidepressant effect and may help improve our understanding of the pathophysiology of MDD.

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

RESULTS

Figure 1

Test items application and voltage protocol diagram. Cells were kept at V = - 60 mV, stepped to V = 40 mV for 2 s, then ramped back to V=-60 mV (2 s). The voltage stimulation was repeated five times, with 15 s interval. Meanwhile, a 120 s perfusion of 1 μM L-glutamate was performed, in presence of 1 μM glycine and 1 mM $MgCl_2$, and the current value measured as the average of last 2 s (dotted box).

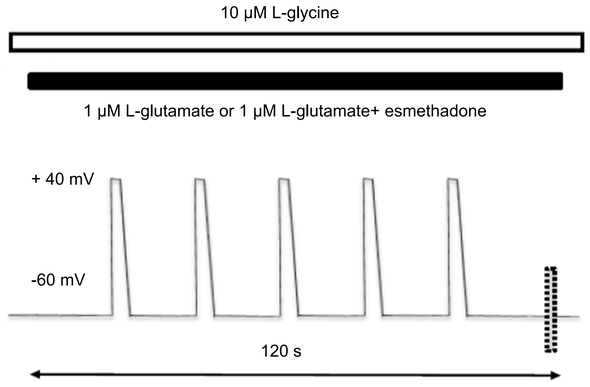


Figure 3

Esmethadone resulted more potent in blocking NMDAR containing hGluN2D subunit (green dots and trace) in described assay conditions. Graph represents % current recorded in the presence of 1, 3, 10, 30, or 100 μM esmethadone and normalized with respect to control, and relative fittings in four different NMDAR cell lines. Recordings were obtained in presence of 1 μM L glutamate,10 μM glycine and 1mM $MgCl_2$, at the end of a 120 s incubation period with L-glutamate and esmethadone. Data are mean ± SEM. IC_{50} and Hill slope values of every fitting are reported in Table 1.

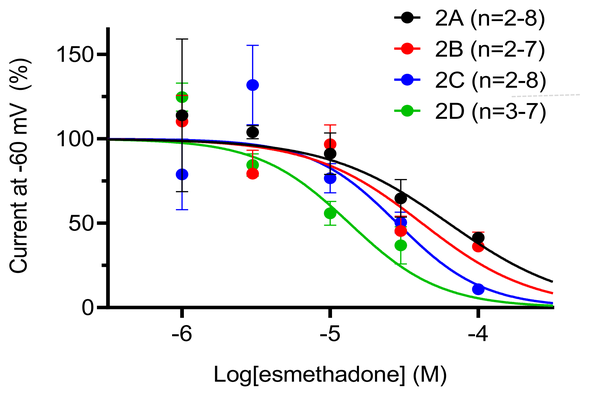


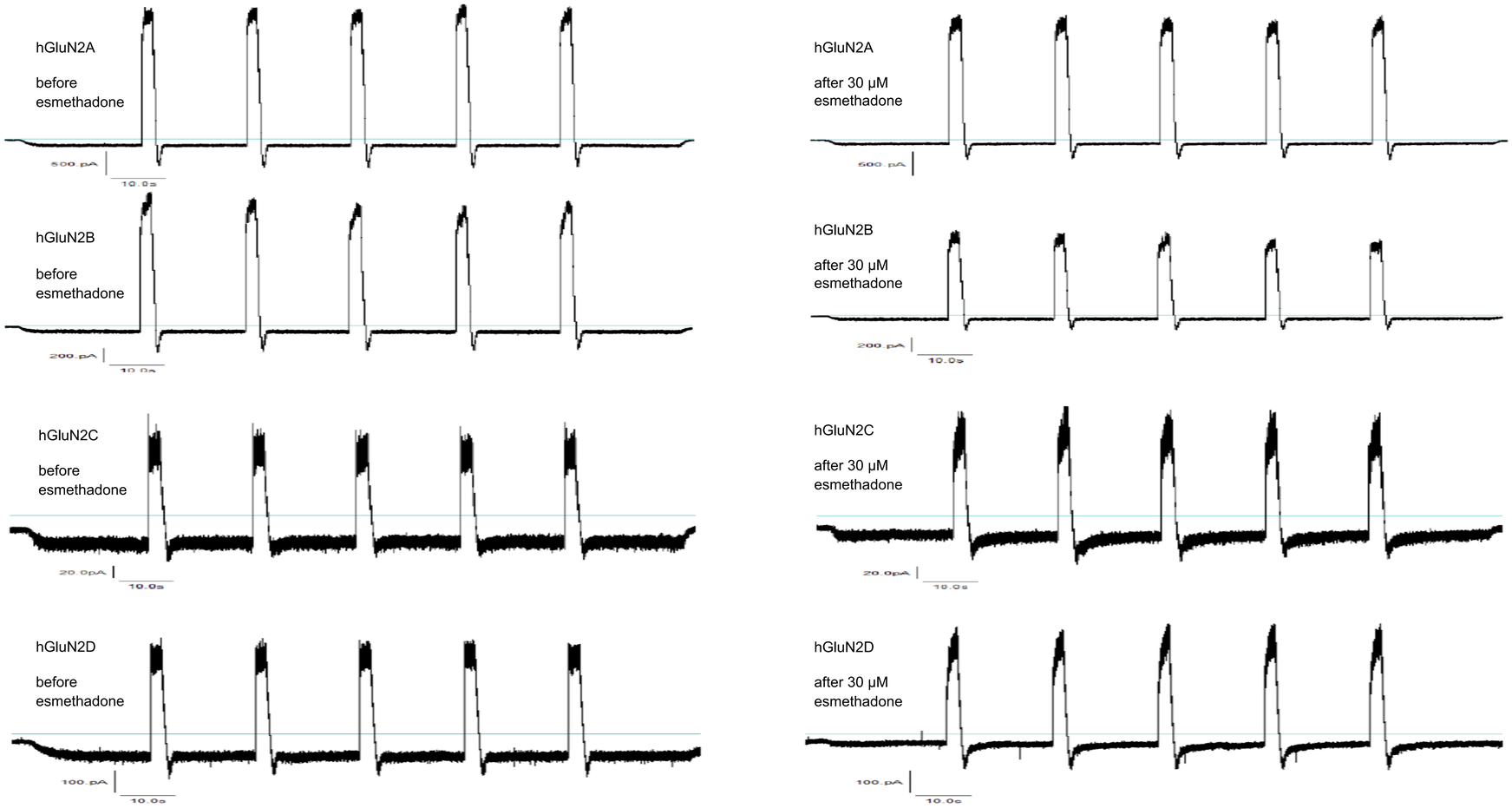
Table 1

Esmethadone resulted more potent in blocking NMDAR containing hGluN2D subunit, about 5-fold more potent than when NMDAR contained hGluN2A subunit. Fittings parameters for esmethadone were obtained from data shown in figure 3, and analysed with GraphPad Prism .

Subunits	IC_{50} (μM)	Hill slope	Cell number
hGluN1-hGluN2A	63.1	1.06	2-8
hGluN1-hGluN2B	41.7	1.17	2-7
hGluN1-hGluN2C	28.4	1.49	2-8
hGluN1-hGluN2D	13.5	1.42	3-7

Figure 2

The effect of different esmethadone concentratations was tested in presence of 1 μM L-glutamate for 120 s. Here, sample traces of recordings from cells before and after addition of 30 μM esmethadone are shown. Normalized data were used for the graphs reported in Figure 3 and then to evaluate esmethadone IC_{50} for the various NMDARs. 1 μM L glutamate was added for 120 s in presence of 10 μM glycine and 1mM $MgCl_2$. Analysed data are presented in Figure 3 and Table 1.



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