Introduction

Esmethadone (REL-1017) is a novel NMDA receptor (NMDAR) channel blocker currently in clinical development for major depressive disorder (MDD).

Objectives

- To better understand esmethadone role as a channel blocker, we examined the functional interaction between esmethadone and quinolinic acid, a brain metabolite and endogenous neurotransmitter acting as a partial agonist at the glutamate binding site of the NMDAR, and esmethadone and gentamicin, an antibiotic showing toxicotic and nephroprotective mediated by NMDAR potentiation.

Methods

- Intracellular calcium levels were measured by fluomimetric imaging plate reader (FLIPR) in 384 well plate format, using Fluo-4 fluorescent free calcium indicator.
- Four CHO cell lines expressing human heterodimeric NMDA receptors were used: hGluN1/hGluN2A, hGluN1/hGluN2B, hGluN1/hGluN2C and hGluN1/hGluN2D.
- Test items were added together with 10 µm glycine and indicated concentration of L-glutamate in magnesium-free buffer.
- Area under the curve (AUC) values of fluorescence readings were measured for 5 minutes after L-glutamate addition.
- Data were normalized to readings obtained in presence of 100 µM L-glutamate plus 10 µM glycine (100%) and buffer (0%).

Results

Figure 1 - Quinolinic acid CRC

Quinolinic acid is an endogenous neurotoxin, acting on NMDAR. Little was known about quinolinic acid effect on various NMDAR isoforms. Therefore, we studied quinolinic acid concentration response curve (CRC) on four NMDA receptor expressing CHO cell lines, by FLIPR assay in presence of 10 µM L-glutamate plus 10 µM glycine (100%) and buffer (0%).

Figure 2a - Esmethadone reduced calcium entry induced by quinolinic acid alone

Esmethadone (50 µM) reduced calcium entry induced by quinolinic acid (0.2 µM) in four NMDAR cell lines. Data are shown as % mean ± SEM. n=42 for each group.

Figure 2b - Esmethadone reduced intracellular calcium entry induced by quinolinic acid in presence of 0.04 µM L-glutamate

Quinolinic acid might be neutralized by achieving NMDAR in presence of low concentrations of ambient L-glutamate. Therefore, 10 µM esmethadone effect on 1000 µM quinolinic acid in presence of 0.04 µM L-glutamate and 10 µM glycine was studied. FLIPR calcium assay was performed in presence of 10 µM glycine, using the 4 NMDAR cell lines. Data are shown as % mean ± SEM. n=42 for each group.

Table 1 - Quinolinic acid EC₅₀

Table showing the EC₅₀ values for esmethadone in presence of different concentrations of quinolinic acid.

Figure 3a - Gentamicin CRC

Gentamicin, an antibiotic with significant renal toxicity, at high concentrations. Therefore, we investigated if gentamicin might activate NMDAR in absence of L-glutamate, gentamicin CRCs were run by FLIPR assay in presence of 10 µM L-glutamate, but in absence of any glycine, using four different CHO cell lines expressing different heterodimeric human NMDA receptors. No effect was observed in any of the cell lines. Data are shown as % mean ± SEM. n=42.

Figure 3b - Gentamicin CRC in 10 µM L-glutamate

To investigate if gentamicin neurotoxicity might be mediated by gentamicin potentiation of L-glutamate induced calcium entry through NMDAR, we performed gentamicin concentration response curve by FLIPR assay in presence of 10 µM L-glutamate with 10 µM glycine. Gentamicin resulted as polar inhibitor only on NMDA receptor containing hGluN2B subunit, with 50% effect at 100 µM.

Figure 4 - Esmethadone reduced gentamicin induced calcium entry in presence of 0.04 µM L-glutamate

Gentamicin might be neutralized by achieving NMDAR in presence of low concentrations of ambient L-glutamate. Therefore, we investigated if esmethadone might counteract such gentamicin effect. 10 µM esmethadone (3X) reduced calcium entry induced by 10 µg/ml gentamicin in presence of 0.04 µM L-glutamate and 10 µM glycine was studied in FLIPR assay, using the 4 NMDAR cell lines. Data are shown as % mean ± SEM. n=42 for each group.

Disclosures

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

Corresponding Author: Paolo L. Manfredi, M.D. Email: pmanfredi@relmada.com