Esmethadone (REL-1017) Shows Slower Onset Kinetics Compared to Ketamine in Manual Patch Clamp Studies

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INTRODUCTION

- (±)-Ketamine and esmethadone have been shown to have robust and rapid antidepressant-like effect in experimental models of depression (Li 2010; Fogaca 2019).
- Esmethadone (REL-1017; dextromethadone; DXT) is in advanced clinical development for major depressive disorder (MDD).

OBJECTIVES

- To evaluate esmethadone and (±)-ketamine in manual patch clamp, and to assess their onset and offset kinetic, as well as their trapping in heterodimeric human hGluN1/hGluN2C NMDAR.

METHODS

- hGluN1/hGluN2C-CHO cells grown on poly-D-lysine coated glass coverslips were studied by manual patch clamp whole cell recording.
- Intracellular solution (in mM): 80 CsF, 50 CsCl, 0.5 CaCl2, 10 HEPES, 11 EGTA, adjusted to pH 7.25 with CsOH.
- Extracellular solution (in mM): 155 NaCl, 3 KCl, 1.5 CaCl2, 10 HEPES, 10 D-glucose adjusted to pH 7.4 with NaOH.
- Recordings occurred at -70 mV fixed voltage equal to holding potential.
- Initial tests established esmethadone and (±)-ketamine concentrations able to inhibit ~ 75% of current elicited by 10/10 µM glutamate/glycine.
- hGluN1/hGluN2C-CHO cells were exposed for 5 s to 10/10 µM L-glutamate/glycine, followed by a 30-s co-application of L-glutamate/ glycine plus test item and a 50 s re-exposure to L-glutamate/glycine, for tau-on and tau-off calculations.
- An intermediate 85-s washing step with 10 µM glycine alone was introduced for trapping studies.
- Test item on-/off-rates were estimated by curve fitting to first order exponential equations (see also Figure 3):

  First order equation for test item onset:

  $$I(t) = I_1 + (I_0 - I_1) \times e^{-t/\tau on} \quad (1)$$

  First order equation for test item offset:

  $$I(t) = I_1 + (I_2 - I_1) \times (1 - e^{-t/\tau off}) \quad (2)$$

- For trapping studies, initial current block was calculated as:

  $$B = [(I - I_B) / I] \times 100 \quad (3)$$

  where I was the current value derived from a linear extrapolation to the end of the L-glutamate antagonist co-application, and I_B was the current measured at the end of L-glutamate/blocker co-application.
- The residual current block was defined as:

  $$B_R = [(I_{1st} - I_{2nd}) / I_{1st}] \times 100 \quad (4)$$

  where I_{1st} was the maximal current measured during 1 s after onset of the first L-glutamate exposure and I_{2nd} was the maximal current measured during 1 s after onset of the delayed second L-glutamate exposure after washout of blocker from the bath.

The trapped block (B_T) was defined as:

$$B_T = B_R / B \times 100 \quad (5)$$

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 - Sample current traces in hGluN1/hGluN2C-CHO cells

Representative current traces recorded from two different cells, added with 10/10 µM L-glutamate/glycine in the absence or in the presence of 10µM esmethadone (left) or 1µM (±)-ketamine (right).

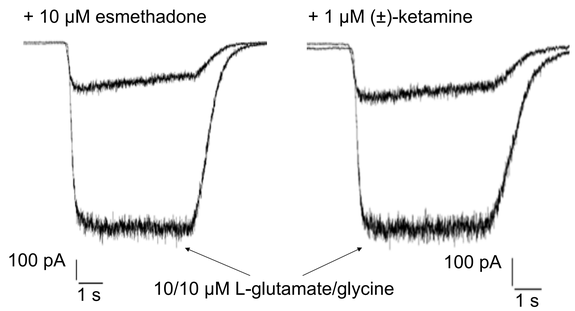

Figure 2 - Esmethadone or (±)ketamine effect on hGluN1/hGluN2C

Initial tests were performed to establish esmethadone and (±)-ketamine concentrations able to inhibit about 75% of current elicited by 10/10 µM glutamate/glycine, as required to carry-out kinetic studies. 10 µM esmethadone and 1 µM (±)-ketamine were used for further kinetic studies, since elicited similar current blockade.
Control current (100%) was induced by 10/10 µM L-glutamate/glycine and resulted of -594.2 ± 103.7 pA (mean ± SEM, n = 28). 10 µM esmethadone and 0.3, 1, 3, 10 (±)-ketamine reduced L-glutamate/glycine elicited current to a residual % current of 74.6 ± 1.9 % (n=12), 97.2 ± 0.3 % (n=3), 89.7 ± 0.6 % (n=3), 74.6 ± 2.2 % (n=3), 44.2 ± 3.0 % (n=7), respectively..

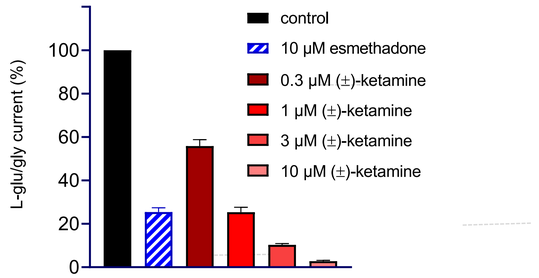

Figure 3 - Sample traces of esmethadone or (±)ketamine onset and offset kinetic experiments

A protocol was set up to evaluates how fast esmethadone blockade of NMDAR mediated currents can be established (onset kinetic), and how fast this blockade can be removed (offset kinetic), by perfusion with a buffer containing agonist L-glutamate but devoid of esmethadone. Test item application protocol diagram (top) and sample traces (bottom) of test item onset and offset kinetic experiments with 10 µM esmethadone treated cell (left), or 1 µM (±)-ketamine treated cell (right). I_0 , I_1 and I_2 were the currents measured at the end of the first 5 s 10 µM/10 µM L-glutamate/glycine application, the 30 s co-application with test item, the final 50 s co-agonists application, respectively.

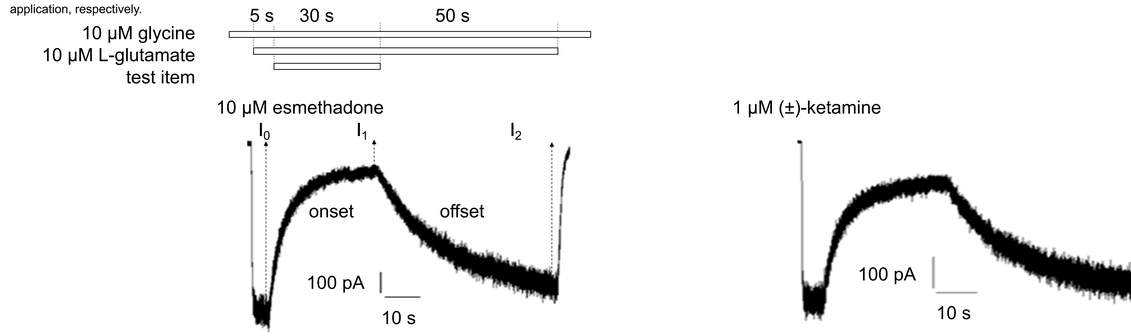

Figure 4 - Onset kinetic fitting

Current values obtained from different experiments during the onset phase were normalized and averaged, to derive tau-on parameters reported in table 1. Traces represent % mean ± standard error of current after addition of 10 µM esmethadone (blue, n= 11), 1 µM (±)-ketamine (red, n=10) and 10 µM (±)-ketamine (violet, n=4), while internal black lines are relative fittings.

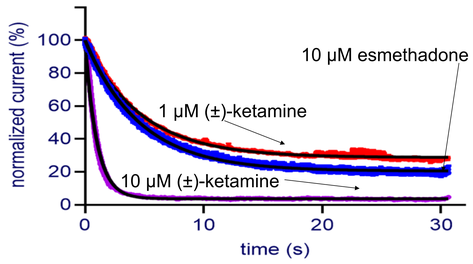

Figure 5 - Offset kinetic fitting

Current values obtained from different experiments during the offset phase were normalized and averaged, to derive tau-off parameters reported in table 2. Traces represent % mean ± standard error of current after removal of 10 µM esmethadone (blue, n=11), 1 µM (±)-ketamine (red, n=10) and 10 µM (±)-ketamine (violet, n=4), while internal black lines are relative fittings.

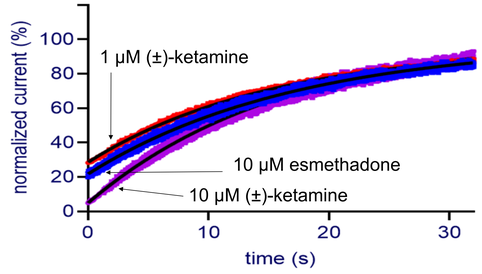

Figure 6 - Sample traces of esmethadone or (±)ketamine trapping experiments

Trapping experiments were designed to measure trapped block (B_T) of 10 µM esmethadone or 1 µM (±)-ketamine, that is % ratio between residual block (B_R) after extensive (85 s) cell wash with glycine alone and initial test item block (B). Test item application protocol diagram (top) and sample traces (bottom) of trapping experiments with 10 µM esmethadone treated cell (left), or 1 µM (±)-ketamine treated cell (right). I_{1st} and I_{2nd} were the peak currents measured within 1 s after onset of the first and second 10 µM/10µM L-glutamate/glycine addition, respectively.

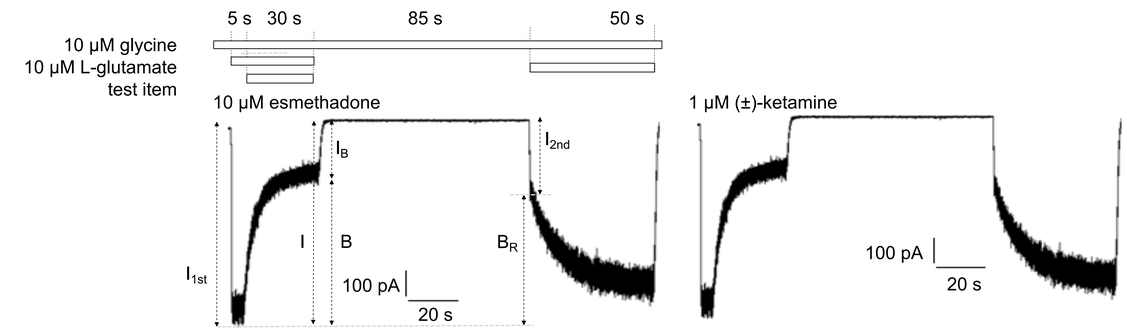

Table 1 - Onset kinetic parameters

Onset kinetic constant (tau-on) of a NMDA receptor antagonist represents the time required for the test item to reach approximately 63.2% of its current blocking effect. Tau-on (s) is reported as mean ± SEM., while I_1 is % current measured at the end of test item addition, and normalized respect to I_0 current, the current value before test item addition, which is set equal to 100%. **** is P<0.0001 from Tukey's test after one-way ANOVA.

Test item	Tau-on (s)	I_1 (% current)	Cell number
10 µM esmethadone	4.7 ± 0.21	20.4 %	11
1 µM (±)-ketamine	4.7 ± 0.14 .	28.7 %	10
10 µM (±)-ketamine	0.99 ± 0.05****	3.6 %	4

Table 2 - Offset kinetic parameters

Offset kinetic constant (tau-off) of a NMDA receptor antagonist represents the time required for the removal of approximately 63.2% of test item current blocking effect, in continuous perfusion with a buffer containing the agonist L-glutamate, but not the test item. Tau-off (s) is reported as mean ± SEM, while I_2 (% current) is measured at the end of test item removal, and normalized respect to I_0 current, the current value before test item addition, which is set equal to 100%. Tau-off values were not significantly different, by Tukey's test after one-way ANOVA.

Test item	Tau-off (s)	I_2 (% current)	Cell number
10 µM esmethadone	17.7 ± 1.1	98.5 %	11
1 µM (±)-ketamine	15.2 ± 0.63 .	95.5 %	10
10 µM (±)-ketamine	17.2 ± 3.0	102.9%	4

Table 3 – Trapping parameters

Trapped block (B_T) resulted similar comparing 10 µM esmethadone and 1 µM (±)-ketamine, although their initial block (B) and residual block (B_R) were significantly different. Experiments were carried out as exemplified in figure 6. Table data are mean ± SEM, and statistic was performed with two-tailed unpaired T test: **** is P < 0.0001; *** is P < 0.001

Test item	B	B_R	B_T	N
10 µM esmethadone	83.8 ± 1.2 ****	71.8 ± 1.1 ***	85.9 ± 1.9	13
1 µM (±)-ketamine	74.0 ± 1.2	64.1 ± 1.3	86.7 ± 1.8	11

CONCLUSIONS

- Slow channel blocking and unblocking kinetics were detected with NMDAR including hGluN2C subunit, in agreement with published observations. [2]
- Recorded data suggest that about 10-fold higher potency of (±)-ketamine respect to esmethadone is due to (±)-ketamine faster onset kinetic when tested at same esmethadone concentration, with no significantly different offset kinetic.
- Comparison of 10 µM esmethadone and 1 µM (±)-ketamine, which elicited similar % inhibition of 10/10 µM L-glutamate/glycine induced current, showed that esmethadone and (±)-ketamine have similar onset and offset kinetics (tau values).
- Slow esmethadone onset kinetic, taking several seconds to block hGluN1/hGluN2C receptor, suggests esmethadone may not affect phasic synaptic activity of NMDA receptor, while it could modulate tonic ambient L-glutamate effects, which might influence activity of brain cells expressing NMDAR composed of specific subunits, preferentially. [3, 4]
- 10 µM esmethadone and 1 µM (±)-ketamine also showed similar trapped current block.
- Esmethadone trapped block might be functional to inhibition of ambient glutamate effect on specific NMDAR, which in turn might be relevant for its antidepressant effect without cognitive side effects.

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