Pharmacological characterization of recombinant cell lines expressing human di-heteromeric NMDA receptors

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N-methyl-D-aspartate receptors (NMDARs) are glutamate and glycine-gated Ca_2-permeable channels highly expressed in the central nervous. crucial to brain functions such as circuit development and learning and memory, and dysfunction of NMDAR activity has been implicated in a variety of neuropathological conditions, including stroke, epilepsy, schizophrenia, depression, Huntington's disease, Alzheimer's disease, Parkinson's disease, and multiple sclerosis. The majority of NMDARs are tetrameric complexes, consisting of two glycine-binding GluN1 subunits and two glutamate-binding GluN2 subunits (Lee et al., 2014). GluN1 is coded by a single gene with at least eight different splice variants; four different Glu2 genes originate GluN2A, GluN2B, GluN2C, and GluN2D subunits (Paoletti, 2011). NMDARs containing different NR2 subunits have different pharmacological and kinetic properties (Willie et al., 2013).
Four different recombinant CHO cell lines have been generated expressing human NMDA receptors composed of GluN1-3 subunit (one of the eight different splice variants of NR1 subunit) in combination with one of the following: GluN2A, GluN2B, GluN2C, GluN2D.
FLIPR (Fluorescence Imaging Plate Reader) calcium mobilization assays have been set up using the four different cell lines. Cell lines have been pharmacologically validated in FLIPR with a set of compounds including negative modulators (NAMs), competitive antagonists, pore blockers. As example, glycine site antagonist 5,7-DCKA was able to inhibit all four different cell lines, albeit with different potencies, as expected, while selective NAM TCN-201 (Bettini et al., 2010) was able to inhibit GluN1-GluN2A cell line with an IC50 of 0.5 uM, but not the other three cell lines. GluN1-GluN2B cell line was also profiled in manual and in automated QPatch. The four cell lines can constitute an useful tool to profile compounds acting at various isoform of NMDARs.

Bettini E, Sava A, Griffante C, Carignani C, Buson A, Capelli AM, Negri M, Andreetta F, Senar-Sancho SA, Guiral L, Cardullo F. Identification and characterization of novel NMDA receptor antagonists selective for NR2A- over NR2B-containing receptors. J Pharmacol Exp Ther. 2010 Dec;335(3):636-44.
Lee CH, Lü W, Michel JC, Goehring A, Du J, Song X, Gouaux E. NMDA receptor structures reveal subunit arrangement and pore architecture. Nature. 2014 Jul 10;511(7508):191-7.
Paoletti P. Molecular basis of NMDA receptor functional diversity. Eur J Neurosci. 2011 Apr;33(8):1351-65.
Wyllie DJ, Livesey MR, Hardingham GE. Influence of GluN2 subunit identity on NMDA receptor function. Neuropharmacology. 2013 Nov;74:4-17.