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Adapting an in vitro neuronal excitotoxicity model to elucidate the novel toxic mechanism of a positive allosteric modulator of the metabotropic glutamate receptor 5

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Metabotropic glutamate receptor 5 (mGlu5) has a prominent role in excitatory neurotransmission in the mammalian CNS and has been shown to have a potential involvement in neurological disorders, such as fragile X disease and schizophrenia. Recently, allosteric ligands for mGlu5 have been described. As well as providing increasing prospects for the regulation of these receptors and use as potential therapeutics, some positive allosteric modulators have been reported to display receptor-dependent neurotoxicity (Parmentier-Batteur, Hutson et al. 2013). We aimed to test an in vitro system to discern the underlying mechanisms of this toxicity, using a neurotoxic positive allosteric modulator (LSN-2814617) (Gilmour, Broad et al. 2013) and the prototypical negative allosteric modulator MPEP.

Rat cortical neurons were derived from embryonic cortical samples from E18 wistar rats and plated onto poly-D-lysine coated plates at a density of 4 x 105 cells well-1 for all experiments, which were performed after 7 days in vitro (DIV). Cell death was measured at a 24 hour time-point using lactate dehydrogenase (LDH) activity as a marker. Firstly, the endogenous ligand of mGlu5, L-glutamate, was added to a 96 well plate at a range of concentrations (1 µM to 10 mM) either alone, or in the presence of MPEP (100 µM) or LSN-2814617 (100 µM). L-glutamate produced cell death across the range of concentrations (pLD50 = 4.80 ± 0.08, n=3). No significant change in total cell death or pLD50 was observed at any concentration of L-glutamate in the presence of LSN-2814617, however, MPEP caused a decrease in total cell death (65 ± 3% of L-glutamate max, n=3), and shifted the pLD50 for L-glutamate (3.71 ± 0.07, n=3). Next, we investigated the time-course of cell death in this system, to ascertain if LSN-2814617 or MPEP affected the rate of neuronal cell death. No significant change in the rate of cell death occurred in the presence of LSN-2814617 or MPEP; however, MPEP again reduced total cell death. Finally, we tested MPEP at a range of concentrations (100 nM to 100 µM) to investigate whether the neuroprotective effect of MPEP occurred in a concentration-dependent manner. Surprisingly, MPEP was only neuroprotective at a concentration of 100 µM, implying that the neuroprotective effect of MPEP is not mediated through mGlu5.

These data show that the neuronal cell death reported for LSN-2814617 cannot be investigated in primary pure cortical neuron preparations using this assay, perhaps implying that it is unlikely the neurotoxic effect is mediated directly by mGlu5 action in neurons. Further pharmacological profiling and comparison of the ligand LSN-2814617 with non-toxic mGlu5 PAMs will be essential in elucidating the underlying mechanism of toxicology.

Gilmour, G., et al. (2013). "In vitro characterisation of the novel positive allosteric modulators of the mGlu(5) receptor, LSN2463359 and LSN2814617, and their effects

on sleep architecture and operant responding in the rat." Neuropharmacology 64: 224-239.

Parmentier-Batteur, S., et al. (2013). "Mechanism based neurotoxicity of mGlu5 positive allosteric modulators - Development challenges for a promising novel antipsychotic target." Neuropharmacology.