Selective blockade of NR2B subunit containing NMDA receptors enhances AMPA receptor-mediated EPSPs and sub-maximal LTP in hippocampal CA1 pyramidal neurons through disinhibitory effects

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Introduction
NR2B subtype selective NMDA receptor antagonists, unlike non-selective NMDA receptor antagonists, do not produce marked stimulant or stereotypical behaviours in rodents, which are considered to reflect psychotomimetic properties in humans. In man, this has translated to high exposures being achieved without marked CNS and psychiatric side-effects (Preskorn et al., 2000; poster 641.22 this session). Furthermore, NR2B subtype selective antagonists have been shown to enhance performance in rats in certain cognitive tests of attention and working memory (Higgins et al., 2005). We sought to examine the potential synaptic mechanisms that may underlie these behavioural findings by studying the effects of EVT 101 (poster 641.22 this session), and the related compound, EVT 105, novel NR2B subtype selective NMDA antagonists currently in clinical development, on synaptic transmission and long term potentiation in hippocampal slices from adult rats.

Methods
Sagittal hippocampal slices of 400µm thickness were prepared from male Wistar rats (5-8 weeks, 150-250g) in cooled artificial cerebrospinal fluid (aCSF; 3°C) using a microslicer (Leica VT1000S). Slices were subsequently maintained in oxygenated (95% O2 - 5% CO2) aCSF at room temperature for at least 1 hour to allow electrophysiological recording. Patch pipettes were pulled using a horizontal puller (Sutter Instrument Co, Novato, Ca, USA) from thin-walled borosilicate glass capillaries (Clarke Electromedical) and had resistances between 4-10 MΩ when filled with recording solution of the following composition (mM): KCl 140, K2EGTA 0.5, HEPES 10, NaATP 2, pH 7.2. Recordings were obtained with a patch clamp amplifier (Axopatch-1D, Axon Instruments). The extracellular aCSF had the following composition (mM): NaCl 127, KCl 1.9, KH2PO4 1.2, MgCl2 1.3, CaCl2 2.4, NaHCO3 26 and D-glucose 10.

Compounds were bath-applied from reservoirs connected to the aCSF flow line by manually operable three-way valves. Generally, antagonists were applied for at least 10 minutes to ensure equilibration in the recording chamber. Synaptic currents and potentials were evoked by electrical stimulation of the Schaffer collateral-commissural pathway with concentric bipolar stimulating electrodes (1-15V, 0.02ms, 0.03-0.05Hz).

Results

CA1 pyramidal neurones: EVT 101 and EVT 105 produced a concomitant increase in peak amplitude of EPSPs and a suppression of IPSPs evoked by stimulation of the Schaffer collateral pathway. Concentrations of 100 nM and 1 μM were used, approximately 10- and 100-fold above their IC50 for blockade of NMDA receptors. A, B show at least 6 superimposed EPSPs and D show the superimposed averages of at least 16 evoked responses.

CA1 pyramidal neurones: EVT 101 and EVT 105 had no effect on the isolated NMDA receptor-mediated EPSP in A CA1 pyramidal cells but produced a profound inhibition of the evoked EPSP EPSP in B stratum radiatum interneurones.

Stratum Radiatum interneurones: EVT 101 and EVT 105 did not produce the same increase in size of the potentiating effect of EVT 105 on EPSPs but produced a profound inhibition of the evoked EPSP in both CA1 pyramidal cells and stratum radiatum interneurones.

As these findings indicated that the NR2B subtype selective antagonists produced disinhibition without blocking NMDA receptor-mediated transmission on CA1 pyramidal cells, we examined the effect of EVT 101 and EVT 105 on the induction and maintenance of submaximal LTP following high frequency stimulation (HFS) of the Schaffer collateral pathway.

Conclusions
1. EVT 101 and EVT 105 increased fast excitatory synaptic responses in CA1 pyramidal neurones following stimulation of the Schaffer collateral pathway through a disinhibitory effect
2. EVT 105 markedly inhibited NMDA receptor-mediated EPSPs and EPSCs on stratum radiatum interneurones but was without effect on the evoked EPSP in CA1 pyramidal cells
3. In contrast to non-selective NMDA antagonists, EVT 101 & 105 potentiated submaximal LTP induction
4. These effects may underlie the cognitive enhancing effects of NR2B subtype selective antagonists seen in certain behavioural tests

References