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Selective blockade of NR2B subunit containing NMDA receptors enhances AMPA receptor-mediated EPSPs and sub-maximal LTP in hippocampal CA-1 pyramidal neurons through disinhibitory effects

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Introduction

Unlike non-selective NMDA receptor antagonists, NR2B subtype selective NMDA receptor antagonists do not produce marked stimulant or stereotypical behaviours in rodents, which are considered to reflect psychotomimetic properties in humans, and high exposures can be achieved in man without producing marked psychotomimetic side-effects (Preskorn *et al*., 2008; poster 641.22 this session). Furthermore, NR2B subtype selective antagonists have been shown to enhance performance in certain cognitive tests of attention and working memory (Higgins *et al*., 2005).

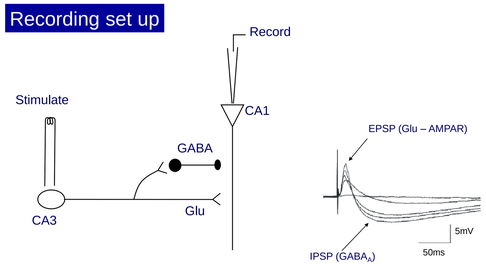
We have sought to examine the potential synaptic mechanisms that may underlie these behavioural findings by examining the effects of EVT 101 (poster 641.22 this session) and EVT 105, novel NR2B subtype selective NMDA antagonists currently in clinical development, on synaptic transmission and LTP in hippocampal slices from adult rats.

Methods

Saggital hippocampal slices of 400μm thickness were prepared from male Wistar rats (5 - 8 weeks, 150-250g) in cooled artificial cerebrospinal fluid (aCSF; <4ºC) using a microslicer (Leica VT1000S). Slices were subsequently maintained in oxygenated (95% O_2 – 5% CO_2) aCSF at room temperature for at least 1 hr prior to 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); 140 Kgluconate, 10 KCl, 0.5 K_2EGTA, 10 HEPES, 2 Na_2ATP, pH 7.2. Recordings were obtained with a patch clamp amplifier (Axopatch-1D, Axon Instruments). The extracellular aCSF had the following composition (in mM): NaCl 127, KCl 1.9, KH_2PO_4 1.2, MgCl 1.3, $CaCl_2$ 2.4, $NaHCO_3$ 26 and glucose 10.

All drugs 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).

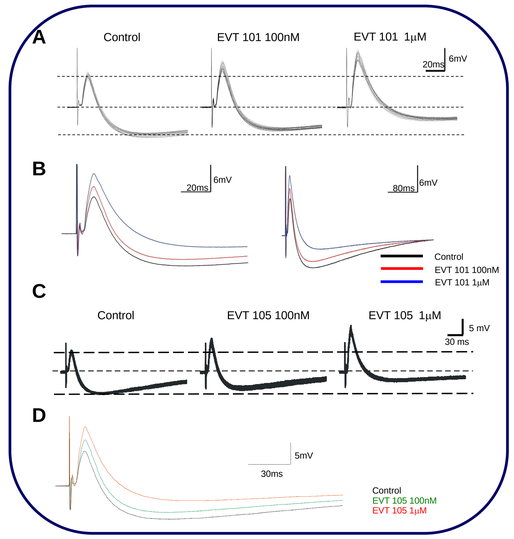
Recording set up



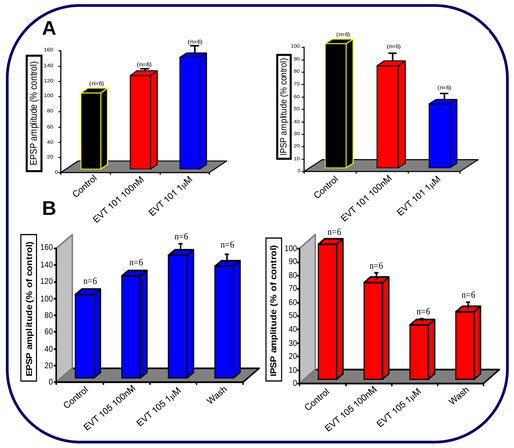
Results

CA-1 pyramidal neurones: EVT 101 and EVT 105 produced a concomitant increase in peak amplitude of EPSPs and a suppression of IPSPs in evoked by stimulation of the Schaffer collateral pathway.

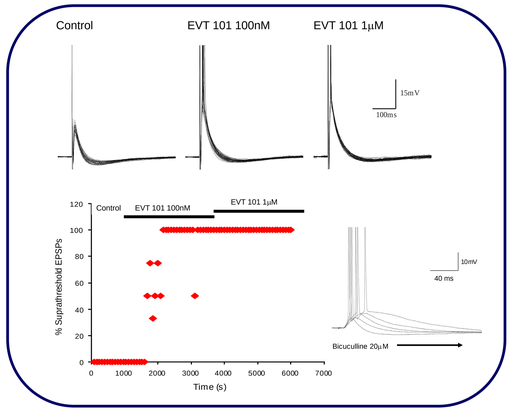
A & **C** show at least 6 superimposed EPSP/IPSPs. **B** & **C** show the overlapping averages of at least 16 evoked responses



CA-1 pyramidal neurones: Mean data of the effects of (A) EVT 101 and (B) EVT 105 on evoked EPSPs and IPSPs in CA1 pyramidal neurones.

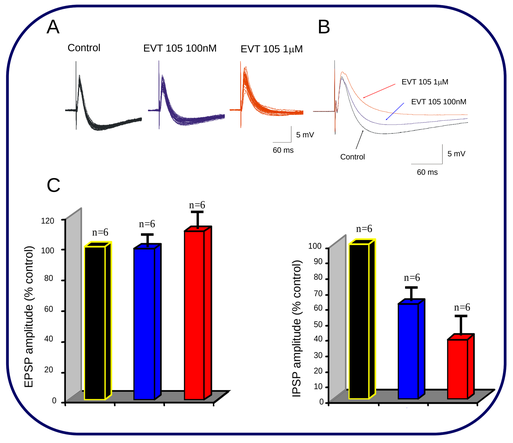


CA-1 pyramidal neurones: EVT 101 and EVT 105 increased the probability of sub-threshold EPSPs reaching threshold for action potential firing but never induced burst firing or spontaneous burst firing, unlike the $GABA_A$ receptor antagonist, bicuculline

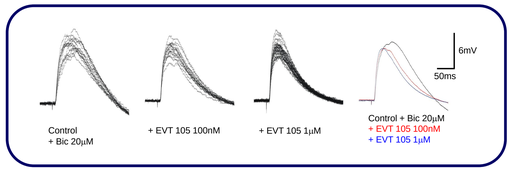


Stratum Radiatum interneurones: EVT 101 and EVT 105 did not produce the same increase in size of EPSPs as in CA-1 cells although they produced a similar decrease in the size of the IPSP

A shows at least 8 superimposed EPSP/IPSPs, **B** shows the overlapping averages of at least 16 evoked responses and **C** shows the mean data from 6 interneurones



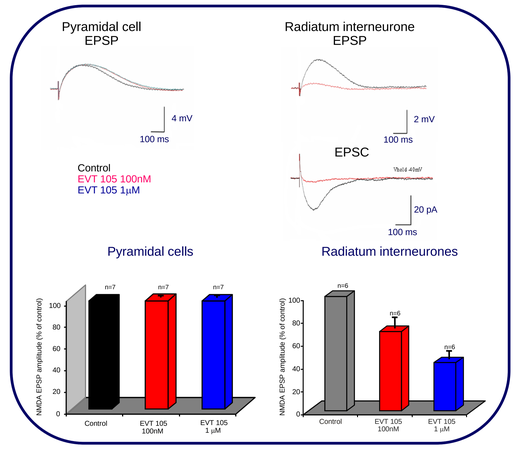
CA-1 pyramidal neurones: Blockade of fast GABAergic inhibition with bicuculline (20 μM) occluded the potentiating effect of EVT 105 on EPSPs



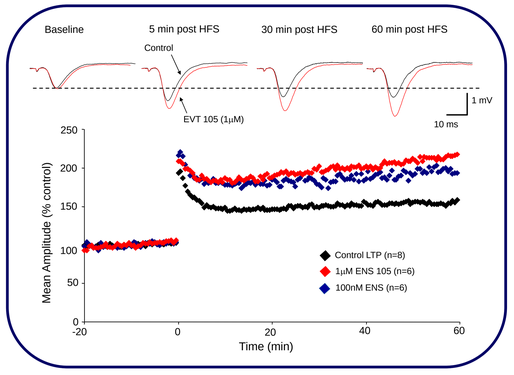
These results were consistent with EVT 101 and EVT 105 having disinhibitory effects on synaptic transmission in the CA-1 region of the hippocampus, indicating that NR2B receptors may play a more predominant role in the synaptic excitation of inhibitory interneurones than of CA-1 pyramidal neurones. We investigated this directly by examining the effects of EVT 105 on isolated NMDA receptor-mediated synaptic responses in both CA-1 pyramidal cells and stratum radiatum interneurones.

NMDA receptor-mediated responses were isolated by perfusion with the $GABA_A$ receptor antagonist, bicuculline (20μM), the $GABA_B$ receptor antagonist, CGP55845 (200nM), the non-NMDA receptor antagonist, NBQX (10μM) and depolarisation of the membrane potential to -48 to -40mV.

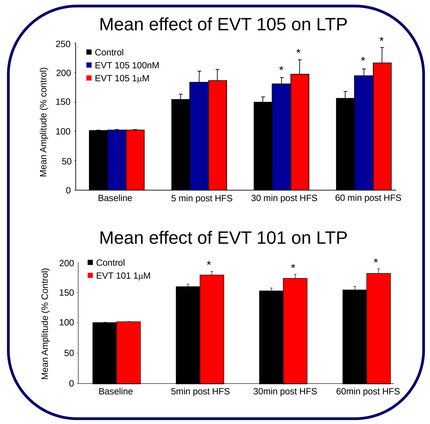
EVT 105 had no effect on the isolated NMDA receptor-mediated EPSP in CA-1 pyramidal cells but produced a profound inhibition of the evoked EPSP and EPSC in stratum radiatum interneurones.



As these findings indicated that the NR2B subtype selective antagonists produced disinhibition without blocking NMDA receptor-mediated transmission on CA-1 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.



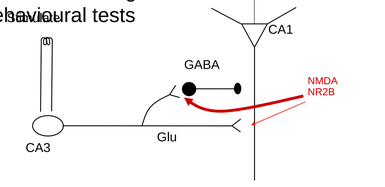
EVT 101 and EVT 105 produced a potentiation of submaximal LTP in CA-1 pyramidal cells.



These results are consistent with previous studies indicating that, under the conditions used here of single shock stimulation, NR2A subunit containing receptors predominate in the mediation of NMDA receptor responses in mature Schaffer collateral – CA-1 synapses and, furthermore, that NR2B antagonists do not block the induction of LTP. Our findings also suggest that the disinhibitory effects induced by NR2B subtype selective antagonists can lead to an enhancement submaximal LTP formation.

Conclusions

1. EVT 101 and EVT 105 produced an increase in fast excitatory synaptic transmission in the CA-1 region of the hippocampus
2. This is due to a disinhibitory effect, caused by a more pronounced effect on inhibitory interneurone synaptic excitation
3. EVT 101 & 105 potentiated sub-maximal LTP induction. This is in contrast to non-selective NMDA antagonists which inhibit LTP induction
4. These effects may underlie the cognitive enhancing effects of NR2B subtype selective antagonists seen in certain behavioural tests



References

Higgins, G.A., Ballard, T.M, Enderlin, M., Haman, M., Kemp, J.A. (2005) Evidence for improved performance in cognitive tasks following selective NR2B NMDA receptor antagonist pre-treatment in the rat. Psychopharmacology, 179: 85–98

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