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L-687,414, a low efficacy NMDA receptor glycine site partial agonist *in vitro*, does not prevent hippocampal LTP *in vivo* at plasma levels known to be neuroprotective

[1,2]Tony Priestley, [1]George R. Marshall, [1,4]Raymond G. Hill & [1,3]John A. Kemp

[1]Merck, Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR

1 *N*-methyl-D-aspartic acid (NMDA) receptors are known to play a key role in the induction phase of long-term potentiation (LTP) at certain hippocampal synapses and to represent some component of spatial learning in animals. The ability of NMDA receptor antagonists (or gene knockout) to impair LTP has led to the suggestion that the therapeutic use of such antagonists may impair cognitive function in humans. The present study compares the effects on LTP of NMDA receptor ion channel block by MK-801 and glycine-site antagonism by 3R(+)*cis*-4-methyl-pyrrollid-2-one (L-687,414).

2 *In vitro* experiments using rat cortical slices revealed L-687,414 to be $\sim$3.6 fold more potent than its parent analogue, R(+)HA-966 at antagonizing NMDA-evoked population depolarizations (apparent K_bs: 15 μM and 55 μM, respectively).

3 Whole-cell voltage-clamp experiments using rat cultured cortical neurones revealed L-687,414 to shift to the right the concentration-response relationship for NMDA-evoked inward current responses ($pK_b = 6.2 \pm 0.12$). L-687,414 affinity for the glycine site on the NMDA receptor complex was also determined from concentration-inhibition curves, $pKi = 6.1 \pm 0.09$. In the latter experiments, L-687,414 and R(+)HA-966 were unable to completely abolish inward current responses suggesting each compound to be a low efficacy partial agonist (estimated intrinsic activity = $\sim$10 and 20% of glycine, respectively).

4 L-687,414 and MK-801 were compared for their effects on NMDA receptor-dependent LTP in the dentate gyrus of anaethestized rats following high frequency stimulation of the medial perforant path (mPP) afferents. Control rats, administered saline (0.4 ml kg^{-1} followed by 0.0298 ml min^{-1}), showed a robust augmentation of the population e.p.s.p. risetime (LTP) recorded in the dentate hilus following tetanic stimulation of the mPP. LTP was effectively abolished in a separate group of rats treated with an MK-801 dosing regimen (0.12 mg kg^{-1} i.v. followed by 1.8 μg kg^{-1} h^{-1}) known to produce maximal neuroprotection in a rat stroke model but, by contrast, remained largely intact in a third group of animals given a similarly neuroprotective L-687,414 treatment (28 mg kg^{-1} i.v. followed by 28 mg kg^{-1} h^{-1}).

5 These experiments suggest that a low level of intrinsic activity at the glycine site may be sufficient to support NMDA receptor-dependent LTP but in circumstances where there is likely to be an excessive NMDA receptor activation the agonism associated with a low efficacy partial agonist, such as L-687,414, is dominated by the antagonist properties. Thus, an NMDA receptor partial agonist profile may offer a therapeutic advantage over neutral antagonists by permitting an acceptable level of 'normal' synaptic transmission whilst curtailing excessive receptor activation.

Keywords: Glutamate receptor; *N*-methyl-D-aspartate; glycine; hippocampus; LTP; partial agonist; L-687,414; synaptic transmission; patch-clamp; *in vivo*

Introduction

Long-term potentiation (LTP) is an activity-dependent increase in synaptic efficacy which has been studied most extensively in the hippocampus. The intense interest in LTP, at least in part, stems from the suggestion that it forms the physiological basis of learning and memory (see Collingridge & Bliss, 1995 for recent review) and the phenomenon is often used as a physiological 'barometer' for the mnemonic properties of drugs. Whilst this may represent a somewhat unrealistic simplification, there is a wealth of evidence to suggest that the hippocampus is fundamentally involved in memory, in particular, lesions of the hippocampal formation are known to result in marked memory deficits in monkeys (Zola-Morgan *et al*., 1991). Similar deficits, particularly in spatial memory, can be induced pharmacologically by *N*-methyl-D-aspartic acid (NMDA) receptor blockade and, as has been more recently demonstrated, by targeted disruption of the gene encoding the NMDAR1 subunit (Tsien *et al*., 1996a,b).

Two key properties of the NMDA receptor are thought to be fundamental to LTP expression. Firstly, the ability of the receptor to function as a coincidence detector requiring the presynaptic release of glutamate and the simultaneous depolarization of the postsynaptic terminal (see Seeburg *et al*., 1995; Collingridge & Bliss, 1995) and secondly, the high calcium permeability of the NMDA receptor (Jahr & Stevens, 1993). It is the elevation of intracellular free calcium levels in the postsynaptic cell and its downstream consequences which are thought to culminate in a long-lasting augmentation of synaptic transmission at that synapse. However, calcium influx

[2]Present addresses: Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, New Jersey 07033, U.S.A;
[3]Pharma Division, Hoffman-La Roche Ltd., CH-4002 Basel, Switzerland.
[4]Author for correspondence.

via the NMDA receptor, which may be involved in plastic processes following controlled, physiological receptor activation, undoubtedly plays a key role in the pathophysiology of excitotoxicity following excessive NMDA receptor activity. Inappropriate, excessive stimulation of NMDA receptors may occur, for example, following an ischaemic insult to the brain. Strategies aimed at attenuating NMDA receptor function in order to achieve neuroprotection during periods of excessive NMDA receptor activation may, therefore, also compromise normal aspects of receptor function, including LTP and, possibly, memory formation.

In the present paper we describe the *in vitro* pharmacological profile of a functional antagonist of NMDA receptor activation, L-687,414, which is the 3**R**(+)*cis*-4-methyl analogue (Williams *et al*., 1989; Leeson *et al*., 1993) of an older generation compound, HA-966. Both HA-966 (specifically the R(+) enantiomer) and L-687, 414 antagonize NMDA receptor function by competing for the glycine co-agonist recognition site on the receptor complex (Fletcher & Lodge, 1988; Foster & Kemp, 1989; Danyz *et al*., 1989; Kloog *et al*., 1990; Hood *et al*., 1990; Compton *et al*., 1990; Foster *et al*., 1992). L-687,414 has improved affinity (~9 fold) over R(+)HA-966 (Foster *et al*., 1991 and 1992) and, like the parent compound, it is a partial agonist with a slightly lower level of intrinsic activity.

Despite being low efficacy partial agonists, R(+)HA-966 and L-687,414 retain the key behavioural properties associated with other NMDA receptor antagonists. Thus, each ligand shows both anticonvulsant (Williams *et al*., 1989; Tricklebank *et al*., 1994) and anxiolytic (Dunn *et al*., 1992) profiles in rodent models but have the added advantage of being devoid of the more prominent pathological effects associated with NMDA receptor ion channel blockers (Hargreaves *et al*., 1993a,b). Furthermore, L-687,414 and the more robust NMDA receptor ion channel blocker, MK-801, have previously been shown to be neuroprotective in a rat model of focal ischaemia (Gill *et al*., 1991; 1995). NMDA receptor partial agonism may offer potential clinical benefits over complete receptor antagonism by permitting enough ongoing receptor activity to sustain normal physiological processes yet curtailing excessive receptor activation such as might occur during pathological states (Leeson & Iversen, 1994). In order to address this issue, we have compared the effects on hippocampal LTP, *in vivo*, of L-687,414 and MK-801 at doses previously shown to produce comparable reductions in lesion volume in the focal ischaemia paradigm. A preliminary account of some of the *in vitro* data contained in this paper has been published previously (Kemp *et al*., 1991a).

Methods

In vitro *cortical slice experiments*

The NMDA receptor antagonist properties of L-687,414 were examined using excitatory amino acid-induced depolarizations of rat cortical slices using the previously described (Kemp *et al*., 1991b) greased-gap technique. Briefly, coronal slices (~500 μm thick) were cut from a 3–4 mm thick block of cerebral cortex/striatum using a vibratome. The tissue was submerged, at all times in an oxygenated artificial cerebrospinal fluid comprising (mM): NaCl 124.0; KCl 2.0; $CaCl_2$ 2.0; $MgCl_2$ 2.0; KH_2PO_4 1.25; $NaHCO_3$ 25.0; D-glucose, 11.0. Tissue wedges, approximately 1 mm wide, consisting of frontoparietal motor cortex, corpus callosum and underlying striatal matter were dissected from the cortical slices. The wedges were mounted in a perspex perfusion chamber and continuously perfused with a modified aCSF which had the same composition described above but which was supplemented with tetrodotoxin (TTX, 300 nM) and which lacked $MgCl_2$ (further details in Kemp *et al*., 1991b). Population depolarizations of the cortical tissues were evoked by 1 min duration bath applications of NMDA or quisqualic acid and were recorded using Ag/AgCl electrodes connected to the d.c. amplifier of a Kipp and Zonen chart recorder. All experiments were performed at room temperature.

Whole-cell voltage-clamp experiments

In order to determine, with more precision, the functional affinity and efficacy of L-687,414 a combination of whole-cell voltage-clamp and rapid application of drugs to individual rat cultured cortical neurones was used. Cell culture techniques have been described in detail elsewhere (Priestley *et al*., 1990). Cortical neurones were grown on poly-lysine coated glass coverslips and were used for electrophysiological experiments between 8 and 21 days *in vitro*. Coverslips were submerged in a perspex chamber and continuously perfused with a salt solution of the following composition (in mM): NaCl, 149.0; KCl, 3.25; CaC_2, 2.0; $MgCl_2$, 2.0; D-glucose, 11.0; tetrodotoxin (TTX), 0.0003; HEPES buffer, 10. The pH of the perfusate was adjusted to 7.35 using NaOH and osmolarity to 350 mOsm using sucrose.

Drugs were diluted from concentrated stock solutions into a modified salt solution which lacked $MgCl_2$, all drug solutions were made up in ultra pure HPLC grade water and all glassware was rinsed extensively in the same water to reduce residual glycine contamination.

Patch pipettes with a tip diameter of ~2–3 μm (resistance ~2–3 MΩ) were formed from borosilicate glass (1.2 mm o.d., 0.94 mm i.d, Clark Electromedical), they were filled with a solution which comprised (in mM): CsF, 120.0; CsCl 10.0; HEPES 10.0; EGTA 10.0, $CaCl_2$ 0.5, pH adjusted to 7.25 with CsOH and osmolarity to 330 mOsm with sucrose. Whole-cell currents were recorded at a holding potential of -60 mV using standard electrophysiological procedures.

In vivo *recording of long-term potentiation*

Male Sprague-Dawley rats were anaesthetized with Sagatal (60 mg kg^{-1}, i.p. and subsequently supplemented as required by injection into a previously cannulated femoral vein) and placed in a stereotaxic frame with the incisor bar positioned 3 mm below interaural zero. A bipolar stimulating electrode was placed in the angular bundle, for stimulation of the medial perforant path (mPP); coordinates (from bregma): -7.7 mm, lateral 3.8 mm, depth 2.5–2.8 mm. A tungsten recording electrode was placed in the hilus of the dentate gyrus at the coordinates: -3.5 mm, lateral 2.3 mm, depth 2.2–2.8 mm. Stimulus intensity and duration were chosen such that the e.p.s.p. risetime was well submaximal and just below the threshold for the generation of a population spike. Stimuli were delivered every 30 s and an average of 4 e.p.s.ps taken every 10 min and used to determine 10–90% risetime. LTP was induced by a tetanic stimulus comprising a train of 4×400 Hz, 20 ms duration at 1 s intervals and at $3 \times$ control stimulus intensity. This procedure was repeated four times at 15 min intervals in order to ensure maximal LTP.

In order to assess the effects of NMDA receptor antagonism on LTP a stable baseline e.p.s.p. response was first obtained for a period of at least 1 h. Animals were then administered either L-687,414 (28 mg kg^{-1} i.v. followed by 28 mg kg^{-1} h^{-1}), MK-801 (0.12 mg kg^{-1} i.v. followed by

1.8 μg kg^{-1} h^{-1}) or saline (0.4 ml kg^{-1} followed by 0.0298 ml min^{-1} – the same infusion rate as used for MK-801). These dosing regimes for L-687,414 and MK-801 were selected on the basis that they achieved comparable levels of neuroprotection in the rat middle cerebral artery occlusion (MCAO) stroke model (Gill *et al.*, 1991; 1995). The concentration of drug in plasma was determined by HPLC during detailed pharmacokinetic studies in which arterial blood samples were taken from anaesthetized rats undergoing MCAO surgery. The constant infusion schedule produced steady-state plasma concentrations between 24–27 μg free base ml^{-1} for L-687,414 (see Gill *et al.*, 1995) and 18.9 ng ml^{-1} for MK-801 (Gill *et al.*, 1991). The first tetanic stimulus was applied 1 h after commencing drug or saline infusion and the infusion was maintained until after the final tetanus. The e.p.s.p. risetime was monitored for at least 1 h after the final tetanus to ensure that any increase represented true LTP as opposed to post-tetanic potentiation.

Drugs

Bulk chemicals used in the preparation of buffered salt solutions and enzymes were obtained from either BDH (NaCl, NaOH, KCl, HEPES, $MgCl_2$), Sigma (EGTA, CsCl, CsF, CsOH, DMSO, glycine, D-serine, L-glutamate) or Fisons (D-glucose, sucrose). NMDA was purchased from RBI, AMPA from Tocris-Cookson, TTX from Calbiochem and Sagatal (pentobarbitone sodium) from Rhone-Poulenc. R(+)HA-966 and L-687,414 were synthesized by Dr B.J. Williams, Medicinal Chemistry Department, MSDRL, Terlings Park, U.K.

Results

Cortical slice experiments

Bath application of increasing concentrations of NMDA to cortical wedges evoked increasing amplitude depolarizations of the cortical matter; in control conditions maximal applied NMDA concentrations were restricted to 20 μM to avoid irreversible excitotoxic damage to the tissue. Perfusion of L-687,414 (30 μM) resulted in a rightward shift in the concentration-response relationship for NMDA-induced depolarization (peak amplitudes, Figure 1); the antagonistic effects of L-687,414 were reversed by co-perfusion of the glycine site agonist, D-serine (100 μM). The apparent affinity of L-687,414 for the glycine site was compared to that of R(+)HA-966 by determining the concentration ratio (the ratio of concentrations of NMDA required to produce depolarizations of equal amplitude in the absence and presence of antagonist) at different antagonist concentrations. These experiments suggested L-687,414 to have an apparent $K_b = 15.3 \pm 2.0$ μM (mean of K_b values measured in triplicate at five different antagonist concentrations: 3, 10, 30, 100 and 300 μM) compared to R(+)HA-966 (100 μM), apparent $K_b = 66 \pm 6$ μM ($n = 5$). At concentrations up to 1 mM, the highest tested, L-687,414 had no effect on AMPA-mediated depolarizations. Also apparent from these experiments was that NMDA antagonist effect of R(+)HA-966 saturated at around 500 μM, i.e. further increases in antagonist concentration failed to produce any further attenuation of the NMDA

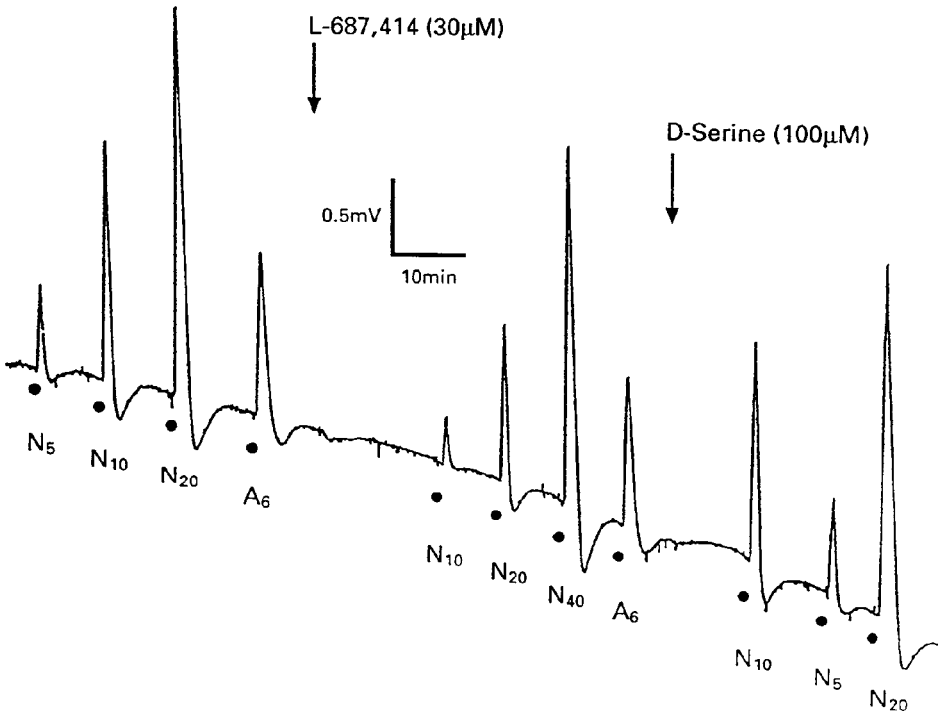

Figure 1 NMDA-evoked population depolarizations of rat cortical slices are antagonized by L-687,414. Bath application of increasing concentrations of NMDA ('N' followed by concentration in μM) produced concentration-related depolarizations, the figure shows a continuous record from a single slice preparation. L-687,414 was bath perfused for ~15 min, higher concentrations of NMDA were required to elicit depolarizations of comparable amplitude to control when the agonist was applied in the presence of L-687,414. The NMDA antagonist effects of L-687,414 were reversed by coperfusion of the glycine site agonist, D-serine (compare N_{20} response for each of the three conditions). Bath application of AMPA (6 μM; A_6) also depolarized the slice but the amplitude of the response was unaffected by L-687,414.

response. This is particularly obvious when log concentration-ratio is plotted against antagonist concentration (Figure 2) and appeared also to be a feature of L-687,414 antagonism. Although less marked than seen with R(+)HA-966, concentrations of L-687,414 above 100 μM produced only marginal increases in antagonism (Figure 2).

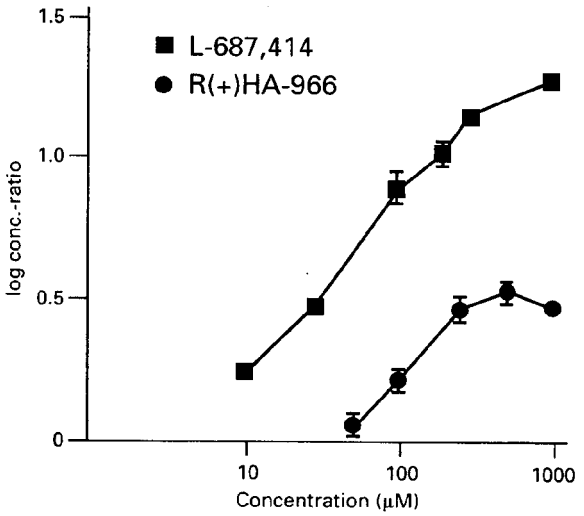

Figure 2 'Pseudo' Schild plot for the NMDA receptor antagonism by the glycine site ligands L-687,414 and R(+)HA-966. The graph shows antagonism, quantified as a concentration-ratio (log scale), as a function of concentration. Concentrations of R(+)HA-966 over the range 30-500 μM produce a progressively increasing rightward shift in the concentration-response curve for NMDA-evoked depolarization which reach a maximal concentration-ratio of $\sim$3. Higher concentrations do not produce further antagonism. By comparison, L-687,414 is capable of producing a more significant antagonist effect resulting in an $\sim$17 fold concentration-ratio at 1 mM (the highest concentration tested). Note that relatively low concentrations (10–100 μM) of L-687,414 produce substantial concentration-ratios whereas concentrations above 100 μM elicit less marked antagonism suggesting saturability of the antagonist effect.

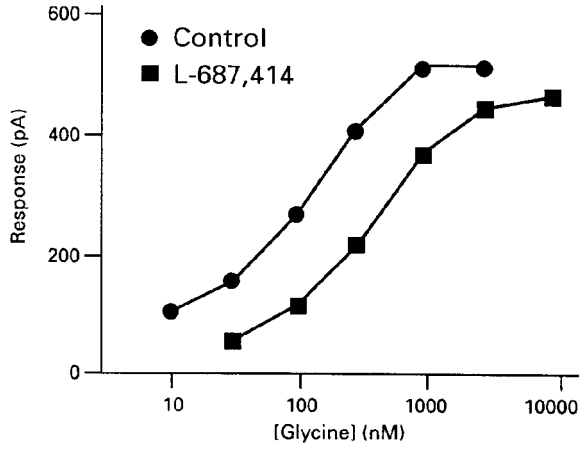

Figure 3 L-687,414 is a competitive glycine site antagonist. The graph shows a concentration-response curve for glycine potentiation of NMDA-evoked whole-cell peak currents recorded from a voltage-clamped rat cortical neurone. The concentration-response relationship for glycine was shifted to the right in the presence of L-687,414 (3 μM) in an apparently parallel manner, suggesting competitive antagonism.

Whole-cell voltage-clamp experiments

Concentration-response curves for NMDA-evoked inward current (peak) responses in voltage-clamped rat cultured cortical neurones were shifted to the right in a parallel manner by L-687,414 (3 μM; Figure 3). These experiments enabled receptor occupancy to be more accurately determined with respect to agonist and antagonist concentrations. The dose-ratios obtained from several such experiments yielded an apparent $pK_b = 6.2 \pm 0.12$ (mean $\pm$ s.e.mean; $n = 5$) for L-687,414.

The cortical slice experiments, described above, suggested that both R(+)HA-966 and L-687,414 were partial agonists with different intrinsic activities. This was also investigated in more detail on voltage clamped cortical neurones. Discrete, 5 s duration, applications of NMDA (30 μM) in the continuous

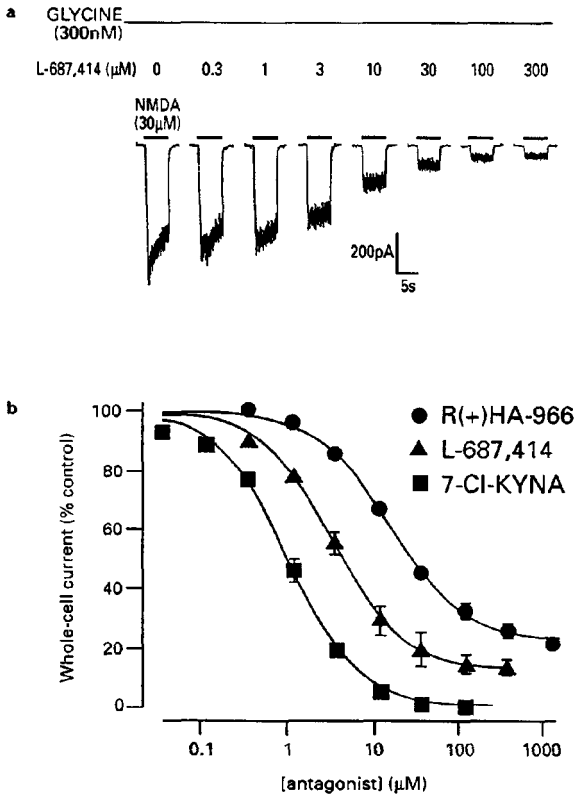

Figure 4 L-687,414 produces substantial but incomplete antagonism of NMDA receptor activation. (**a**): Discrete, fast application of NMDA (30 μM, duration shown by horizontal bars) in the continuous presence of glycine (300 nM) evoked typical inward whole-cell currents in a voltage-clamped rat cortical neurone. Peak current amplitudes were attenuated in a concentration-related manner by coapplication of L-687,414, each concentration was equilibrated for a 30 s period prior to NMDA application. Note that NMDA-evoked currents were not completely abolished even at 300 μM L-687,414. (**b**): Data from several experiments showing inhibition curves for the antagonism of NMDA-evoked whole-cell peak current responses by R(+)HA-966, L-687,414 and 7-Cl-KYNA. The prototypical antagonist, 7-Cl-KYNA is capable of producing complete antagonism of receptor function at concentrations of 30–100 μM. By contrast, antagonism by R(+)HA-966 appears to plateau at around 80% and that by L-687,414 at around 90%, suggesting that these latter compounds retain a degree of intrinsic activity which is greater for R(+)HA-966 than for L-687,414. Curves fitted through data points obtained from individual cells revealed the following affinities (K_i – geometric mean, – s.e.mean + s.e.mean) for each glycine site antagonist: 7-CL-KYNA, 0.46 μM (0.43, 0.49); R(+)HA-966, 5.90 μM (4.90, 7.10); L-687,414, 0.98 μM (0.89, 1.07).

presence of glycine (300 nM) evoked inward current responses which were attenuated in a concentration-related manner by L-687,414 (Figure 4). Concentration-inhibition curves, generated from these experiments, revealed a $plC_{50} = 5.6 \pm 0.09$ (mean ± s.e.mean; $n = 6$) for L-687,414, using the Cheng-Prusoff relationship (Cheng & Prusoff, 1973) this corresponded to a calculated $pKi = 6.1 \pm 0.09$. However, like R(+)HA-966 but unlike 7-chlorokynurenic acid, L-687,414 failed to produce complete antagonism of the NMDA response and the inhibition curve reached a plateau at ~90% inhibition. By comparison, the inhibition produced by R(+)HA-966 reached a plateau at 79%, thus, both L-687,414 and R(+)HA-966 are partial agonists with different intrinsic activities and different affinities for the glycine site of the NMDA receptor.

In vivo LTP experiments

In control animals ($n = 4$), saline infusion had no effect on the e.p.s.p. risetime and tetanic stimulation resulted in a significant ($P < 0.05$; 2-tailed paired *t*-test) increase in e.p.s.p. risetime of 38% over pre-infusion level measured 1 h after tetanus (Figure 5). Infusion of MK-801 ($n = 4$) appeared to produce a slight, but non statistically significant, reduction in e.p.s.p. risetime measured 1 h after commencing the infusion and immediately prior to the tetanic stimulation. Tetanic stimulation in the presence of MK-801 resulted in a small increase in e.p.s.p. risetime which was, again, non-significant with respect to control level but was significantly different to that obtained after 1 h infusion with MK-801 pre-tetanus. More relevant, however, the tetanus-induced increase in e.p.s.p. risetime in the presence of MK-801 was significantly attenuated ($P < 0.05$, 2-tailed, unpaired *t*-test) compared to saline-infused control animals (Figure 5). Thus, MK-801 significantly impaired the activity-dependent increase in synaptic efficacy at the mPP to dentate gyrus synapse. Infusion of L-687,414 ($n = 4$) had no significant effect on the risetime of the e.p.s.p. measured prior to tetanus. The risetime in the e.p.s.p. following tetanic stimulation in the L-687,414 infused animals was 34% greater than control pre-infusion level ($P < 0.02$) and this increase was not significantly different to that produced in saline infused control animals (Figure 5).

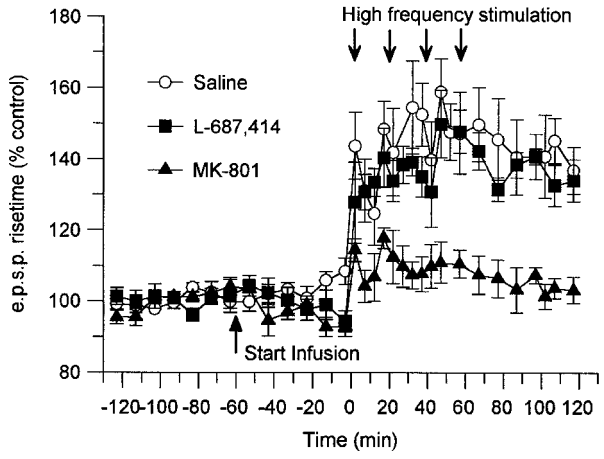

Figure 5 Frequency-dependent LTP at the perforant path to dentate granule cell synapse is attenuated by MK-801 but not by L-687,414. Synaptic efficacy was quantified by measuring the risetime of e.p.s.ps evoked by regular stimuli applied at low frequency. Trains of tetanic stimuli produce an essentially immediate and sustained augmentation of e.p.s.p. risetime, 'LTP', in rats administered saline i.v. (control, 0.4 ml kg^{-1} followed by 0.0298 ml min^{-1}). The response to high frequency stimulation was unaffected by the i.v. infusion of L-687,414 (28 mg kg^{-1} bolus followed by 28 mg kg^{-1} h^{-1}) but was substantially reduced by an infusion of MK-801 (0.12 mg kg^{-1} bolus followed by 1.8 μg kg^{-1} min^{-1}).

Discussion

This paper describes the pharmacological profile of the novel NMDA receptor glycine site ligand, L-687,414. L-687,414 displaces [^{3}H]glycine (IC50 = 1.37 μM) and [^{3}H]L-689,560 (a glycine site antagonist; IC50 = 2.72 μM) radioligand binding to P2 cortical membranes (Grimwood *et al*., 1992). In cortical slice experiments, L-687,414 showed absolute selectivity for the NMDA receptor over AMPA receptors, as far as solubility constraints allowed. Complete reversal of the NMDA receptor antagonist effects of L-687,414, by the glycine site agonist D-serine and the rightward parallel shift of glycine concentration-response curves by L-687,414 in voltage-clamped cortical neurones provide additional irrefutable evidence for the site of action being the glycine site on the NMDA receptor complex. Inhibition curves describing the incomplete antagonism of NMDA responses, even at high concentrations of L-687,414, confirm that this compound is a partial agonist and indicate a level of intrinsic activity approximately 10% of glycine. This profile might reasonably be expected to result in substantial NMDA receptor antagonism *in vivo*. Indeed, L-687,414 dose-dependently antagonizes photostimulation-induced seizure activity in baboons (Smith & Meldrum, 1992) and that evoked in mice by either NMDLA, pentylenetetrazol or electroshock – in the latter experiments the anticonvulsant effects of L-687,414 were reversed by i.c.v. administration of the glycine site agonist, D-serine (Tricklebank *et al*., 1994).

NMDA receptors are known to be fundamentally involved in the processes leading to the induction of LTP at certain synapses in the hippocampus (Harris *et al*., 1984). Complete NMDA receptor antagonism might be expected, therefore, to result in a cognitive deficit. In rats this appears to be the case, at least in relation to aspects of spatial learning (Morris, 1989; Bannerman *et al*., 1995, Tsien *et al*., 1996b but see Saucier & Cain, 1995). The fundamental role of NMDA receptors in mPP to dentate gyrus synaptic plasticity is demonstrated in the present study by the ability of MK-801 to greatly reduce LTP at these synapses as has previously been found to be the case in conscious rabbits (Robinson & Reed, 1992). However, an infusion regime designed to establish a maintained plasma level of ~25 μg ml^{-1} for L-687,414 resulted in no significant effect on tetanus-evoked LTP in the same brain region. This plasma concentration of L-687,414 is significant as it has previously been shown to be sufficient to produce a highly significant 41% reduction in cortical ischaemic volume following permanent occlusion of the left middle cerebral artery, when compared to saline control-treated rats (Gill *et al*., 1995). The molecular weight (free base) of L-687,414 is ~130, a plasma level of 25 μg ml^{-1} would, therefore, be equivalent to a plasma concentration of 190 μM, if it were possible to simply assume efficient brain penetration this would be likely to approach a near maximally effective concentration (see Figure 4b). Also significant is the fact that the infusion regime employed for MK-801 in the present study was chosen so as to achieve essentially comparable levels of neuroprotection in the same animal model of focal ischaemic brain damage (Gill *et al*., 1991). The results suggest that, in contrast to MK-801, a neuroprotective dosing regime for L-687,414 does not block the induction of LTP in the hippocampus and, by extrapolation, that L-687,414 may not produce marked cognitive impairments at neuroprotective doses. The ability of L-

687,414 to sustain LTP may reflect the partial agonist profile of the compound and, if so, suggests that only a low level of NMDA receptor activity is required to enable LTP induction in the dentate gyrus of the hippocampus. In contrast, complete antagonism at the glycine site on the NMDA receptor complex has been shown previously to prevent LTP in rat hippocampal slices (Izumi *et al*., 1990; Bashir *et al*., 1990; Oliver *et al*., 1990) although other strategies which result in incomplete NMDA receptor blockade permit LTP (Frankiewicz *et al*., 1996). The fact that 'full' glycine site antagonists, such as 7-Cl-KYNA are effective in preventing hippocampal LTP argues against the effects of L-687,414 being the result of a receptor subtype preference towards NMDA receptors activated during ischaemia and not those involved in synaptic plasticity in hippocampus. Firstly, there is no evidence to support a substantial difference in NMDA receptor subtype expression patterns between cortical and hippocampal areas. Secondly, experiments in our laboratory have revealed an essentially identical pharmacological profile for 7-Cl-KYNA and L-687,414 at the likely predominating NR1/NR2A and NR1/NR2B subunit combinations found in the respective brain regions (Priestley *et al*., 1995).

In summary, the data presented here suggest that a low efficacy partial agonist profile might present a clinical advantage in the context of neuroprotection, by permitting enough ongoing receptor activity to sustain normal physiological processes and thereby limit side effect liability, yet at the same time retaining sufficient antagonism to effectively curtail excessive receptor activation such as might occur during pathological states.

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