British Journal of Pharmacology (1996) 117, 1487–1492

Effects of a partial agonist and a full antagonist acting at the glycine site of the NMDA receptor on inflammation-induced mechanical hyperalgesia in rats

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1 NMDA receptor antagonists have previously been shown to have antinociceptive effects in behavioural experiments, but controversy remains as to the role of NMDA receptors in mechanical hyperalgesia. We have studied the effects on mechanical nociceptive thresholds in rats with carrageenin-induced paw inflammation of L-687,414, a low efficacy partial agonist which acts as a functional antagonist at the glycine modulatory site of the NMDA receptor and of L-701,324, a structurally novel, highly selective, full antagonist at this site.

2 Mechanical thresholds were measured for both hind paws 1 h before and 3 h after carrageenin or saline was injected into 1 hind paw. Dose-response curves were constructed for each test compound in separate experiments, with test compound or vehicle being given i.p. 1 h before the final test.

3 Both compounds produced selective dose-dependent and statistically significant reversal of mechanical hyperalgesia, with minimum effective doses of 100 mg kg^{-1} L-687,414 and 3 mg kg^{-1} L-701,324. Neither L-687,414 nor L-701,324 affected the response threshold of the contralateral non-inflamed paw over the dose-range producing reversal of carrageenin-induced hyperalgesia. Neither compound had any effect on the paw oedema produced by carrageenin injection.

4 These results show that both a full antagonist and a low efficacy partial agonist at the glycine modulatory site of the NMDA receptor complex reverse inflammation-induced mechanical hyperalgesia, thus supporting the argument that maximal activation of the glycine site is required for transmission via NMDA receptors, and showing that NMDA receptor-mediated actions are important in mechanical hyperalgesia induced by inflammation

Keywords: N-methyl D-aspartate; glycine B receptor; nociception; analgesia; pain; carrageenin

Introduction

It has been proposed that the N-methyl-D-aspartate (NMDA) glutamate receptor subtype has a particular role in mediating persistent pain and hyperalgesia (for review see Dickenson, 1990; Woolf & Thompson, 1991; Dubner & Ruda, 1992; Urban *et al.*, 1994). Consistent with this view, in behavioural tests NMDA receptor antagonists attenuate or reverse the thermal hyperalgesia evoked by local inflammation (Ren *et al.*, 1992a, b; Yamamoto *et al.*, 1993; Eisenberg *et al.*, 1994) or by peripheral neuropathy (Mao *et al.*, 1992; 1993; Yamamoto & Yaksh, 1992; Tal & Bennett, 1993; Eisenberg *et al.*, 1995) without having any analgesic effect on the response to noxious stimulation of the contralateral, non-affected limb. In contrast, the effect of NMDA receptor antagonists on mechanical hyperalgesia in animals is less clear cut, since whilst Ren & Dubner (1993) found that blockers of the NMDA receptor ion channel attenuated mechanical hyperalgesia evoked by inflammation, others have found that NMDA receptor antagonists had no effects on mechanical hyperalgesia evoked by neuropathy (Tal & Bennett 1993) and that intrathecal administration of NMDA provokes thermal but not mechanical hyperalgesia (Mellor *et al.*, 1993). However, in electrophysiological studies, NMDA receptor antagonists reverse the increased mechanical responsiveness of spinal neurones induced by arthritis (Schaible *et al.*, 1991; Neugebauer *et al.*, 1993), by intradermal capsaicin (Dougherty *et al.*, 1992), or by intraplantar injection of Freund's adjuvant (Ren *et al.*, 1992a) and also reduce the hyper-reflexia to mechanical stimuli induced by mustard oil or bradykinin (Woolf & Thompson, 1991; Ma & Woolf, 1995).

The observation that ketamine, an NMDA receptor ion channel blocker, reduces mechanical hyperalgesia induced by intradermal capsaicin in human subjects (Park *et al.*, 1994) is also consistent with a role for NMDA receptors in mechanical hyperalgesia. In man, both ketamine (Stannard & Porter, 1993; Backonja *et al.*, 1994; Park *et al.*, 1994; Arendt-Neilson *et al.*, 1995; Cherry *et al.*, 1995; Persson *et al.*, 1995) and the competitive glutamate recognition site antagonist, CPP (Kristensen *et al.*, 1992) have been shown to have analgesic properties in a variety of experimental and clinical pain states. However, even in studies in which the drug is given intrathecally, psychotomimetic effects are seen with these agents (White *et al.*, 1982; Kristensen *et al.*, 1992; Backonja *et al.*, 1994; Park *et al.*, 1994). In order to exploit the therapeutic potential of this class of compounds it is therefore important to develop NMDA antagonists with reduced side-effect liability.

The NMDA receptor is now known to contain several recognition sites, giving rise to at least 4 distinct classes of NMDA receptor antagonists that act competitively at the glutamate recognition site, by antagonizing the modulatory actions of glycine or of polyamines, or by blocking the associated ion channel (for review, see Wong & Kemp, 1991). The use of agents that act at the glycine modulatory site (see Leeson & Iversen, 1994, for review) may be more attractive clinically since such agents appear to have a lower potential for adverse CNS side-effects as indicated by the lack of changes in cerebral glucose metabolism and cortical morphology seen at effective doses (Hargreaves *et al.*, 1993a, b; Kemp & Leeson, 1993) and by their ability to antagonize the behavioural effects

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of psychostimulant agents without themselves activating mesolimbic dopamine systems (Hutson *et al.*, 1991; 1995; Bristow *et al.*, 1993; 1995).

Antagonists acting at the NMDA/glycine modulatory site have been found to have antinociceptive effects in most of the studies in which they have been tested. They reduce the response to formalin injection in some studies (Dickenson & Aydar, 1991; Millan & Seguin, 1993; 1994) although not in all (Coderre, 1993; Coderre & Van Empel, 1994). NMDA/glycine site antagonists reverse the enhanced neuronal responses seen after u.v.-induced inflammation (Chapman & Dickensen, 1994), and reverse thermal hyperalgesia evoked by inflammation or peripheral neuropathy in behavioural experiments (Mao *et al.*, 1992; Ren *et al.*, 1992b). Furthermore, the NMDA/glycine site partial agonist, (+)-HA-966, inhibits spinal c-fos expression in the spinal dorsal horn after intraplantar carrageenin in rats (Chapman *et al.*, 1995). However, to our knowledge, there are no published results on the effects of NMDA/glycine modulatory site antagonists on mechanical hyperalgesia.

In the present studies we have tested the effects of two agents with antagonist actions at the glycine modulatory site of the NMDA receptor on mechanical nociceptive thresholds in rats with carrageenin-induced inflammation of one hind paw. The aims of the study were to examine further the role of the NMDA receptor in mechanical hyperalgesia, and specifically to test the effects of antagonism of the glycine modulatory site on mechanical hyperalgesia. To control for the possibility that any effects seen were due to non-specific actions, two structurally different compounds were used: L-687,414, a partial agonist with lower efficacy and higher affinity at the glycine modulatory site than (+)-HA-966 (Kemp & Leeson, 1993), and L-701,324 (Kulagowski *et al.*, 1994) a structurally novel, high affinity, selective full antagonist for the NMDA/glycine site (Table 1). Some of these results have been published in abstract form (Laird *et al.*, 1994).

Methods

Male Sprague-Dawley rats (Bantin & Kingman, 100–190 g body weight) were acclimatized to the testing room for at least 1 h before behavioural testing on the day of the experiment. The tests were performed by an experimenter who was 'blind' to the drug treatment given. Each animal was used once only and was killed humanely on completion of testing. The threshold of the response to a noxious mechanical stimulus was measured with a modified Ugo Basile algesiometer. The animal's hind paw was positioned over a convex surface (radius 2.5 mm) and gradually increasing pressure applied to the dorsal surface until the animal vocalized. The mechanical threshold was determined for both hind paws to provide a baseline for subsequent comparison before the injection of one hind paw with carrageenin. Carrageenin (0.15 ml of a solution of 30 mg ml^{-1} in saline; salt weight) or an equivalent volume of vehicle was injected into the plantar surface of one hind paw, and inflammation allowed to develop for 3 h, when the mechanical threshold for each hind paw was again determined. Vehicle or test compounds were administered i.p. at a dose volume of 1 ml kg^{-1} 1 h before the final test. The volume of each hind paw was also measured after each paw pressure test with a plethysmometer.

Two compounds were tested in separate experiments. In each experiment, one group of rats was given an injection intraplantar with saline and i.p. with vehicle to provide a control for the effects of carrageenin. The remaining groups were injected intraplantar with carrageenin and i.p. with vehicle or the test compound. L-687,414 ((+)-*cis*-4-methyl- HA 966) was dissolved in saline, and its effects tested in 2 separate experiments from which the data were combined. In the first, groups of carrageenin-injected rats were treated with vehicle, 10 or 50 mg kg^{-1} L-687,414. In the second, 3 groups were treated with vehicle, 10 or 100 mg kg^{-1} L-687,414. L-701,324 (7-chloro-4-hydroxy-3-(3-phenoxy) phenylquinolin-2-(1H)-one) was prepared in 0.5% methocel and 6 groups of carrageenin-injected rats treated with vehicle, 0.3, 1, 3, 10 or 30 mg kg^{-1} L-701,324. Doses are free base weight.

The data were analysed as differences from the baseline scores prior to carrageenin injection. Analysis of variance was performed, with post-hoc Newman-Keuls multiple comparison tests when significant main effects were seen. Differences for which $P<0.05$ were considered to be statistically significant.

Results

Intraplantar injection of carrageenin produced a marked inflammation of the hind paw characterized by reddening and an increase in paw volume (oedema). There was a statistically significant decrease in the mean mechanical threshold of the injected paw in the group with carrageenin-injected paws (and no drug treatment) compared to the group with saline-injected paws in all experiments, confirming the induction of mechanical hyperalgesia by carrageenin. However, there was no significant difference in the mechanical threshold of the non-injected non-inflamed contralateral hind paws between these 2 groups (Figures 1 and 2).

Treatment with L-687,414 (Figure 1) or with L-701,324 (Figure 2) produced a statistically significant (ANOVA $P<0.01$) dose-dependent reversal of mechanical hyperalgesia in the carrageenin-injected hind paws. The minimum effective dose ($P<0.05$) that was significantly different from that of the vehicle-treated group was 100 mg kg^{-1} of L-687,414 and 3 mg kg^{-1} of L-701,324 in line with their functional activity in NMDA assays (Table 1). There were no significant differences

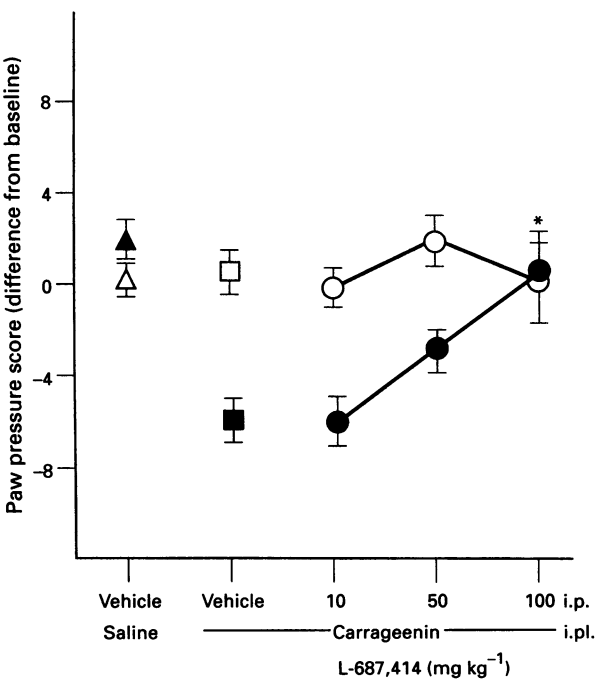

Figure 1 The inhibitory effect of the NMDA/glycine site partial agonist, L-687,414, on mechanical pain threshold in the hind paw of rats only after the induction of inflammation by intraplantar (i.pl.) injection of carrageenin; (△, ▲) the negative control responses in vehicle (i.p.)-treated rats with (▲) and without (△) i.pl. injection of saline into one paw; (□, ■) positive control responses in the inflamed carrageenin-injected paw (■) and the contralateral non-inflamed paw (□) in vehicle (i.p.)-treated rats; (○, ●) responses in the inflamed carrageenin injected paw (●) and the contralateral non-inflamed paw (○) in L-687,414 treated rats. Points are mean ± s.e.mean for $n=10$. *$P<0.05$ with respect to inflamed carrageenin-injected paw in vehicle i.p. treated group.

in the mean change in mechanical threshold of the non-inflamed contralateral hind paw in groups treated with any dose of L-687,414 (Figure 1) examined, indicating that L-687,414 reversed mechanical hyperalgesia at doses that were not frankly analgesic. A similar profile was observed with L-701,324 in the range 0.3–10 mg kg^{-1} but at the highest dose tested (30 mg kg^{-1}) a significant increase in mechanical threshold was also seen in the non-inflamed hind paw (analgesia: Figure 2).

There was no overt behavioural disruption with L-687,414 although mild ataxia was observed after the highest (100 mg kg^{-1}, i.p.) dose. Similarly with L-701,324 mild ataxia was observed at 10 mg kg^{-1}, i.p., with overt behavioural effects typical of the NMDA antagonist class (lateral head movements and side to side movements of the hind quarters) becoming evident after the 30 mg kg^{-1}, i.p., dose.

Neither L-687,414 nor L-701,324 had any effect on the increase in volume of the carrageenin-injected hind paws (Table 2).

Discussion

The present results show that both an antagonist and a low efficacy partial agonist at the glycine modulatory site of the NMDA receptor are able to reverse inflammation-induced mechanical hyperalgesia. The difference in the minimum effective doses of L-687,414 and L-701,324 in the current study (100 versus 3 mg kg^{-1}) can largely be explained by the approximately 20 fold difference in their functional activity as NMDA receptor antagonists (see Table 1), and is thus unlikely to be due to a large difference in brain penetration. Both compounds have been found to be very active as anticonvulsants in mice when administered i.p. (see Table 1), thus indicating good brain penetration (see Kulagowski *et al.*, 1994, for discussion of the relative brain penetration and *in vivo* activity of these and related compounds). Neither L-687,414 nor L-701,324 had any effect on the volume of the injected

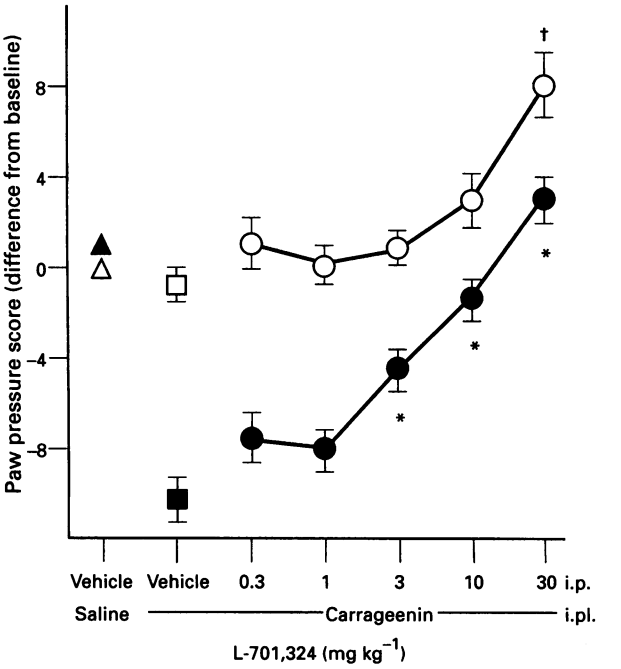

Figure 2 The potent selective inhibitory effect of the high affinity NMDA/glycine site antagonist, L-701,324, on mechanical pain threshold in the hind paws of rats after the induction of inflammation induced by intraplantar (i.pl.) injection of carrageenin; (△, ▲) negative control responses in vehicle (i.p.)-treated rats with (▲) and without (△) i.pl. injection of saline into one paw; (□, ■) positive control responses in the inflamed carrageenin injected paw (■) and the contralateral non-inflamed paw (□) in vehicle (i.p.)-treated rats; (○, ●) responses in the inflamed carrageenin injected paw (●) and the contralateral non-inflamed paw (○) in L-701,324-treated rats. Points are mean±s.e.mean for $n=10$. $*P<0.05$ with respect to inflamed carrageenin-injected paw in vehicle i.p. treated group; $\dagger P<0.05$ with respect to non-inflamed paw in vehicle i.p. treated group.

Table 1 Radioligand binding, functional NMDA receptor antagonist properties and anticonvulsant activities of the glycine site antagonists

Compound	*IC_{50} (μM) [^{3}H]-L-689,560*[a]	K_b (μM) *NMDA*[b]	*ED_{50}* (mg kg^{-1}, i.p.) *anticonvulsant activity*[c]	
L-687,414	1.6	0.63	5.1	Partial agonist
L-701,324	0.002	0.028	0.9	High affinity antagonist

[a]Inhibition of binding of [^{3}H] -L-689,560 to the strychnine-insensitive glycine binding site on rat cortex/hippocampal brain membranes. IC_{50} is the concentration of ligand (nM) required to inhibit 50% of specific binding.
[b]Blockade of NMDA-induced depolarizations in rat cortical slices. Apparent K_b calculated from rightward shift in NMDA concentration-response curves.
[c]Anticonvulsant activity characterized by degree of protection (absence of tonic seizures during 30 s of sound exposure in audiogenic seizure prone DBA/2 mice. Data taken from Saywell *et al.* (1991) and Kulagowski *et al.* (1994).

Table 2 Effect of the glycine site antagonists on change in volume of the injected paw

L-687,414	*Change in volume* (ml)	*L-701,324*	*Change in volume* (ml)
Saline + vehicle	0.09±0.02	Saline + vehicle	−0.03±0.03
Carra + vehicle	0.70±0.04	Carra + vehicle	1.00±0.07
Carra + 10 mg kg^{-1}	0.70±0.04	Carra + 0.3 mg kg^{-1}	0.96±0.12
Carra + 50 mg kg^{-1}	0.67±0.07	Carra + 1 mg kg^{-1}	0.97±0.10
Carra + 100 mg kg^{-1}	0.67±0.07	Carra + 3 mg kg^{-1}	0.90±0.12
		Carra + 10 mg kg^{-1}	0.90±0.08
		Carra + 30 mg kg^{-1}	NT

Saline + vehicle groups had saline injected intraplantar and the test vehicle i.p. 'Carra' groups had carrageenin injected intraplantar, and vehicle or the test drug (at the dose indicated) injected i.p. Each value is the mean change in paw volume (±s.e.mean) of 10 rats. NT indicates not tested

paw, indicating that the actions of these compounds were not due to an effect on peripheral inflammatory processes.

NMDA receptor antagonists are known to disrupt motor function (Kemp & Leeson, 1993) and thus it could be argued that the effects of the test compounds in the current study were due to effects on the motor system rather than true antinociceptive effects. However, L-701,324 had significant effects in the current study at doses 10 fold below those which affect motor function (Kulagowski *et al.*, 1994), so the actions of this compound are very likely to be antinociceptive. In contrast, for L-687,414 there was no separation between doses having a significant antinociceptive effect in the current study and those known to affect motor function (Saywell *et al.*, 1991). Nonetheless, we believe that the effects of L-687,414 in the current study were likely to be antinociceptive, since both L-687,414 and L-701,324 (at doses <30 mg kg^{-1}) affected the response to stimulation of the inflamed paw, and not the response to stimulation of the contralateral paw of the same animal, and thus the effect cannot be ascribed to general impairment of motor function. Furthermore, the nociception endpoint used was vocalization which is a response requiring central integration, unlike spinally-mediated withdrawal reflexes that could be disrupted by depression of spinal reflexes or drug-induced ataxia (see Cahusac *et al.*, 1984, for discussion of this endpoint). Both test compounds in the present study had significant antinociceptive effects at doses well below those which provoked the overt PCP-like behavioural disruption (head-weaving, body rolls etc.) characteristic of NMDA receptor antagonists. The actions of the 2 test compounds were thus very similar, with the exception that the therapeutic window appears to be greater for L-701,324. This compound also has the advantage that it shows high oral availability (Kulagowski *et al.*, 1994).

In the present experiments neither L-687,414 nor L-701,324 had any effect on responses to mechanical stimulation of the contralateral, non-inflamed paw, at doses that completely reversed carrageenin-induced hyperalgesia. However, at 30 mg kg^{-1}, L-701,324 produced a significant increase in the mechanical threshold of the non-inflamed contralateral paw, which may be due to the overt PCP-like behavioural disruption also seen at this dose. Intrathecal NMDA/glycine site antagonists have no effect on the thermal response threshold in the unaffected paw in rats with inflammation or neuropathy (Mao *et al.*, 1992; Ren *et al.*, 1992b). This lack of effect on the unaffected limb at doses that significantly attenuate hyperalgesia in the affected limb is also reported for antagonists at the NMDA receptor ion channel and at the glutamate recognition site (Mao *et al.*, 1992; Ren *et al.*, 1992a, b; Yamamoto & Yaksh, 1992; Ren & Dubner, 1993; Tal & Bennett, 1993; Eisenberg *et al.*, 1994; 1995) and so appears to be a general feature of antagonism at the NMDA receptor.

Partial reversal ($\sim 60\%$) of mechanical hyperalgesia evoked by intraplantar Freund's adjuvant injection in rats has been observed after intrathecal (i.t) administration of the NMDA receptor ion channel blocker, dizocilpine and the glutamate recognition site antagonist, AP-5 (Ren & Dubner, 1993). In the present experiments, full reversal of mechanical hyperalgesia was achieved with systemic NMDA glycine site antagonists. In a human study, mechanical hyperalgesia evoked by intradermal capsaicin injection was also partially reversed by ketamine (Park *et al.*, 1994). A range of inflammatory stimuli also produce NMDA receptor antagonist-sensitive increases in the mechanical responses of dorsal horn neurones (Schaible *et al.*, 1991; Dougherty *et al.*, 1992; Ren *et al.*, 1992a; Neugebauer *et al.*, 1993) and of spinal reflexes (Woolf & Thompson, 1991; Ma & Woolf, 1985). However, mechanical hyperalgesia evoked by peripheral neuropathy in rats was not affected by treatment with dextrorphan, an NMDA receptor ion channel blocker, although thermal hyperalgesia was reversed in the same study (Tal & Bennett, 1993). Further, i.t. NMDA administration in rats failed to produce mechanical hyperalgesia, although heat hyperalgesia was seen (Mellor *et al.*, 1993). This suggests that there may be differences in the mechanisms producing mechanical hyperalgesia following tissue injury or inflammation as compared with that following peripheral nerve injury or acute pharmacological manipulation.

NMDA/glycine site antagonists have been reported to show antinociceptive effects on the responses evoked by formalin (Dickenson & Aydar, 1991; Millan & Seguin, 1993; 1994), a procedure which provokes on-going pain without central sensitization (Xu *et al.*, 1995), although other studies do not show such effects (Coderre, 1993; Coderre & Van Empel, 1994) for reasons that are not clear. NMDA/glycine site antagonists also block the increased neuronal responsiveness evoked by repetitive electrical stimulation of C-fibres, known as 'wind-up' (Dickenson, 1990), as do other classes of NMDA receptor antagonists (Davies & Lodge, 1987; Dickenson & Sullivan, 1987). Wind-up is thought to be a substrate for central sensitization and thus hyperalgesia (Dickenson, 1990; Woolf & Thompson, 1991; Urban *et al.*, 1994), although it is unlikely to be involved in all forms of hyperalgesia, for example, in pain and hyperalgesia as a result of visceral stimuli or damage, since visceral nociceptive pathways show little or no evidence of wind-up (Alarcon & Cervero, 1990; Laird *et al.*, 1995). Furthermore, NMDA/glycine site antagonists reduce the increased neuronal excitability (Chapman & Dickenson, 1994) and also the mechanical and thermal hyperalgesia evoked by peripheral inflammation (Ren *et al.*, 1992b, present results), and the thermal hyperalgesia evoked by neuropathy (Mao *et al.*, 1992).

Thus, the available evidence suggests that NMDA/glycine site antagonists are effective (as are other classes of NMDA receptor antagonists) in inhibiting the behavioural expression of hyperalgesia and the increased responsiveness of nociceptive neurones in animal models of inflammatory and neuropathic pain which closely mirror human pain states. In human clinical studies, other classes of NMDA receptor antagonists have been shown to have analgesic properties (Kristensen *et al.*, 1992; Stannard & Porter, 1993; Backonja *et al.*, 1994; Cherry *et al.*, 1995; Persson *et al.*, 1995). This suggests that NMDA/glycine site antagonists are likely to show similar analgesic effects in clinical pain states.

The potential use of novel NMDA receptor antagonists for the treatment of hyperalgesia and pain in man is dependent upon the demonstration of an acceptable therapeutic window. This appears to be a more realistic goal with the NMDA glycine site antagonists than with the non-competitive ion channel blocking agents or the competitive glutamate recognition site antagonists (see Introduction). The recent description of NMDA glycine receptor full antagonists with good oral availability and brain penetration, such as L-701,324 (Kulagowski *et al.*, 1994), coupled with the present results showing that this agent is effective in reversing experimental hyperalgesia, suggest that it would be interesting and feasible to test the analgesic effects of full antagonists of the glycine site in man.

We are grateful to our colleagues in the Medicinal Chemistry Department, Merck Sharp and Dohme Research Laboratories, Harlow, for the syntheses of L-687,414 and L-701,324.

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(Received September 1, 1995
Revised December 7, 1995
Accepted December 19, 1995)