The anticonvulsant and behavioural profile of L-687,414, a partial agonist acting at the glycine modulatory site on the N-methyl-D-aspartate (NMDA) receptor complex

M.D. Tricklebank, L.J. Bristow, P.H. Hutson, P.D. Leeson, M. Rowley, K. Saywell, L. Singh, F.D. Tattersall, L. Thorn & B.J. Williams

Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR

1 The anticonvulsant and behavioural effects of the glycine/NMDA receptor partial agonist, L-687,414 (**R**(+)-*cis*- β -methyl-3-amino-1-hydroxypyrrolid-2-one) have been investigated in rodents.

2 L-687,414 dose-dependently antagonized seizures induced by N-methyl-D,L-aspartic acid (NMDLA, $ED_{50} = 19.7 \text{ mg kg}^{-1}$), pentylenetetrazol (PTZ, $ED_{50} = 13.0 \text{ mg kg}^{-1}$) and electroshock ($ED_{50} = 26.1 \text{ mg kg}^{-1}$) when given intravenously 15 min before test, in male Swiss Webster mice but was most potent against audiogenic seizures induced by a 120 dB bell in DBA/2 mice ($ED_{50} = 5.1 \text{ mg kg}^{-1}$, i.p., 30 min before test).

3 L-687,414 also induced impairments of performance in a rotarod test in both Swiss Webster and DBA/2 mice and the ratio [rotarod MED:anticonvulsant ED_{50}] varied between 0.9 and 5, depending on the convulsant used.

4 Similar behaviours to those seen after administration of the non-competitive NMDA receptor antagonist, MK-801 (head weaving, body rolling, hyperlocomotion) were seen in the mouse after giving L-687,414, although the peak effect occurred at a dose (100 mg kg^{-1}) which was 5–20 times the anticonvulsant ED_{50} s, depending on the convulsant used. Unlike MK-801, however, doses of L-687,414 that were behaviourally stimulant did not increase dopamine turnover in the nucleus accumbens.

5 Consistent with the interaction of L-687,414 with the glycine/NMDA receptor, the anticonvulsant, ataxic and motor stimulant effects of the compound were significantly attenuated by the glycine/NMDA receptor agonist, D-serine ($10\text{--}100 \mu\text{g}$ per mouse, i.c.v.).

6 The results show that L-687,414 is a potent, orally active anticonvulsant with a more benign pharmacological profile than antagonists acting at the ion channel of the NMDA receptor complex. The compound is a useful tool with which to probe the functional role of the glycine co-agonist site *in vivo*.

Keywords: Glycine co-agonist site; N-methyl-D-aspartate (NMDA) receptor; L-687,414; anticonvulsant; behaviour; PCP-like effects; dopamine turnover

Introduction

The N-methyl-D-aspartate (NMDA) subtype of excitatory amino acid receptors is thought to consist of a multiple grouping of modulatory sites in addition to a recognition site for the neurotransmitter itself. This has given rise to at least four distinct classes of NMDA receptor antagonists: those acting (i) competitively at the neurotransmitter recognition site (e.g. CPP, (3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid, Davies *et al.*, 1986); (ii) by blocking the associated ion channel (e.g. phencyclidine, (PCP, Anis *et al.*, 1983) and dizocilpine (MK-801, Wong *et al.*, 1987)); (iii) at a site mimicked by combination of the NMDAR1 and NMDAR2B subunits of the NMDA receptor complex (e.g. ifenprodil, Williams, 1993) and (iv) by antagonizing the co-agonist actions of glycine (e.g. 7-chlorokynurenic acid (Kemp *et al.*, 1988) and **R**(+)-1-hydroxy-3-aminopyrrolid-2-one ((+)-HA-966, Singh *et al.*, 1990a).

Each of the four classes of NMDA antagonist has neuroprotective properties. However, in rodent, NMDA channel blockers are also characterized by their ability to induce 'PCP-like' behavioural effects that include the induction of a complex motor syndrome that progresses to ataxia with increasing dose, and cross-generalisation in drug discrimination

studies (for review, see Willetts *et al.*, 1991). Competitive NMDA receptor antagonists share many of these behavioural properties, although they are generally less potent (relative to anticonvulsant doses) and less 'PCP-like' than the channel blockers (Tricklebank *et al.*, 1989; Willetts *et al.*, 1991).

Compounds that attenuate the actions of NMDA via the glycine and ifenprodil recognition sites are currently few in number and less well-characterized behaviourally. However, initial studies indicate that both ifenprodil and (+)-HA-966 are devoid of PCP-like properties, suggesting that antagonists acting at these modulatory sites may have distinct advantages over NMDA channel blockers. In this study, the anticonvulsant and behavioural effects of the low efficacy glycine/NMDA receptor partial agonist, L-687,414 (**R**(+)-*cis*- β -methyl-3-amino-1-hydroxypyrrolid-2-one), a more potent derivative of (+)-HA-966 (Leeson *et al.*, 1993), have been investigated. *In vitro*, L-687,414 inhibits strychnine-insensitive [^3H]-glycine binding to rat cerebral cortex synaptic membranes ($pIC_{50} = 5.8$) and glycine-potentiated NMDA responses with a pK_b of 6.2 in cultured cortical neurones and is thus about 10 fold more potent than (+)-HAA-966 (Leeson *et al.*, 1993). However, like (+)-HA-966, L-687,414 has some intrinsic activity at the glycine receptor having an efficacy level of approximately 7% of the maximum response to glycine in cortical neurones.

¹ Author for correspondence.

Methods

Animals

Experiments were performed on male Swiss Webster (20–30 g) or DBA/2 (8–10 g, 22–23 days of age) mice or Sprague Dawley rats (350–450 g) obtained from Bantin and Kingman, Hull, U.K. Animals were housed in groups of five under a 12 h light/dark cycle (lights on at 07 h 00 min) with food and water freely available. All procedures described below followed the laws governing animal experimentation in the United Kingdom. Animals subjected to techniques inducing convulsions were killed humanely within 2 min of experiencing a tonic seizure.

Intracerebroventricular injections

For the intraventricular (i.c.v.) injection of compounds, mice were anaesthetized with isoflurane and the skull exposed. Injections (5 μ l) were made at lambda with a 3.5 mm long, 27 gauge needle attached to a 10 μ l Hamilton microsyringe and the wound sealed with epoxy resin. Injections of dye by this procedure led to a uniform distribution of injectate throughout the ventricular system within 10 min.

Audiogenic seizures

Exposure of DBA/2 mice at 22–23 days of age to a 120 dB, 1.4 kHz bell induced wild running progressing to tonic extension of the limbs within 7–10 s of the onset of the sound. Groups of 8 mice were injected intraperitoneally with 4–5 doses of the test compound, 30 min before test. Animals not convulsing after exposure to the sound for 30 s were considered protected. The dose protecting 50% of the mice (ED_{50}) was calculated by probit analysis.

Seizures induced by *N*-methyl-D,L-aspartic acid or pentylenetetrazol

Groups of 8 Swiss Webster mice were injected subcutaneously with either *N*-methyl-D,L-aspartic acid (NMDLA, 500 mg kg⁻¹) or pentylenetetrazol (PTZ, 120 mg kg⁻¹) and the latency to the onset of tonic seizures noted over the following 30 min. Test compounds were given 15 min (intravenously) or 30 min (orally) before administration of the convulsant. Animals not convulsing within the 30 min observation period were considered protected. ED_{50} values were determined by probit analysis.

Anticonvulsant effects were also examined in the rat. For these studies, test compounds were injected i.v., 15 min before the i.v. injection of PTZ (80 mg kg⁻¹). In control animals, tonic seizures were induced within 1 min of injection of PTZ. Animals not convulsing were considered protected.

Seizures induced by electroshock

Tonic seizures were induced in Swiss Webster mice by application of electroshock (0.5 mA, 0.5 s) via corneal electrodes. Test compounds were given intravenously to groups of 8 mice, 30 min before application of shock. The number of mice protected from tonic limb extension was determined in each dose group.

Motor co-ordination

Animals were first trained to remain for 120 s on a rotarod apparatus revolving at 15 (mouse) or 8 (rat) r.p.m. Animals falling several times were discarded. The time animals were subsequently able to remain on the rod was determined 15 or 30 min after injection of the test compounds. The duration of each trial was 120 s.

The PCP-like behavioural syndrome

Quantification of lateral head movements (head weaves), side-to-side movements of the hind quarters (body rolls) and locomotion, behaviours characteristic of the syndrome induced in rodents by non-competitive NMDA receptor antagonists such as phencyclidine (PCP) and MK-801 (Tricklebank *et al.*, 1989), was carried out in Swiss Webster mice. Animals were first habituated to a perspex observation box (250 $\times$ 250 $\times$ 250 mm high) for 30 min. The test compound was then injected i.v. and the animal replaced in the cage. Fifteen min later, the above behaviours were scored (number of head weaves, body rolls and quadrants of the cage entered) for 5 min by an observer unaware of the drug treatment administered.

Locomotor activity

Swiss Webster mice were placed in individual perspex cages (230 $\times$ 200 $\times$ 210 mm high) lined with wire grids (pore size $\times$ 10 $\times$ 10 mm) to permit climbing. Each cage contained two parallel infrared photocells at the base of the cage to record cage crossing (number of consecutive beam breaks). Output from the photocells was linked to a BBC Master microcomputer and recorded at 10 min intervals. Following a 2 h habituation period, the animals were injected intravenously with either MK-801 (0.0625–1 mg kg⁻¹) or L-687,414 (75–200 mg kg⁻¹) and activity recorded for the following 2 h.

Determination of dopamine, HVA and DOPAC in mouse brain

Mice were injected with either MK-801 (0.05–1 mg kg⁻¹, i.v.), L-687,414 (5–106 mg kg⁻¹, i.v.) or 0.9% NaCl (10 ml kg⁻¹). Thirty min later, the animals were decapitated. Brains were removed and the striatum and nucleus accumbens dissected using a steel punch. Following storage at –70°C, tissue was homogenised in 10 volumes of 0.4 M perchloric acid containing 0.1% sodium metabisulphite, 0.01% cysteine and 0.0035% EDTA and centrifuged at 3,000 g for 20 min. Monoamines in the supernatant were separated by high pressure liquid chromatography and the concentrations of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) determined electrochemically as described by Hutson *et al.* (1991).

Drugs

L-687,414 (**R**(+)-*cis*- β -methyl-3-amino-1-hydroxypyrrolid-2-one maleate) and dizocilpine (MK-801) were synthesized by Merck Sharp and Dohme Research Laboratories. Phencyclidine HCl was obtained from Ultrafine Chemicals Ltd (Salford, U.K.). Compounds were dissolved in 0.9% NaCl and injected in a volume of 10 ml kg⁻¹ (mouse) or 1 ml kg⁻¹ (rat) by routes specified in the text. Doses of L-687,414 refer to the free base. Corrections for salt forms were not made for MK-801 maleate or phencyclidine HCl.

Results

Anticonvulsant studies in the mouse

Given intravenously, L-687,414 dose-dependently inhibited seizures induced in the mouse by NMDLA (ED_{50} = 19.7 mg kg⁻¹, Figure 1), PTZ (ED_{50} = 13.0 mg kg⁻¹, Figure 2) and electroshock (ED_{50} = 26.1 mg kg⁻¹, Figure 2). The compound was also orally active against seizures induced by NMDLA (Figure 1) and by electroshock (ED_{50} s = 32.8 mg kg⁻¹ and 44 mg kg⁻¹ respectively, Figure 1 and Table 1). L-687,414 was most potent when administered by the i.p. route against seizures induced by sound in DBA/2 mice (ED_{50} = 5.1 mg kg⁻¹, Figure 3).

The involvement of the glycine/NMDA receptor in the anticonvulsant effect of L-687,414 in the mouse was determined by examining the ability of the i.c.v. administration of the glycine receptor agonist, D-serine (3–100 μg per mouse), to reverse the anticonvulsant effect of a 60 mg kg^{-1} (i.v.) dose of L-687,414 against seizures induced by NMDLA. When co-administered with L-687,414 15 min before injection of NMDLA, D-serine (100 μg per mouse, i.c.v.) attenuated the increase in seizure latency to a value not significantly different from that of animals given only drug vehicles (Figure 4).

Figure 1 The effect of L-687,414 on seizures induced by N-methyl-D,L-aspartate (NMDLA) in the mouse. Male Swiss Webster mice were injected intravenously (○) or orally (●) with L-687,414 15 or 30 min respectively before the subcutaneous injection of NMDLA (500 mg kg^{-1}) and the latency to tonic seizure noted over the following 30 min. The results are shown as the mean ($\pm$ s.e.mean) latency to convulse of 8 animals per dose group.

Figure 2 The effect of L-687,414 on seizures induced by pentylenetetrazol (PTZ) or electroshock in the mouse. Male Swiss Webster mice were injected intravenously with L-687,414 15 min before the subcutaneous injection of 120 mg kg^{-1} PTZ (○) or application of electroshock (0.5 mA, 0.5 s) via corneal electrodes (●). Protection was defined as the absence of tonic seizures within 30 min of injection of PTZ or immediately after exposure to electroshock. There were 8 animals in each dose group.

Anticonvulsant studies in the rat

In the rat, L-687,414 was somewhat weaker against seizures induced by an intravenous bolus injection of PTZ (ED_{50} = 61.3 mg kg^{-1} , i.v., Table 2) than against seizures induced by the subcutaneous injection of PTZ in the mouse (Table 1). In contrast, the non-competitive NMDA receptor antagonist, MK-801, potently antagonized PTZ-induced seizures in the rat with an ED_{50} (0.2 mg kg^{-1} , Table 2) similar to that seen in the subcutaneous PTZ model in the mouse.

The overall anticonvulsant profile of L-687,414 is summarized in Table 1.

Effects on motor co-ordination

L-687,414 dose-dependently induced impairments of performance on the rotarod in the mouse (Figures 3 and 5). However, in both DBA/2 and Swiss Webster mice, the ratio [rotarod MED: anticonvulsant ED_{50}] varied between 0.9 and 5, depending on the convulsant used (Table 1). In the rat, the lowest dose of L-687,414 to decrease the latency to fall from the rotarod significantly was 20 mg kg^{-1} , giving a ratio of 0.3 (Table 1).

In the Swiss Webster mouse, administration of D-serine (10–100 μg per mouse, i.c.v.) immediately before injection of L-687,414 (60 mg kg^{-1} , i.v.) prevented the impairment of rotarod performance determined 15 min later (Figure 6). D-Serine itself was without effect on rotarod performance (results not shown).

The PCP-like behavioural syndrome

MK-801 (0.063–1 mg kg^{-1}) induced head weaving, body rolling and hyperlocomotion in the mouse although, with the

Figure 3 The effect of L-687,414 on seizures induced by sound and on rotarod performance in DBA/2 mice. Groups of 8 mice were trained to remain for 120 s on a rotarod revolving at 15 r.p.m. Thirty min after the i.p. injection of L-687,414 the animals were re-tested for their latency to fall from the rotarod (maximum 120 s) and immediately exposed to a 120 dB, 1.4 kHz bell and the latency to the onset of a tonic seizure noted (maximum 30 s). Results are shown as the mean ($\pm$ s.e.mean) latency to convulse (○) or the mean ($\pm$ s.e.mean) percentage deficit in rotarod performance (●) calculated as $100 \times (120 - \text{latency to fall (s)})/120$.

Table 1 Summary of the anticonvulsant and ataxic properties of L-687,414

	Test	Strain	Route	Anticonvulsant ED_{50} (mg kg^{-1})	Rotarod MED (mg kg^{-1})	Rotarod MED/Anticonvulsant ED_{50}
Mouse	Audiogenic	DBA/2	i.p.	5.1	27.3	5.3
	NMDLA	Swiss W.	i.v.	19.7	44.0	2.2
	NMDLA	Swiss W.	p.o.	32.8	40.0	1.2
	PTZ	Swiss W.	i.v.	13.0		3.4*
	Electroshock	Swiss W.	i.v.	26.1		1.5*
Rat	PTZ	S. Dawley	p.o.	44.0		0.9*
			i.v.	61.3	20.0	0.3

*Calculated assuming the minimum effective dose to be 40 mg kg^{-1} . For experimental details, see Methods. For abbreviations, see text.

exception of body rolling, the dose-response relationship was biphasic (Figure 7 and Table 3), probably reflecting the progressive inability of the animals to move as ataxia increased in severity and the righting reflex was lost.

A similar, though less intense behavioural profile was seen with L-687,414 (Figure 7 and Table 3) although the dose required for the peak effect (100 mg kg^{-1}) was 5–20 times greater than the dose required to block audiogenic or NMDLA-induced seizures. The maximum head weaving score obtained in mice given L-687,414 was approximately 50% of that achieved with MK-801 but the maximum scores for locomotion and body rolling were similar. Each of these behaviours was attenuated by the i.c.v. administration of D-serine, given 15 min before injection of L-687,414 (100 mg kg^{-1} , i.v., Figure 8).

Locomotor activity

MK-801 increased locomotor activity over the dose range of $0.0625 \text{ mg kg}^{-1}$ to 0.25 mg kg^{-1} . Higher doses of MK-801 induced much lower but longer lasting levels of hyperactivity, the effect of 1 mg kg^{-1} still being significant at the end of the 2 h observation period (Figure 9).

L-687,414 also increased locomotor activity, the peak effect being about 75% of the maximum score seen with MK-801

Figure 4 Effect of D-serine on the anticonvulsant action of L-687,414. D-Serine or saline was injected i.c.v. under isoflurane anaesthesia. The mice were then immediately given L-687,414 (60 mg kg^{-1} , i.v.). Fifteen min later, N-methyl-D,L-aspartate (NMDLA) was injected and the animals were observed for 30 min for the onset of a tonic seizure. The results are shown as the mean ($\pm$ s.e.mean) latency to convulse. *Significantly different from animals given only L-687,414, $P < 0.05$ (ANOVA followed by Dunnett's *t* test).

Figure 5 The effect of L-687,414 on seizures induced by N-methyl-D,L-aspartate (NMDLA) and on rotarod performance in Swiss Webster mice. Groups of 8 mice were trained to remain for 120 s on a rotarod revolving at 15 r.p.m. Fifteen min after the i.v. injection of L-687,414 the animals were re-tested for their latency to fall from the rotarod (maximum 120 s) and immediately injected with NMDLA (500 mg kg^{-1} , s.c.) and the latency to the onset of a tonic seizure noted (maximum 30 min). Results are shown as the mean ($\pm$ s.e.mean) latency to convulse (○) or the mean ($\pm$ s.e.mean) percentage deficit in rotarod performance (●) calculated as $100 \times (120 - \text{latency to fall (s)})/120$.

Figure 6 Effect of D-serine on the rotarod deficit induced by L-687,414. Swiss Webster mice were first trained to remain on a rotarod revolving at 15 r.p.m. D-Serine or saline was then injected i.c.v. under isoflurane anaesthesia. The mice were then immediately given L-687,414 (60 mg kg^{-1} , i.v.). Fifteen min later, the mice were re-tested on the rotarod and the latency to fall noted. The results are shown as the mean ($\pm$ s.e.mean) latency to fall from the rotarod ($n = 8$ per group). *Significantly different from animals given only L-687,414, $P < 0.05$ (ANOVA followed by Dunnett's *t* test).

Table 2 The anticonvulsant effects of MK-801 and L-687,414 against seizures induced by pentylenetetrazol in the rat

	Dose (mg kg^{-1} , i.v.)	No. Protected No. Tested	
Saline		0/16	
MK-801	0.0625	2/16	
	0.125	10/16	
	0.25	17/24	
	0.5	13/16	
	1.0	14/15	
	2.0	16/16	$\text{ED}_{50} = 0.2 \text{ mg kg}^{-1}$
Saline		0/8	
L-687,414	20	0/8	
	40	2/8	
	80	5/8	
	100	7/8	
	160	8/8	$\text{ED}_{50} = 61.3 \text{ mg kg}^{-1}$

Male Sprague Dawley rats were given MK-801 or L-687,414. Fifteen minutes later they were injected intravenously with pentylenetetrazol (80 mg kg^{-1}). Animals not convulsing within 1 min of injection were considered protected. ED_{50} values were calculated by probit analysis.

and occurring within minutes of injection at a dose of 100 mg kg^{-1} (Figure 9). The hyperactivity response to L-687,414 was transient however, the scores of drug and vehicle-treated animals being indistinguishable by 40 min after injection. A dose of 200 mg kg^{-1} L-687,414 did not increase motor activity at any time after injection.

Effects on dopamine metabolism

The concentrations of DOPAC, HVA and the ratio $([\text{DOPAC}] + [\text{HVA}]) / [\text{DA}]$, an index of dopamine (DA) turnover, significantly increased in the mouse nucleus accumbens following the intravenous injection of MK-801: alterations in the striatum were smaller in magnitude and not statistically significant (Table 4). With L-687,414 ($5\text{--}106 \text{ mg kg}^{-1}$, i.v.), no significant effects on the concentrations of DOPAC or HVA or on the ratio $([\text{DOPAC}] + [\text{HVA}]) / [\text{DA}]$ were seen in either the nucleus accumbens or the striatum (Table 4).

Discussion

Excitatory amino acid receptor antagonists acting at the ion channel of the NMDA receptor complex are anticonvulsant and neuroprotective agents. However, in rodents these compounds also activate mesocorticolimbic dopamine systems, induce hyperlocomotion, stereotypy and ataxia and cross-generalize with PCP-like drugs of abuse (e.g. ketamine and

Figure 7 The induction of head weaving in the mouse by MK-801 and L-687,414. Male Swiss Webster mice were first habituated to a perspex observation cage for 30 min. MK-801 (○) or L-687,414 (●) were then injected intravenously and the animals returned to the cage. Fifteen min later, the number of lateral head movements (head weaves) was scored for 5 min by an observer unaware of the drug treatments. Results are shown as the mean ($\pm$ s.e.mean) number of head weaves of 8 mice per group.

Figure 8 Effect of D-serine on the behaviours induced by L-687,414. Male Swiss Webster mice were first habituated to a perspex observation cage for 30 min. D-Serine or saline was then injected i.c.v. under isoflurane anaesthesia. Fifteen min later, a dose of 100 mg kg^{-1} L-687,414 was injected intravenously and the animals returned to the cage. After a further 15 min, the number of (a) lateral head movements (head weaves), (b) body rolls and (c) quadrants of the cage entered were scored for 5 min by an observer unaware of the drug treatments. Results are shown as the mean ($\pm$ s.e.mean) number of each behaviour counted ($n = 8$ mice per group). *Significantly different from animals given only L-687,414, $P < 0.05$ (ANOVA followed by Dunnett's t test).

Table 3 Behaviours induced by MK-801 and L-687,414 in the mouse

	Dose (mg kg^{-1} , i.v.)	No. of head weaves	No. of body rolls	No. of quadrants entered
Saline		33 ± 7	0	13 ± 3
MK-801	0.063	60 ± 11	0	64 ± 23
	0.125	159 ± 7	0	$120 \pm 15^*$
	0.25	$205 \pm 18^*$	4 ± 2	$76 \pm 20^*$
	0.5	$137 \pm 23^*$	$10 \pm 5^*$	44 ± 13
	1.0	71 ± 26	6 ± 4	6 ± 4
Saline		28 ± 8	0	18 ± 7
L-687,414	50	46 ± 5	5 ± 2	46 ± 6
	75	78 ± 13	$12 \pm 3^*$	$79 \pm 17^*$
	100	$110 \pm 16^*$	$14 \pm 4^*$	$126 \pm 26^*$
	200	45 ± 26	11 ± 7	3 ± 2

Swiss Webster mice were injected intravenously with MK-801 or L-687,414 and 15 min later observed for 5 min during which time the number of head weaves, body rolls and quadrants of the observation cage entered were counted. Results are the means $\pm$ s.e.mean of 8 animals per group.

* $P < 0.05$ vs saline-treated animals (Dunnett's t test).

the benzomorphans) in drug discrimination paradigms (for review, see Willetts *et al.*, 1991). Antagonism of the actions of NMDA can also be achieved by blocking the glycine co-agonist site on the NMDA receptor complex. Little is known of the behavioural pharmacology of glycine/NMDA receptor antagonists, however, since there are few compounds available that readily penetrate the CNS on systemic injection. Glycine-site antagonists that have been examined in some detail include 7-chlorokynurenic acid and the low efficacy partial agonist, (+)-HA-966 (Koek & Colpaert,

1990; Singh *et al.*, 1990a,b,c; Hutson *et al.*, 1991; Bristow *et al.*, 1993), the general conclusion being that anticonvulsant effects can be maintained in the absence of PCP-like behavioural stimulation.

L-687,414 offers distinct advantages as a tool for investigating the functions of the glycine modulatory site over analogues of kynurenic acid and (+)-HA-966: the compound has higher affinity for the receptor and high bioavailability. These properties in the whole animal are demonstrated by the much lower ED₅₀ compared to (+)-HA-966 to block seizures induced by NMDLA (19.7 mg kg⁻¹ vs. 900 mg kg⁻¹, i.v., Singh *et al.*, 1990a) and the relatively small differences in anticonvulsant potency seen when L-687,414 was given by the oral and intravenous routes.

The large difference in potency between (+)-HA-966 (Singh *et al.*, 1990a) and L-687,414 in blocking NMDLA-induced seizures (45 fold) is, however, greater than would have been anticipated from the approximate 10 fold increase in affinity for the glycine site. In the audiogenic seizure model, the difference in potency between the two compounds does reflect their respective affinities *in vitro* and the discrepancy with NMDLA may be a function of (i) the more powerful convulsant stimulus offered by NMDLA, (ii) the higher degree of intrinsic activity of (+)-HA-966 at the glycine site or, (iii) a combination of these together with pharmacokinetic factors. That the anticonvulsant effects of both L-687,414 and (+)-HA-966 are mediated by the glycine modulatory site is indicated by the ability of similar doses of the glycine receptor agonist, D-serine, to reverse the protective action of both compounds against NMDLA (Figure 4 and Singh *et al.*, 1990a).

L-687,414 was also active against seizures induced by pentylenetetrazol and electroshock, although in the former case, the compound was considerably weaker in the rat than in the mouse while MK-801 was equipotent in both species. The severity of the convulsant stimulus may again be a factor here since in the rat, PTZ was given as an intravenous bolus with seizures developing almost immediately whilst in the mouse the compound was given subcutaneously, taking about 10–12 min for a tonic seizure to occur.

As with the non-competitive NMDA receptor antagonist, MK-801, L-687,414 was found to impair the motor coordination necessary to maintain position on a revolving rotarod. The impairment in rotarod performance was reversed by administration of D-serine, consistent with the effect being mediated by the glycine modulatory site. However, unlike MK-801 (Tricklebank *et al.*, 1989) the ataxia induced by L-687,414 occurred at doses that were often higher than those required for protection from seizures, giving a 'therapeutic window' of between 1 and 5 fold, depending on the seizure model employed for comparison. The largest window was seen with audiogenic seizures which again might be a

Figure 9 Effect of (a) MK-801 and (b) L-687,414 on locomotor activity. Male Swiss Webster mice were habituated for 2 h to automated photocell activity cages. Saline (○), MK-801 (● 0.0625 mg kg⁻¹, □ 0.125 mg kg⁻¹, ■ 0.25 mg kg⁻¹, △ 0.5 mg kg⁻¹, ▲ 1 mg kg⁻¹) or L-687,414 (● 75 mg kg⁻¹, □ 100 mg kg⁻¹) were then injected intravenously and the animals returned to the cages. The number of cage crossings (the number of consecutive beam breaks) were then monitored and recorded at 10 min intervals for a total of 2 h. Results are shown as the mean (± s.e.mean) number of cage crossings (*n* = 8 animals per group). *Significantly different from animals given saline, *P* < 0.05 (ANOVA with repeated measures followed by Dunnett's *t* test).

Table 4 Effect of MK-801 on dopamine metabolism in the striatum and nucleus accumbens of the mouse

	Dose (mg kg ⁻¹)	Nucleus accumbens			Striatum		
		DOPAC (ng g ⁻¹)	HVA (ng g ⁻¹)	$\frac{DOPAC + HVA}{DA}$	DOPAC (ng g ⁻¹)	HVA (ng g ⁻¹)	$\frac{DOPAC + HVA}{DA}$
Saline	—	674 ± 50	764 ± 61	0.29 ± 0.003	917 ± 65	1210 ± 68	0.25 ± 0.005
MK-801	0.05	693 ± 69	776 ± 37	0.30 ± 0.006	1013 ± 93	1427 ± 86	0.27 ± 0.01
	0.01	790 ± 75	964 ± 71	0.33 ± 0.18	1049 ± 144	1668 ± 119	0.29 ± 0.01
	0.5	1032 ± 65*	1133 ± 93*	0.40 ± 0.012*	1236 ± 142	1781 ± 142	0.31 ± 0.02
	1.0	1112 ± 30*	1170 ± 96*	0.41 ± 0.02*	1139 ± 79	1461 ± 111	0.28 ± 0.01
Saline	—	660 ± 45	788 ± 71	0.27 ± 0.02	808 ± 43	1023 ± 75	0.21 ± 0.02
L-687,414	5	727 ± 47	526 ± 47	0.20 ± 0.01	767 ± 52	1044 ± 69	0.21 ± 0.02
	16	652 ± 53	470 ± 23	0.21 ± 0.02	716 ± 92	1085 ± 79	0.20 ± 0.02
	53	723 ± 39	510 ± 44	0.23 ± 0.03	809 ± 101	1025 ± 47	0.20 ± 0.02
	106	960 ± 78	674 ± 109	0.28 ± 0.02	1327 ± 197	1683 ± 448	0.26 ± 0.07

Swiss Webster mice were injected intravenously with either MK-801 or L-687,414 and killed 30 min later. Values shown are the mean concentrations or concentration ratios ± s.e.mean of 4–5 animals per group.

**P* < 0.05 vs saline-treated mice (Dunnett's *t* test).

reflection of a lower relative intensity of the convulsant stimulus to overcome.

With respect to the motor stimulation and stereotypy characteristic of non-competitive NMDA ion channel blockers and absent in animals given (+)-HA-966 or 7-chlorokynurenic acid (Tricklebank & Saywell, 1990; Koek & Colpaert, 1990; Bristow *et al.*, 1993), it is perhaps surprising that such behaviour was clearly seen after giving L-687,414. Head weaving, body rolling and hyperlocomotion were all present after intravenous injection of the compound with maximum intensities that were between 50% and 75% of those seen with MK-801 and occurring within the first 30 min of administration. The intensity of each behavioural component was attenuated by D-serine, again indicating the involvement of the glycine modulatory site in their genesis. However, a significant window between the anticonvulsant and peak stimulant doses was clearly apparent, ranging from 5 to 20 fold, depending on the convulsant used. With MK-801, no such window was found (Tricklebank *et al.*, 1989).

It is likely that the stimulation of motor activity induced by non-competitive NMDA receptor antagonists is mediated by increased release of dopamine, particularly in mesolimbic brain regions (French & Vantini, 1984; Tiedtke *et al.*, 1990; Rao *et al.*, 1990; Criswell *et al.*, 1993). Unlike MK-801, however, L-687,414 did not increase the concentration of HVA or DOPAC or alter dopamine turnover, as expressed by the ratio of ([DOPAC] + [HVA])/[DA], in the nucleus accumbens or striatum, even after administration of high doses. This suggests that changes in dopamine metabolism might be secondary to the increase in motor activity. However, since the behavioural response to L-687,414 was transient, it may be that changes in dopamine release were of insufficient duration and/or magnitude to be reflected in increased metabolite concentrations.

The interoceptive stimulus properties of PCP, as measured in drug discrimination paradigms, are thought to be mediated by blockade of the NMDA receptor (Tricklebank *et al.*, 1987; 1989; Willetts & Balster, 1989; Koek *et al.*, 1990; Singh *et al.*, 1990c). High doses of L-687,414 induced the majority of animals tested to choose the PCP lever, indicating that a degree of stimulus similarity exists between the compounds (Bourson & Tricklebank, 1991). However, the selection of the PCP lever induced by L-687,414 did not occur without a marked reduction in response rate, suggesting that the stimuli were not identical. Indeed, in animals trained to discriminate L-687,414 from saline, PCP did not generalize to the stimulus at doses above or below those reducing response rate whilst a structurally unrelated glycine/NMDA receptor antagonist, L-695,902 (7-chloro-4-hydroxy-3-methoxycarbonyl-2-quinolone, Rowley *et al.*, 1993) completely generalized without alteration of response rate (Bourson & Tricklebank, unpublished observations).

The relatively high degree of PCP-appropriate responding induced by L-687,414 contrasts with that seen with competitive NMDA receptor antagonists acting at the neurotransmitter recognition site. Similarly, the intensity of behavioural stimulation seen with L-687,414 appears to be greater than that seen with some (Tricklebank *et al.*, 1989), but perhaps not all (Koek & Colpaert, 1990; Svensson *et al.*, 1991) competitive NMDA receptor antagonists and is certainly greater than that seen with (+)-HA-966, 7-chlorokynurenic acid (Koek & Colpaert, 1990; Tricklebank & Saywell, 1990; Bristow *et al.*, 1993) and L-695,902 (Tricklebank *et al.*, 1993). It is difficult at the present time to reconcile these differences. Partial agonism does not seem to be a factor given the wholly antagonist actions of 7-chlorokynurenic acid and L-695,902. Some differences may be related to the relatively low affinity of the drugs for the glycine site and/or their slow brain penetration, although the probable existence of subtypes of the NMDA receptor (Moriyoshi *et al.*, 1991; Monyer *et al.*, 1992) raises the possibility that differential receptor subtype selectivity could account for some aspects of the varied profile of competitive, non-competitive and glycine/NMDA receptor antagonists. It is also possible that the intrinsic activities of compounds varies across subtypes so that, for example, an efficacy of 7% obtained with L-687,414 against cortical NMDA receptors (Leeson *et al.*, 1993) may be very different in an area predominantly expressing a non-cortical receptor subtype.

In conclusion, L-687,414 should prove to be a valuable tool for determining the behavioural relevance of the glycine modulatory site on the NMDA receptor complex. It is clearly premature, however, to define the functional properties of glycine site antagonists on the basis of a single compound and the availability of higher affinity ligands is keenly awaited. Nevertheless, in addition to the anticonvulsant properties described above, L-687,414 has also been shown to be neuroprotective in models of cerebral ischaemia (Gill *et al.*, 1991) and at neuroprotective doses did not increase cerebral glucose metabolism, distort cortical neuronal morphology or increase blood pressure, in contrast to MK-801 (Hargreaves *et al.*, 1993). It is also notable that in the photosensitive baboon photo-induced myoclonus was completely antagonized by L-687,414 at doses devoid of motor impairment (Smith & Meldrum, 1992), giving rise to the hope that some forms of epilepsy in man would be amenable to treatment with glycine/NMDA receptor antagonists at doses below those producing adverse behavioural effects.

The authors thank Dr R.G. Hill for advice and critical reading of the manuscript.

References

- ANIS, N.A., BERRY, S.C., BURTON, N.R. & LODGE, D. (1983). The dissociative anaesthetics, ketamine and PCP, selectively reduce excitation of central mammalian neurones by N-methyl-aspartate. *Br. J. Pharmacol.*, **79**, 565–575.
- BOURSON, A. & TRICKLEBANK, M.D. (1991). The discriminative stimulus properties of the glycine/NMDA receptor antagonist, L-687,414. *Fund. Clin. Pharmacol.*, **5**, 443.
- BRISTOW, L.J., HUTSON, P.H., THORN, L. & TRICKLEBANK, M.D. (1993). The glycine/NMDA receptor antagonist, R-(+)-HA-966, blocks activation of the mesolimbic dopaminergic system induced by PCP and dizocilpine (MK-801) in rodents. *Br. J. Pharmacol.*, **108**, 1156–1163.
- CRISWELL, H.E., JOHNSON, K.B., MUELLER, R.A. & BREESE, G.R. (1993). Evidence for involvement of brain dopamine and other mechanisms in the behavioral action of the N-methyl-D-aspartic acid antagonist MK-801 in control and 6-hydroxydopamine-lesioned rats. *J. Pharmacol. Exp. Ther.*, **265**, 1001–1010.
- DAVIES, J., EVANS, R.H., HERLING, P.L., JONES, A.W., OLVERMAN, H.J., POOK, P. & WATKINS, J.C. (1986). CPP, a new potent and selective NMDA antagonist. Depression of central neuron responses, affinity for [³H]D-AP5 binding sites on brain membranes and anticonvulsant activity. *Brain Res.*, **382**, 169–173.
- FRENCH, E.D. & VANTINI, G. (1984). Phencyclidine-induced locomotor activity in the rat is blocked by 6-hydroxydopamine lesions of the nucleus accumbens: comparisons to other psychomotor stimulants. *Psychopharmacol.*, **82**, 83–88.
- GILL, R., HARGREAVES, R.J. & KEMP, J.A. (1991). Neuroprotective effects of the glycine site antagonist (+)cis-4-methyl-HA-966 (L-687,414) in rat focal ischaemia model. *J. Cereb. Blood Flow Metab.*, **11**, Suppl. 2, S304.

- HARGREAVES, R.J., RIGBY, M., SMITH, D. & HILL, R.G. (1993). Lack of effect of L-687,414 ((+)-*cis*-4-methyl-HA-966), an NMDA receptor antagonist acting at the glycine site, on cerebral glucose metabolism and cortical neuronal morphology. *Br. J. Pharmacol.*, **110**, 36–42.
- HUTSON, P.H., BRISTOW, L.J., THORN, L. & TRICKLEBANK, M.D. (1991). R-(+)-HA-966, a glycine/NMDA receptor antagonist, selectively blocks the activation of the mesolimbic dopamine system by amphetamine. *Br. J. Pharmacol.*, **103**, 2037–2044.
- KEMP, J.A., FOSTER, A.C., LEESON, P.D., PRIESTLEY, T., TRIDGETT, R., IVERSEN, L.L. & WOODRUFF, G.N. (1988). 7-Chlorokynurenic acid is a selective antagonist at the glycine modulatory site of the N-methyl-D-aspartate receptor complex. *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 6547–6550.
- KOEK, W. & COLPAERT, F.C. (1990). Selective blockade of N-methyl-D-aspartate (NMDA)-induced convulsions by NMDA antagonists and putative glycine antagonists: relationship with phencyclidine-like behavioral effects. *J. Pharmacol. Exp. Ther.*, **252**, 349–357.
- KOEK, W., WOODS, J.H. & COLPAERT, F.C. (1990). N-methyl-D-aspartate antagonism and phencyclidine-like activity: a drug discrimination analysis. *J. Pharmacol. Exp. Ther.*, **253**, 1017–1025.
- LEESON, P.D., WILLIAMS, B.J., ROWLEY, M., MOORE, K.W., BAKER, R., KEMP, J.A., PRIESTLEY, T., FOSTER, A.C. & DONALD, A.E. (1993). Derivatives of 1-hydroxy-3-aminopyrrolidin-2-one (HA-966). Partial agonists at the glycine site of the NMDA receptor. *Biorg. Med. Chem. Lett.*, **3**, 71–76.
- MONYER, H., SPRENGEL, R., SCHOEPPFER, R., HERB, A., HIGUCHI, M., LOMELI, H., BURNASHEV, N., SAKMANN, B. & SEEBURG, P.H. (1992). Heteromeric NMDA receptors: molecular and functional distinction of subtypes. *Science*, **256**, 1217–1221.
- MORIYOSHI, K., MASU, M., ISHII, T., SHIGEMOTO, R., MIZUNO, N. & NAKANISHI, S. (1991). Molecular cloning and characterization of the rat NMDA receptor. *Nature*, **354**, 31–37.
- RAO, T.S., KIM, H.S., LEHMANN, J., MARTIN, L.L. & WOOD, P.L. (1990). Selective activation of dopaminergic pathways in the mesocortex by compounds that act at the phencyclidine (PCP) binding site: tentative evidence for PCP recognition sites not coupled to N-methyl-D-aspartate (NMDA) receptors. *Neuropharmacol.*, **29**, 225–230.
- ROWLEY, M., LEESON, P.D., STEVENSON, G.I., MOSELEY, A.M., STANSFIELD, I., SANDERSON, I., ROBINSON, L., BAKER, R., KEMP, J.A., MARSHALL, G.R., FOSTER, A.C., GRIMWOOD, S., TRICKLEBANK, M.D. & SAYWELL, K. (1993). 3-Acyl-4-hydroxyquinolin-2(1H)-ones. Systemically active anticonvulsants acting by antagonism at the glycine site of the N-methyl-D-aspartate receptor complex. *J. Med. Chem.*, **36**, 3386–3396.
- SINGH, L., DONALD, A.E., FOSTER, A.C., HUTSON, P.H., IVERSEN, L.L., IVERSEN, S.D., KEMP, J.A., LEESON, P.D., MARSHALL, G.R., OLES, R.J., PRIESTLEY, T., THORN, L., TRICKLEBANK, M.D., VASS, C.A. & WILLIAMS, B.J. (1990a). Enantiomers of HA-966 (3-amino-1-hydroxypyrrolidin-2-one) exhibit distinct central nervous system effects: (+)-HA-966 is a selective glycine/N-methyl-D-aspartate receptor antagonist, but (–)-HA-966 is a potent γ -butyrolactone-like sedative. *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 347–351.
- SINGH, L., MENZIES, R. & TRICKLEBANK, M.D. (1990b). The discriminative stimulus properties of (+)-HA-966, an antagonist of the glycine/N-methyl-D-aspartate receptor. *Eur. J. Pharmacol.*, **186**, 129–132.
- SINGH, L., WONG, E.F.H., KESINGLAND, A.C. & TRICKLEBANK, M.D. (1990c). Evidence against involvement of the haloperidol-sensitive sigma recognition site in the discriminative stimulus properties of (+)-N-allylnormetazocine ((+)-SKF 10,047). *Br. J. Pharmacol.*, **99**, 145–151.
- SMITH, S.E. & MELDRUM, B.S. (1992). The glycine-site NMDA receptor antagonist, R-(+)-*cis*- β -methyl-3-amino-1-hydroxypyrrolidin-2-one, L-687,414 is anticonvulsant in baboons. *Eur. J. Pharmacol.*, **211**, 109–111.
- SVENSSON, A., PILELBLAD, E. & CARLSSON, M. (1991). A comparison between the non-competitive NMDA antagonist dizocilpine (MK-801) and the competitive NMDA antagonist D-CPPene with regard to dopamine turnover and locomotor-stimulatory properties in mice. *J. Neurol. Transm. [Gen Sect]*, **85**, 117–129.
- TIEDTKE, P.I., BISCHOFF, C. & SCHMIDT, W.J. (1990). MK-801-induced stereotypy and its antagonism by neuroleptic drugs. *J. Neural Trans.*, **81**, 173–182.
- TRICKLEBANK, M.D., KEMP, J.A., RIGBY, M., TATTERSALL, D., MARSHALL, G., SAYWELL, K., BOURSON, A., LEESON, P.D., KULAGOWSKI, J.J., ROWLEY, M., MAWER, I.M. & HILL, R.G. (1993). The pharmacological profile of the glycine/NMDA receptor antagonist, L-695,902 and related 4-hydroxy-2-quinolones. Abstracts of the meeting *Focus on Epilepsy II. Antiepileptic Drugs in the 1990s: Current and Investigational Therapies*. Whistler, Canada, p. 24.
- TRICKLEBANK, M.D. & SAYWELL, K. (1990). Behavioural properties of antagonists acting at the glycine modulatory site on the NMDA receptor/ion channel complex. *Neurosci. Abst.*, **16**, 462.
- TRICKLEBANK, M.D., SINGH, L., OLES, R.J., PRESTON, C. & IVERSEN, S.D. (1989). The behavioural effects of MK-801: a comparison with antagonists acting non-competitively and competitively at the NMDA receptor. *Eur. J. Pharmacol.*, **167**, 127–135.
- TRICKLEBANK, M.D., SINGH, L., OLES, R., WONG, E.H.F. & IVERSEN, S.D. (1987). A role for receptors of N-methyl-D-aspartic acid in the discriminative stimulus properties of phencyclidine. *Eur. J. Pharmacol.*, **141**, 497–501.
- WILLETTS, J. & BALSTER, R.L. (1989). Effects of competitive and noncompetitive N-methyl-D-aspartate (NMDA) antagonists in rats trained to discriminate NMDA from saline. *J. Pharmacol. Exp. Ther.*, **251**, 627–633.
- WILLETTS, J., BALSTER, R.L. & LEANDER, J.D. (1991). The behavioural pharmacology of NMDA receptor antagonists. *Trends Pharmacol. Res.*, **11**, 423–428.
- WILLIAMS, K. (1993). Ifenprodil discriminates subtypes of the N-methyl-D-aspartate receptor: selectivity and mechanisms at recombinant heteromeric receptors. *Mol. Pharmacol.*, **44**, 851–859.
- WONG, E.H.F., KEMP, J.A., PRIESTLEY, T., KNIGHT, A.R., WOODRUFF, G.N. & IVERSEN, L.L. (1986). The anticonvulsant MK-801 is a potent N-methyl-D-aspartate antagonist. *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 7104–7108.

(Received March 30, 1994)

Revised June 15, 1994

Accepted June 22, 1994)