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The glycine/NMDA receptor antagonist, R-(+)-HA-966, blocks activation of the mesolimbic dopaminergic system induced by phencyclidine and dizocilpine (MK-801) in rodents

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1 The effects of the glycine/N-methyl-D-aspartate (NMDA) receptor antagonist, R-(+)-HA-966 on the neurochemical and behavioural responses to phencyclidine (PCP) and dizocilpine (MK-801) have been determined in rodents.

2 In rats, pretreatment with PCP (5 and 10 mg kg^{-1}) or MK-801 (0.25 and 0.5 mg kg^{-1}) dose-dependently stimulated dopamine turnover in nucleus accumbens, amygdala and medial prefrontal cortex, but had no effect in striatum. In contrast, pretreatment with (+)-HA-966 (10 and 30 mg kg^{-1}) did not affect dopamine turnover in any brain region investigated.

3 Pretreatment with (+)-HA-966 (10 and 30 mg kg^{-1}) significantly antagonized the stimulation of dopamine turnover induced by both PCP (10 mg kg^{-1}) and MK-801 (0.5 mg kg^{-1}) in rat nucleus accumbens, amygdala and medial prefrontal cortex.

4 Intracerebral dialysis studies in conscious rats demonstrated that systemic injection of PCP (10 mg kg^{-1}) markedly stimulated dopamine release from the nucleus accumbens, an effect that was abolished by pretreatment with (+)-HA-966 (30 mg kg^{-1}).

5 Pretreatment with PCP (3–30 mg kg^{-1}) or MK-801 (0.1–1.6 mg kg^{-1}) significantly increased locomotor activity in mice. In contrast, subcutaneous injection of (+)-HA-966 (10–100 mg kg^{-1}) failed to stimulate activity.

6 Pretreatment with (+)-HA-966 (10 and 30 mg kg^{-1}) dose-dependently antagonized both PCP (10 mg kg^{-1}) and MK-801 (0.4 mg kg^{-1}) induced hyperactivity in mice.

7 Blockade of PCP-induced hyperactivity by (+)-HA-966 is unlikely to be explained by the induction or potentiation of sedation/ataxia since PCP-induced rotarod deficits were not significantly different in mice pretreated with (+)-HA-966 (30 mg kg^{-1}) or saline.

8 The results demonstrate that (+)-HA-966 antagonizes both the neurochemical and behavioural effects of PCP and MK-801, possibly through interactions at the glycine/NMDA receptor.

Keywords: (+)-HA-966; glycine site; NMDA receptor complex; dizocilpine (MK-801); phencyclidine; hyperactivity; dopamine turnover

Introduction

Several studies have demonstrated that phencyclidine (PCP) facilitates dopaminergic transmission in brain (for review see Johnson, 1983). However, whilst stimulation of basal dopamine release from striatal slices by PCP has been reported *in vitro* (Vickroy & Johnson, 1982; Snell *et al.*, 1984), *ex vivo* studies have shown little effect in intact striatum, in contrast to the marked stimulation of release that has been demonstrated from mesocorticolimbic dopaminergic terminals (Bowers & Hoffman, 1984; Deutch *et al.*, 1987; Rao *et al.*, 1989; 1990). Increased dopamine release from limbic brain is thought to mediate PCP-induced hyperactivity since the behaviour is blocked by dopamine receptor antagonists and by 6-hydroxydopamine lesions of the ventral tegmental area or nucleus accumbens (French & Vantini, 1984; French *et al.*, 1985). Given the psychotomimetic effect of PCP in man (Snyder, 1980; Javitt & Zukin, 1991), blockade of PCP-stimulated dopamine release or hyperactivity may be predictive of antipsychotic potential.

Radioligand binding studies have identified three recognition sites in brain membranes which bind PCP with submicromolar affinity: the PCP binding site associated with the ion channel of the N-methyl-D-aspartate (NMDA) receptor complex, the sigma binding site (Largent *et al.*, 1986) and the dopamine uptake site (Vignon *et al.*, 1988). All are pharmacologically distinct: dizocilpine (MK-801) selectively blocks NMDA receptor ion channels (Wong *et al.*, 1986; 1988) whilst haloperidol, 1,3,di-(2-tolyl)guanidine (DTG) and (+)-3-PPP potently bind to sigma sites (Su, 1982; Tam & Cook, 1984; Largent *et al.*, 1984; Weber *et al.*, 1986). PCP and cocaine, but not MK-801 or ketamine, bind to the dopamine uptake site labelled by [^{3}H]-N-[2-benzo(b)thiophenyl)cyclohexyl]piperidine (Vignon *et al.*, 1988). Although recent attention has focused on the involvement of the sigma recognition site in the psychotomimetic effects of PCP (Largent *et al.*, 1988), behavioural data suggest that blockade of NMDA receptors is responsible for the major pharmacological properties of the compound. Thus, in anticonvulsant tests and drug discrimination paradigms, the potency of many PCP-like compounds correlates with their potency in binding assays using [^{3}H]-PCP, [^{3}H]-TCP and [^{3}H]-MK-801 as radioligands (Quirion *et al.*, 1981; Mendelsohn *et al.*, 1984; Hayes & Balster, 1985; Singh *et al.*, 1990c). Furthermore, the highly selective non-competitive NMDA receptor antagonist, MK-801 generalizes to the PCP discriminative stimulus (Tricklebank *et al.*, 1987), is anticonvulsant in mice and induces PCP-like motor stimulation (Tricklebank *et al.*, 1989). Whilst stimulation of mesolimbic dopamine release by PCP could result from inhibition of dopamine uptake (Vignon *et al.*, 1988; Rothman *et al.*, 1989; Maurice *et al.*, 1991), MK-801 has little effect on uptake mechanisms at pharmacologically relevant concentrations (Maurice *et al.*, 1991),

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yet increases dopamine turnover in a manner identical to PCP (Rao *et al.*, 1990).

In addition to compounds blocking the NMDA receptor ion channel, at least three other distinct classes of NMDA receptor antagonists exist: viz, those acting (i) competitively at the neurotransmitter recognition site, (ii) by antagonizing the potentiating effects of polyamines and (iii) by antagonizing the potentiating effects of glycine (for review, see Lodge & Johnson, 1990). R-(+)-HA-966 (R-(+)-3-amino-1-hydroxypyrrolid-2-one) is an antagonist at the glycine/NMDA modulatory site (Singh *et al.*, 1990a) and exhibits a behavioural profile distinct from PCP and other NMDA receptor ion channel blockers: in rodents, (+)-HA-966 does not generalize to the PCP discriminative stimulus, stimulate motor activity or enhance dopamine turnover (Singh *et al.*, 1990a,b; Tricklebank & Saywell, 1990). Previous studies have shown that (+)-HA-966 can selectively modulate the mesolimbic dopaminergic system (Hutson *et al.*, 1991): pretreatment with (+)-HA-966 antagonized amphetamine-induced dopamine synthesis in the nucleus accumbens, but not in the striatum. (+)-HA-966 also antagonized the hyperactivity, but not the stereotypy, induced by infusion of amphetamine into the rat nucleus accumbens and striatum respectively. Thus, since PCP and MK-801 are able to activate selectively mesolimbic dopamine systems, the present studies have examined the effects of (+)-HA-966 on the stimulation of dopamine turnover and motor activity induced in rodents by these compounds.

Methods

Animals

Male Sprague Dawley rats (250–300 g) and BKTO mice (20–30 g) (Bantin and Kingman, Hull) were housed in groups of 5 on a 12 h light/dark cycle (lights on at 06 h 00 min) and allowed standard laboratory diet and tap water *ad libitum*.

Neurochemical studies

Rats were injected with either saline (0.9% NaCl, 1 ml kg^{-1}, i.p.), PCP (5 and 10 mg kg^{-1}, i.p.) or MK-801 (0.25 and 0.5 mg kg^{-1}, i.p.) and killed by stunning and decapitation 60 min later. Brains were removed and the striatum, nucleus accumbens, amygdala and prefrontal cortex dissected and stored at − 70°C.

In a separate study, rats were pretreated with either saline (1 ml kg^{-1}, i.p.) or (+)-HA-966 (10 and 30 mg kg^{-1}, i.p.) followed 20 min later by either saline (1 ml kg^{-1}, i.p.), PCP (10 mg kg^{-1}, i.p.) or MK-801 (0.5 mg kg^{-1}, i.p.). All animals were killed after a further 60 min, brain regions dissected and stored as previously described.

Tissue samples were homogenized in 10 vol 0.4 M perchloric acid containing 0.1% sodium metabisulphite, 0.01% EDTA and 0.1% cysteine and centrifuged at 3000 *g* for 20 min. Aliquots of the supernatant were analysed by high performance liquid chromatography (h.p.l.c.) with electrochemical detection essentially as described by Hutson *et al.* (1991).

Intracerebral dialysis in conscious rats

Rats were anaesthetized with pentobarbitone (60 mg kg^{-1}, i.p.; Sagatal, RMB) and implanted with a concentric dialysis probe in the nucleus accumbens according to coordinates derived from Paxinos & Watson, 1982 (A + 1.7 mm; L 1.5 mm from bregma; V − 8 mm from dura). The probe was essentially as described by Hutson & Suman-Chauhan (1990) except that a 2 mm length of membrane (Filtral 16, Hospal, France) was used. Eighteen h later, the probe was perfused with a physiological salt solution (composition in mM: NaCl 125, KCl 2.5, $MgCl_2$ 1.18, $CaCl_2$ 1.26) at a rate of 2 µl min^{-1}. Samples were collected at 20 min intervals and analysed immediately by h.p.l.c. with electrochemical detection for dopamine content (Hutson *et al.*, 1991). Values were not corrected for *in vitro* recovery of dopamine through the probe. Two h after beginning perfusion, rats were injected with either saline (1 ml kg^{-1}, i.p.) or (+)-HA-966 (30 mg kg^{-1}, i.p.) followed 20 min later by either saline (1 ml kg^{-1}, i.p.) or PCP at a dose (10 mg kg^{-1}, i.p.) that was found in preliminary experiments to give a robust, but submaximal increase in dopamine efflux. Probe placements were confirmed by flushing the probe with indian ink and histological examination at the end of each experiment.

Mouse locomotor activity

Although both rats and mice show marked hyperlocomotion to PCP, mice were used for studying the antagonist properties of (+)-HA-966 because in preliminary studies the dose-response relationship to PCP in this species appeared broader and less likely to be compromised by the onset of ataxia.

Mouse activity was quantified in automated cages (230 × 280 × 210 mm) lined with wire grids and equipped with two parallel infra red photocell beams at the top and base of the cage with input to a BBC Acorn Microcomputer. Whilst a number of behavioural parameters can be measured, cage crossings (i.e. consecutive beam breaks across the base of the cage) have been selected as the measure of locomotion.

Mice were habituated to individual cages for 2 h and then pretreated with either saline (10 ml kg^{-1}, s.c.) or (+)-HA-966 (10 and 30 mg kg^{-1}, s.c.) followed 30 min later by either saline (10 ml kg^{-1}, s.c.), PCP (10 mg kg^{-1}, s.c.) or MK-801 (0.4 mg kg^{-1}, s.c.). The animals were immediately returned to the photocell cages and the number of beam breaks occurring in 10 min intervals recorded for 2 h.

Rotarod performance

The sedative/ataxic effects of (+)-HA-966 and PCP were examined in mice by use of a rotarod apparatus. Animals were trained to remain on a rotarod revolving at 15 r.p.m. for 2 min and then pretreated with either saline (10 ml kg^{-1}, s.c.) or (+)-HA-966 (30 mg kg^{-1}, s.c.). Thirty min later, mice received either saline (10 ml kg^{-1}, s.c.) or PCP (3–30 mg kg^{-1}, s.c.) and the latency to fall off the rotarod determined 30 min later. Animals not falling off within 2 min were given a maximum score of 120 s.

Statistical analysis

Mouse motor activity data were analysed by analysis of variance for repeated measures followed by one way analysis of variance with Tukey's *t* test for pair-wise comparisons at individual time points. Neurochemical data were also analysed by analysis of variance followed by Tukey's test.

Drugs

Phencyclidine HCl (Ultrafine Chemicals), MK-801 (dizocilpine, Merck Sharp and Dohme Research Laboratories) and (+)-HA-966 (R-(+)-3-amino-1-hydroxypyrrolid-2-one, Merck, Sharp and Dohme Research Laboratories) were dissolved in 0.9% NaCl.

Results

Effect of PCP and MK-801 on regional brain dopamine turnover in the rat

Pretreatment with PCP (5 and 10 mg kg^{-1}, i.p.; Figure 1a) dose-dependently increased dopamine turnover, as defined by the ratio of [DOPAC + HVA]/[DA], in the medial prefrontal

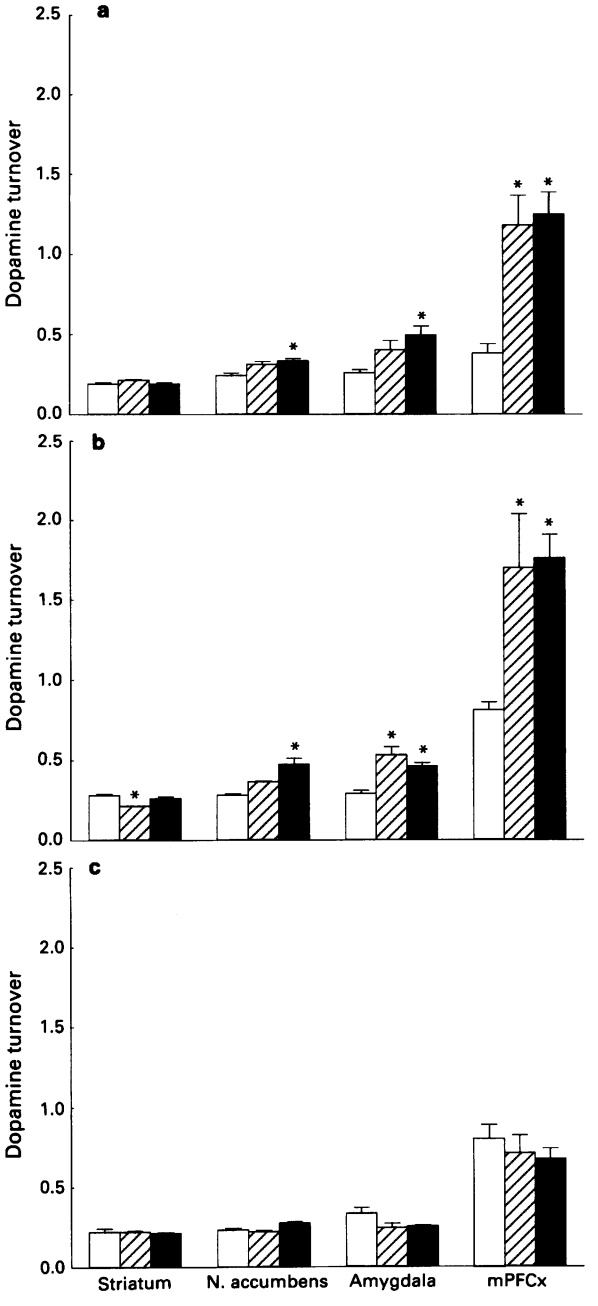

Figure 1 Effect of (a) phencyclidine (PCP, 5 mg kg^{-1}, i.p., hatched columns and 10 mg kg^{-1}, i.p., solid columns), (b) dizocilpine (MK-801, 0.25 mg kg^{-1}, i.p., hatched columns and 0.5 mg kg^{-1}, i.p., solid columns) and (c) (+)-HA-966 (10 mg kg^{-1}, i.p., hatched columns and 30 mg kg^{-1}, i.p., solid columns) on dopamine turnover in rat striatum, nucleus accumbens (N. accumbens), amygdala and medial prefrontal cortex (mPFCx). Open columns show the effects of pretreatment with saline (1 ml kg^{-1}, i.p.). Each column shows the mean for 4–12 rats (± s.e.mean). Dopamine turnover is expressed as the ratio of the levels of [DOPAC + HVA]/[dopamine] measured by h.p.l.c. with electrochemical detection. Data were analysed by one way analysis of variance followed by Tukey's t test; $^*P<0.05$ compared with saline-treated rats.

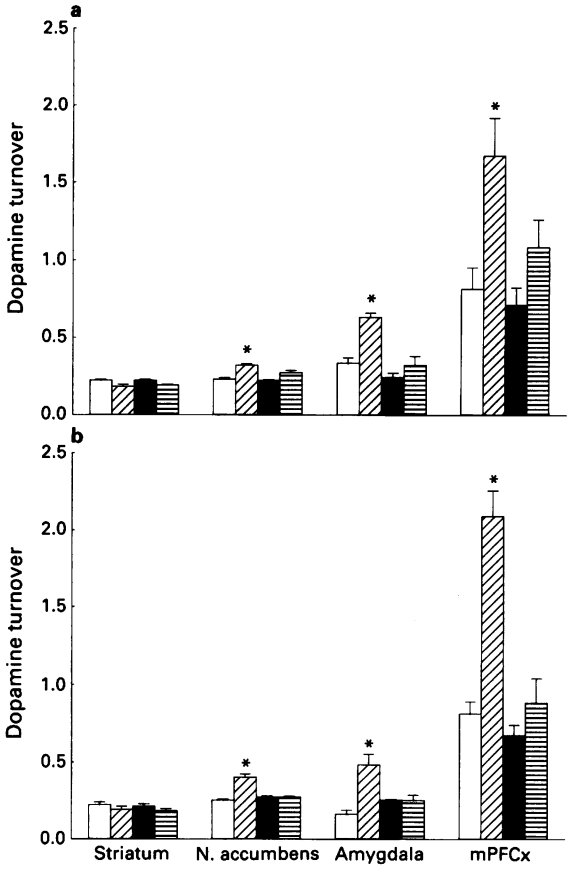

Figure 2 Effect of (a) (+)-HA-966 (10 mg kg^{-1}, i.p.) and (b) (+)-HA-966 (30 mg kg^{-1}, i.p.) on dopamine turnover stimulated by phencyclidine (PCP, 10 mg kg^{-1}, i.p.) in rat striatum, nucleus accumbens (N. accumbens), amygdala and medial prefrontal cortex (mPFCx). Each column shows the mean for 6–12 rats (± s.e.mean); open columns: saline (1 ml kg^{-1}, i.p.) + saline (1 ml kg^{-1}, i.p.); columns with diagonal hatching: saline (1 ml kg^{-1}, i.p.) + PCP (10 mg kg^{-1}, i.p.); solid columns: (+)-HA-966 (10 mg kg^{-1}, i.p (a), 30 mg kg^{-1}, i.p. (b)) + saline (1 ml kg^{-1}, i.p.); columns with horizontal hatching: (+)-HA-966 (10 mg kg^{-1}, i.p. (a) or 30 mg kg^{-1}, i.p. (b)) + PCP (10 mg kg^{-1}, i.p.). Dopamine turnover is expressed in individual rats as the ratio of [DOPAC + HVA]/[DA] measured by h.p.l.c. with electrochemical detection. Data were analysed by two way analysis of variance followed by Tukey's t test. $^*P<0.01$ compared with appropriate control group.

cortex (210% and 229% of controls), amygdala (54% and 88%) and nucleus accumbens (29% and 38% respectively). In contrast, PCP (5 and 10 mg kg^{-1}) did not significantly alter dopamine turnover in rat striatum (Figure 1a). Stimulation of dopamine turnover was also observed in the medial prefrontal cortex (110 and 106%), amygdala (83% and 59%) and nucleus accumbens (28% and 68%) following the intraperitoneal injection of MK-801 (0.25 and 0.5 mg kg^{-1} respectively; Figure 1b). A small, but significant reduction in dopamine turnover was demonstrated in the striatum following the lowest dose of MK-801 (0.25 mg kg^{-1}, Figure 1b). The stimulation of dopamine turnover by PCP and MK-801 reflected an increase in metabolite concentrations since in all experiments, dopamine concentration was not significantly altered (Table 1). Pretreatment with (+)-HA-966 (10 and 30 mg kg^{-1}, i.p.) was without effect on dopamine turnover in any brain region investigated (Figure 1c).

Effect of (+)-HA-966 on the stimulation of dopamine turnover by PCP and MK-801 in corticolimbic brain regions

Pretreatment with (+)-HA-966 (10 and 30 mg kg^{-1}, i.p.) 20 min prior to injection of PCP (10 mg kg^{-1}, i.p.) significantly and dose-dependently attenuated the increase in dopamine turnover induced by PCP in rat nucleus accumbens (reduced by 55% and 87%), amygdala (reduced by 100% and 72%) and medial prefrontal cortex (reduced by 69% and 94% respectively, Figure 2a,b). Similar effects were also observed in rats treated with MK-801 (0.5 mg kg^{-1}, i.p.; Figure 3). Thus, MK-801-stimulated dopamine turnover was

Table 1 Effect of phencyclidine (PCP), dizocilpine (MK- 801) and (+)-HA-966 on dopamine concentration (ng g^{-1}; mean ± s.e.mean, $n = 4-12$) in rat brain regions

Dose (mg kg^{-1})	*Striatum*	*N. accumbens*	*Amygdala*	*mPFCx**
PCP				
0	8569 ± 350	5877 ± 350	475 ± 22	126 ± 12
5	8766 ± 222	6452 ± 445	621 ± 55	97 ± 20
10	8508 ± 496	5562 ± 186	397 ± 27	73 ± 10
MK-801				
0	7354 ± 354	5573 ± 273	447 ± 18	101 ± 7
0.25	9339 ± 654	5407 ± 361	376 ± 55	92 ± 6
0.5	8962 ± 623	5872 ± 143	343 ± 40	102 ± 9
(+)-HA-966				
0	8075 ± 291	6933 ± 227	469 ± 38	76 ± 8
10	8070 ± 508	7192 ± 475	525 ± 46	88 ± 5
30	9404 ± 342	5796 ± 489	577 ± 74	242 ± 35

*Medial prefrontal cortex.

significantly attenuated in the nucleus accumbens (59%), amygdala (87%) and medial prefrontal cortex (76%) following pretreatment with (+)-HA-966 (30 mg kg^{-1}, i.p.; Figure 3). The effect of (+)-HA-966 on dopamine turnover was mediated solely via attenuation of the increase in dopamine metabolites induced by PCP or MK-801 (data not shown).

Effect of (+)-HA-966 on PCP-stimulated dopamine efflux from the nucleus accumbens in conscious rats

Intraperitoneal injection of PCP (10 mg kg^{-1}) markedly stimulated dopamine efflux from the nucleus accumbens of conscious rats which reached a maximum value of 480 fmol 40 μl^{-1}, 20 min after injection and declined over the next 4 h (Figure 4). (+)-HA-966 (30 mg kg^{-1}, i.p.) did not alter basal dopamine release, but when given 20 min prior to PCP (10 mg kg^{-1}, i.p.) markedly attenuated PCP-stimulated dopamine efflux (Figure 4).

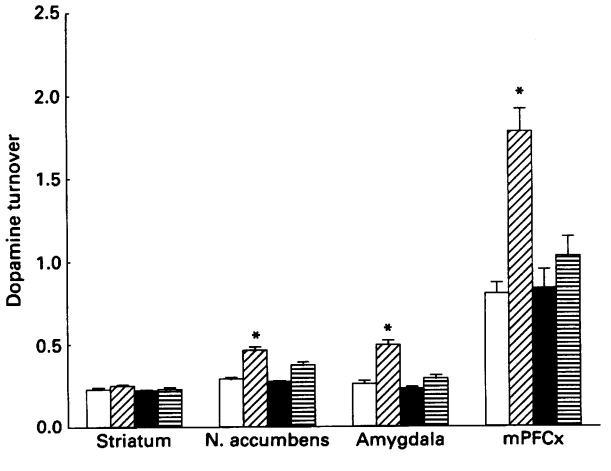

Figure 3 Effect of (+)-HA-966 (30 mg kg^{-1}, i.p.) on the stimulation of dopamine turnover induced by dizocilpine (MK-801, 0.5 mg kg^{-1}, i.p.) in rat striatum, nucleus accumbens (N. accumbens), amygdala and medial prefrontal cortex (mPFCx). Each column shows the mean for 6–12 rats (± s.e.mean); open columns: saline (1 ml kg^{-1}, i.p.) + saline (1 ml kg^{-1}, i.p.); columns with diagonal hatching: saline (1 ml kg^{-1}, i.p.) + MK-801 (0.5 mg kg^{-1}, i.p.); solid columns: (+)-HA-966 (30 mg kg^{-1}, i.p.) + saline (1 ml kg^{-1}, i.p.); columns with horizontal hatching: (+)-HA-966 (30 mg kg^{-1}, i.p.) + MK-801 (0.5 mg kg^{-1}, i.p.). Dopamine turnover is expressed as the ratio of [DOPAC + HVA]/[DA] measured by h.p.l.c. with electrochemical detection. Data were analysed by two way analysis of variance followed by Tukey's t test. *$P < 0.05$ compared with appropriate control group.

Effect of PCP, MK-801 and (+)-HA-966 on spontaneous activity in mice

Administration of PCP (1–30 mg kg^{-1}, s.c.) or MK-801 (0.1–1.6 mg kg^{-1}, s.c.) to mice previously habituated for 2 h to individual photocell activity cages significantly and dose-dependently increased the frequency of cage crossings compared to saline-pretreated animals (Figure 5). Peak hyperactivity responses occurred following 0.4 mg kg^{-1} MK-801 and 10 mg kg^{-1} PCP, the biphasic dose-response curves probably resulting from the marked ataxia induced by the higher doses (Figure 5).

Pretreatment with (+)-HA-966 (10–100 mg kg^{-1}, s.c.) did not alter motor activity in mice (Figure 5).

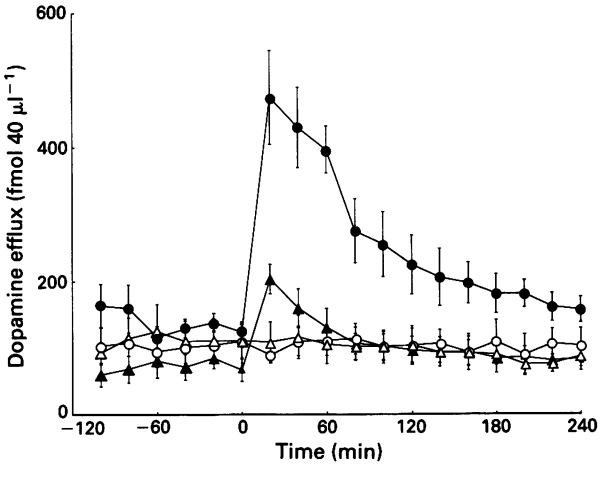

Figure 4 Effect of (+)-HA-966 (30 mg kg^{-1}, i.p.) on phencyclidine (PCP, 10 mg kg^{-1}, i.p.)-stimulated dopamine efflux from nucleus accumbens in conscious rats. Results are expressed as the mean concentration of dopamine (fmol) per 40 µl aliquot collected every 20 min (± s.e.mean, $n = 5-6$); (○) saline (1 ml kg^{-1}, i.p.) + saline (1 ml kg^{-1}, i.p.); (●) saline (1 ml kg^{-1}, i.p.) + PCP (10 mg kg^{-1}, i.p.); (△) (+)-HA-966 (30 mg kg^{-1}, i.p.) + saline (1 ml kg^{-1}, i.p.); (▲) (+)-HA-966 (30 mg kg^{-1}, i.p.) + PCP (10 mg kg^{-1}, i.p.). Areas under the curves were analysed by 2-way analysis of variance followed by Tukey's t test. Basal efflux (−120 min–0 min) did not differ significantly between groups. The area under the curve for animals given only PCP during the time period 0 min–240 min (262 ± 29 fmol per 40 µl per min) was significantly different from animals given saline (105 ± 27 fmol per 40 µl per min), (+)-HA-966 (96 ± 20 fmol per 40 µl per min), or (+)-HA-966 + PCP (110 ± 20 fmol per 40 µl per min), $P < 0.05$.

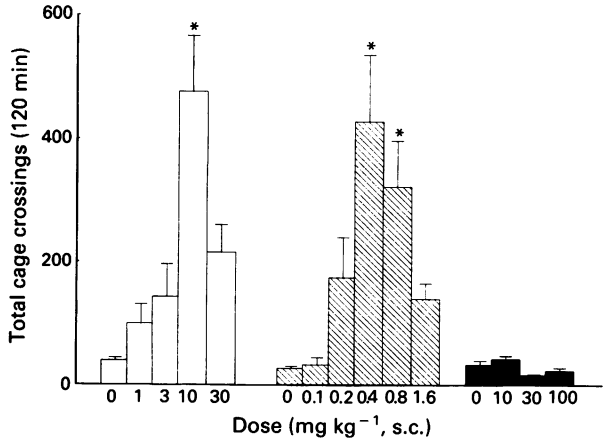

Figure 5 Effect of phencyclidine (PCP, 1–30 mg kg^{-1}, s.c., open columns), dizocilpine (MK-801, 0.1–1.6 mg kg^{-1}, s.c., hatched columns) and (+)-HA-966 (1–100 mg kg^{-1}, s.c., solid columns) on cage crossings recorded from 0–120 min following drug administration in mice. Results are expressed as mean activity counts (± s.e.mean, $n = 7$–13). Data were analysed by one way analysis of variance followed by Tukey's t test. *$P < 0.05$ compared to saline-pretreated mice.

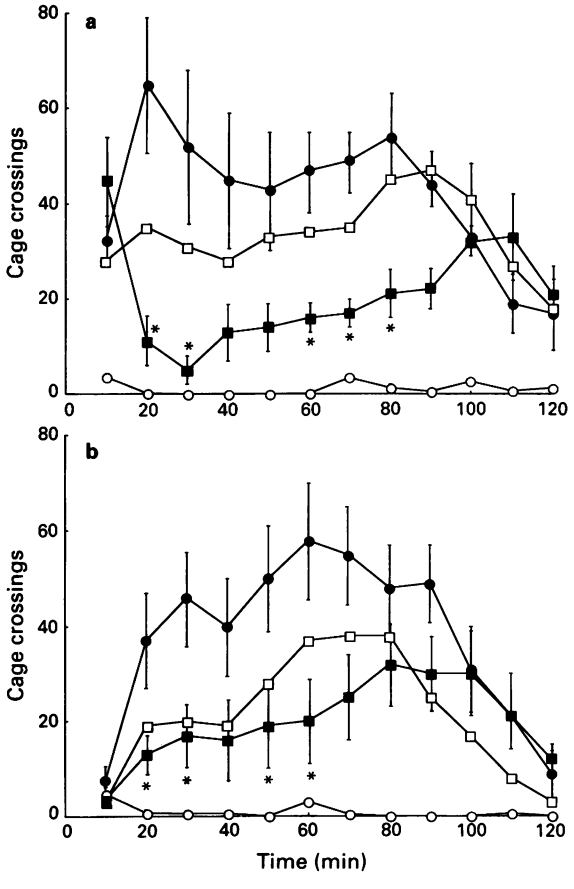

Figure 6 Effect of 30 min pretreatment with (+)-HA-966 (10 or 30 mg kg^{-1}, s.c.) on the stimulation of cage crossing induced by (a) phencyclidine (PCP, 10 mg kg^{-1}, s.c.) and (b) dizocilpine (MK-801, 0.4 mg kg^{-1}, s.c.) in mice. Results are expressed as mean activity counts (± s.e.mean, $n = 8$–11); (a) (○) saline (10 ml kg^{-1}, s.c.) + saline (10 ml kg^{-1}, s.c.); (●) saline (10 ml kg^{-1}, s.c.) + PCP (10 mg kg^{-1}, s.c.); (□) (+)-HA-966 (10 mg kg^{-1}, s.c.) + PCP (10 mg kg^{-1}, s.c.); (■) (+)-HA-966 (30 mg kg^{-1}, s.c.) + PCP (10 mg kg^{-1}, s.c.); (b) (○) saline (10 ml kg^{-1}, s.c.) + saline (10 ml kg^{-1}, s.c.); (●) saline (10 ml kg^{-1}, s.c.) + MK-801 (0.4 mg kg^{-1}, s.c.); (□) (+)-HA-966 (10 mg kg^{-1}, s.c.) + MK-801 (0.4 mg kg^{-1}, s.c.); (■) (+)-HA-966 (30 mg kg^{-1}, s.c.) + MK-801 (0.4 mg kg^{-1}, s.c.). Data were analysed by analysis of variance for repeated measures followed by Tukey's t test. *$P < 0.05$ compared to saline/PCP or saline/MK-801-treated mice.

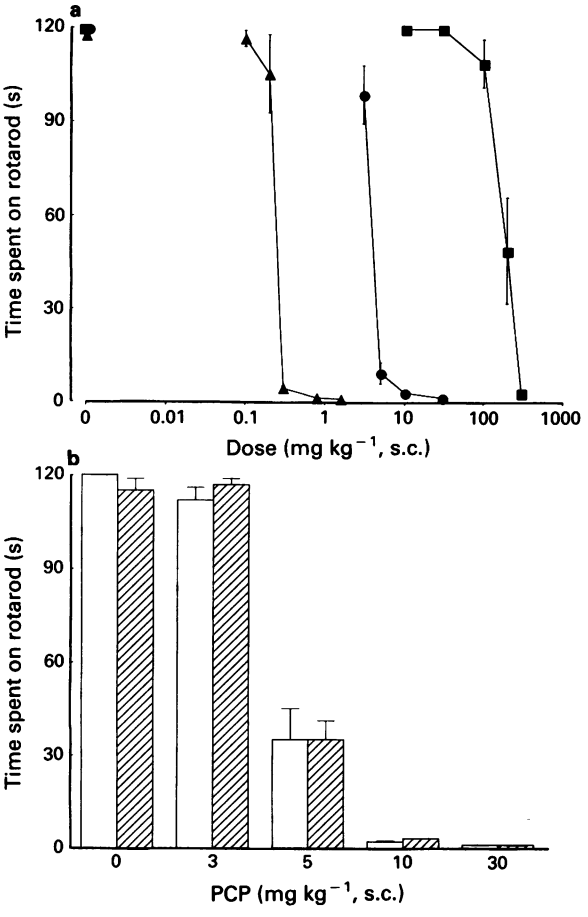

Figure 7 (a) Effect of (▲) dizocilpine (MK-801, 0.1–1.6 mg kg^{-1}, s.c.), (●) phencyclidine (PCP, 3–30 mg kg^{-1}, s.c.) and (■) (+)-HA-966 (10–300 mg kg^{-1}, s.c.) on rotarod performance tested 30 min after injection in mice. Results are expressed as the mean latency to fall from a rotarod revolving at 15 revolutions per min (± s.e.mean, $n = 8$). (b) Effect of 30 min pretreatment with (+)-HA-966 (30 mg kg^{-1}, s.c., hatched columns) or saline (10 ml kg^{-1}, s.c., open columns) on PCP (3–30 mg kg^{-1}, s.c.)-induced rotarod impairment in mice. Results are expressed as the mean latency to remain on a rotarod (15 r.p.m.); data were analysed by analysis of variance followed by Tukey's t test and were not significantly different.

Effects of (+)-HA-966 on PCP and MK-801-induced hyperactivity in mice

The pretreatment of mice with (+)-HA-966 (10 and 30 mg kg^{-1}, s.c.) dose-dependently antagonized the increase in locomotor counts recorded following the injection of PCP (10 mg kg^{-1}, s.c., Figure 6a) and MK-801 (0.4 mg kg^{-1}, s.c., Figure 6).

Effect of (+)-HA-966 on PCP-induced impairments in rotarod performance in mice

Both PCP and MK-801 significantly reduced the ability of mice to remain on a rotarod revolving at 15 r.p.m. at doses similar to those also stimulating motor activity in mice (Figure 7a). Pretreatment with (+)-HA-966 also induced a rotarod deficit, although the minimum effective dose was considerably greater than those attenuating PCP or MK-801 induced hyperactivity (200 mg kg^{-1}, s.c., Figure 7a cf. 10 and 30 mg kg^{-1}, s.c.). Administration of (+)-HA-966 (30 mg kg^{-1}, s.c.) 30 min prior to injection of PCP did not alter the PCP-induced motor deficit (Figure 7b).

Discussion

The present study extends our previous observations (Hutson *et al.*, 1991) that the glycine/NMDA receptor antagonist, (+)-HA-966 is able to modulate the activity of mesolimbic dopamine neurones in rodent brain. Thus, in the absence of any direct effect on brain dopamine metabolism or release, (+)-HA-966 significantly attenuated the increase in dopamine turnover induced in rat nucleus accumbens, amygdala and medial prefrontal cortex by the systemic injection of PCP and MK-801. Furthermore, the PCP-induced increase in dopamine outflow in the nucleus accumbens of the conscious rat, measured by intracerebral dialysis, was almost completely abolished by pretreatment with (+)-HA-966, suggesting an action on presynaptic dopaminergic systems.

In behavioural studies, (+)-HA-966 was also able to attenuate the actions of PCP and MK-801: whilst (+)-HA-966 was without effect on motor activity, it significantly and dose-dependently attenuated the increase in cage crossings induced by both stimulants. It is unlikely that this behavioural antagonism results from a sedative action of (+)-HA-966 since neither spontaneous activity (Hutson *et al.*, 1991) nor rotarod performance (present study) were impaired by (+)-HA-966 at doses inhibiting PCP or MK-801-induced hyperlocomotion. Since the motor hyperactivity response to both compounds declines at high doses as ataxia becomes predominant (Tricklebank *et al.*, 1989), the failure of (+)-HA-966 to enhance the rotarod deficit induced by PCP demonstrates that the blockade of hyperactivity by (+)-HA-966 is mediated by a true antagonism rather than by a leftward shift in the dose-response relationship to the stimulants.

The molecular mechanism by which (+)-HA-966 inhibits the activation of dopamine systems by PCP and MK-801 is clearly difficult to understand if it is assumed that all three compounds are acting as NMDA receptor antagonists (Wong *et al.*, 1986; 1988; Singh *et al.*, 1990a). Although PCP has appreciable affinity for the sigma recognition site (Largent *et al.*, 1986) and inhibits dopamine re-uptake (Vignon *et al.*, 1988), in addition to its ability to block the ion channel on the NMDA receptor, MK-801 shares only the latter property, consistent with other evidence indicating that blockade of NMDA receptors is responsible for the neurochemical and behavioural effects of PCP (Bowers & Hoffman, 1984; Hayes & Balster, 1985; Deutch *et al.*, 1987; Willetts & Balster, 1988; Tricklebank *et al.*, 1987; 1989; Rao *et al.*, 1989; 1990; Singh *et al.*, 1990c). (+)-HA-966 also antagonizes the actions of NMDA, but via blockade of the glycine modulatory site on the NMDA receptor (Singh *et al.*, 1990a), yet, paradoxically, attenuates the behavioural and neurochemical actions of the non-competitive NMDA receptor antagonists. It is, of course, possible that (+)-HA-966 does not attenuate drug-induced dopaminergic hyperactivity via glycine receptors, but via some other as yet unidentified pharmacological property. However, the structurally distinct glycine/NMDA receptor antagonist, 5,7-dichlorokynurenic acid, has also been found to attenuate the stimulation of dopamine synthesis induced by amphetamine (Hutson *et al.*, 1991), suggesting that anti-dopaminergic effects may be a general property of glycine/NMDA receptor antagonists. Alternatively, the results could indicate that the activation of dopamine systems by PCP and MK-801 does not involve the NMDA receptor, although as noted above, there is, at present, little other evidence consistent with this hypothesis.

In support of the ability of NMDA receptor blockade to attenuate dopaminergic hyperactivity, competitive NMDA receptor antagonists have been reported to antagonize the locomotor response to the intra-accumbens injection of amphetamine (Pulvirenti *et al.*, 1991) and to attenuate the increase in firing rate of dopaminergic neurones in the ventral tegmentum induced by PCP (French, 1992). Taken together, these results suggest the presence of a glutamatergic system that enhances mesolimbic dopaminergic functioning. Indeed, NMDA itself stimulates [^{3}H]-dopamine efflux from accumbens and striatal slices and stimulates locomotor activity following infusion into rat nucleus accumbens, ventral tegmental area or substantia nigra (Pycock & Dawbarn, 1980; Donzanti & Uretsky, 1983; 1984; Hamilton *et al.*, 1986; Jones *et al.*, 1987; O'Neill *et al.*, 1989; Ransom & Deschenes, 1989). It is possible that stimulation and blockade of dopaminergic systems by NMDA receptor-mediated events can occur at different loci in the brain. The paradox may not, therefore, lie in why NMDA/glycine receptor antagonists are able to inhibit the effects of NMDA receptor ion channel blockers, but in why the different classes of NMDA receptor antagonists differ in their abilities to stimulate dopamine systems (Kabuto *et al.*, 1989; French *et al.*, 1991; Hutson *et al.*, 1991).

It is pertinent to note that others have shown that the glycine receptor agonists, D-serine and D-alanine, also antagonize PCP-induced stereotypy (Contreras, 1990; Tanii *et al.*, 1991). Whilst functional assays *in vitro* have demonstrated that (+)-HA-966 predominantly antagonizes responses to NMDA, blockade is not complete, consistent with the compound being a partial agonist with a level of efficacy of about 10% of that of glycine (Singh *et al.*, 1990a). Thus, it is possible that the antagonism by (+)-HA-966 of the behavioural and neurochemical effects of non-competitive NMDA receptor antagonists reflects an agonist action at the glycine modulatory site.

In conclusion, it is clear that (+)-HA-966 is able to modulate both the neurochemical and behavioural response to the non-competitive NMDA receptor antagonists, PCP and MK-801. The involvement of the glycine modulatory site in mediating these effects, though likely, will remain difficult to prove until other, structurally unrelated glycine receptor antagonists, or partial agonists have been identified as having similar properties. Nevertheless, the mesolimbic selectivity of non-competitive NMDA receptor antagonists and of the antagonism by (+)-HA-966 of dopaminergic hyperlocomotion, but not stereotypy induced by amphetamine (Hutson *et al.*, 1991) lends the compound aspects of the preclinical pharmacological profile of atypical neuroleptic compounds. Given the strong resemblance of PCP intoxication in man to paranoid schizophrenia (Javitt & Zukin, 1991), the anti-psychotic potential of glycine/NMDA receptor antagonists seems worthy of investigation.

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