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Effects of and interactions between antagonists for different sites on the NMDA receptor complex on hippocampal and striatal acetylcholine efflux in vivo

Peter H. Hutson *, Joanne E. Hogg

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

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Abstract

Intraperitoneal administration of the non-competitive NMDA receptor antagonists (5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (MK-801, 0.25 and 0.5 mg/kg) and 1-(1-phenylcyclohexyl)piperidine (PCP, 5 and 10 mg/kg) increased the extracellular concentration of acetylcholine in rat hippocampus but not striatum. In contrast, *R*-(+)-3-amino-1-hydroxypyrrolid-2-one (R(+)-HA-966, 30 and 60 mg/kg), an antagonist at the glycine modulatory site of the NMDA receptor, did not affect acetylcholine efflux in either region. (±)-3-[2-Carboxypiperazin-4-yl]-propyl-1-phosphonic acid ((±)CPP, 10 mg/kg) and *cis*-4-(phosphonomethyl)piperidine-2-carboxylic acid (CGS19755, 5 mg/kg), competitive antagonists at the glutamate agonist site of the NMDA receptor, marginally increased hippocampal acetylcholine efflux. Pretreatment with R(+)-HA-966 (60 mg/kg) or (±)CPP (10 mg/kg) attenuated the increase of hippocampal acetylcholine efflux by MK-801 (0.5 mg/kg). However, prior administration of CGS19755 (5 mg/kg) prolonged the MK-801-induced increase of hippocampal acetylcholine efflux. Results demonstrate differential effects on hippocampal and striatal acetylcholine efflux of antagonists at different sites on the NMDA receptor complex and are discussed in relation to previously described effects of these drugs on mesolimbic dopamine function.

Keywords: Hippocampus; Striatum; Acetylcholine; Microdialysis; NMDA receptor; NMDA receptor antagonist

1. Introduction

Of the three ionotropic excitatory amino acid receptor subtypes, characterised by the selectivity of electrophysiological responses to the agonists kainate, AMPA/quisqualate and *N*-methyl-D-aspartate (NMDA) (Watkins and Evans, 1981), the latter is perhaps the most extensively studied. It is now generally accepted that the NMDA receptor is comprised of several modulatory sites regulated by H^+ ions (Tang and Aizenman, 1993; Traynelis and Cull-Candy, 1990), polyamines (Ransom and Stec, 1988; McGurk et al., 1990), zinc (Peters et al., 1987; Westbrook and Mayer, 1987) and redox agents (Aizenman et al., 1989; Lazarewicz et al., 1989). Three other modulatory sites have been more extensively studied due, in part, to the development of selective ligands i.e. the glutamate agonist site for which antagonists (±)-3-[2-carboxypiperazin-4-yl]-propyl-1-phosphonic acid ((±)CPP) (Murphy et al., 1987) and *cis*-4-(phosphonomethyl)piperidine-2-carboxylic acid (CGS19755) (Murphy et al., 1988) have good affinity and selectivity, the glycine co-agonist site which is blocked by *R*-(+)-3-amino-1-hydroxypyrrolid-2-one (R(+)-HA-966) (Singh et al., 1990) and 5,7-dichlorokynurenic acid (Baron et al., 1990) with good selectivity, and finally the ion channel which is blocked in a non-competitive manner by 1-(1-phenylcyclohexyl)piperidine (PCP; phencyclidine) (Kemp et al., 1987) and (5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (MK-801; dizocilpine) (Wong et al., 1986, 1988).

We have previously demonstrated that antagonists at different sites on the NMDA receptor complex differentially affect dopamine metabolism in various

* Corresponding author. Tel.: 44 279 440460; fax: 44 279 440390.

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regions of the rat brain and consequently may or may not enhance locomotor activity. Thus PCP and MK-801 enhance locomotor activity and mesocorticolimbic dopamine metabolism but not in the striatum (Bristow et al., 1993). In contrast, R(+)-HA-966 and 5,7-dichlorokynurenic acid do not affect dopamine metabolism in these brain regions, neither do they affect locomotor activity (Hutson et al., 1991; Bristow et al., 1993). A similar lack of effect on behaviour and dopamine synthesis in the striatum and nucleus accumbens was also observed following administration of (±)CPP and CGS19755 (Bristow et al., 1994). Moreover and somewhat surprisingly, we have also found that pretreatment with glycine/NMDA receptor antagonists prevents the increase of locomotor activity and mesocorticolimbic dopamine metabolism induced by either PCP or MK-801 (Bristow et al., 1993). The mechanism(s) by which this interaction occurs is as yet unknown. However, it is clear that neuronal pathways other than the mesocorticolimbic dopamine system are modulated by the action of glutamate/aspartate at the NMDA receptor complex e.g. the forebrain cholinergic system. NMDA receptors are present in reasonably high density within the rat cortex, hippocampus and to a lesser extent in the caudate nucleus (Maragos et al., 1988), regions which contain either cholinergic interneurones and/or receive cholinergic afferents from cell bodies within the diagonal band of Broca or basal nucleus of Meynert/substantia inominata. In vitro studies have demonstrated that NMDA at high micromolar concentrations evokes the release of [^{3}H]acetylcholine from striatal, cortical and septal slices but not from slices of the hippocampus (Lodge and Johnston, 1985; Ulus et al., 1992; Lehmann and Scatton, 1982). These effects of NMDA are blocked by both competitive and non-competitive NMDA receptor antagonists at concentrations which had no effects on [^{3}H]acetylcholine release per se.

In the light of our previous results which illustrated neurochemical and behavioural differences between different classes of NMDA receptor antagonists and a recent study (Hasegawa et al., 1993) which showed an increase of cortical acetylcholine efflux in vivo following the systemic administration of MK-801, we have examined the effects of and possible interactions between antagonists acting at the glutamate agonist, glycine co-agonist and ion-channel modulatory sites of the NMDA receptor complex on acetylcholine efflux in the hippocampus and striatum in vivo.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Bantin and Kingman, UK), weight range 250–350 g, were housed in groups of five, maintained on a 12 h light:dark cycle (lights on 07:00 h, off 19:00 h) and allowed standard laboratory diet and water ad libitum.

2.2. Surgery and dialysis

Rats were implanted under isofluorane anaesthesia with a concentric dialysis probe (Hutson et al., 1990) incorporating a 5 mm membrane (GFE9, Gambro) in the hippocampus (coordinates A −4.8 mm from bregma; L 5.0 mm; V −8.0 mm from dura) or striatum (A +0.2 mm from bregma; L 3.0 mm; V −7.5 mm from dura according to Paxinos and Watson, 1982). On the following day, when the animals had recovered from surgery, the probe was perfused with Ringer solution (composition in mM: NaCl 125, KCl 2.5, $MgCl_2$ 1.18, $CaCl_2$ 1.26, pH 7.5) containing neostigmine (2 μM, Sigma) at a rate of 2 μl/min.

2.3. Neurochemical analysis

Samples were collected at 20 min intervals and frozen at −70°C until required for analysis of acetylcholine by HPLC with a post-column enzyme reactor and electrochemical detection as previously described (Hutson et al., 1990). Briefly, the system used a Chromspher 5 μm C18 reverse phase column (100 × 4.6 mm) as a pre-column to saturate the mobile phase with silica, a Chromspher guard column (10 × 2.1 mm) and a Chromspher 5 μm C18 reverse phase analytical column (100 × 3 mm), pre-loaded with lauryl sulphate (5 mg/ml in H_2O). The enzyme reactor (10 × 3 mm) was loaded with 1 ml mobile phase containing 160 units acetylcholine esterase (EC 3.1.1.7 type VI-5 from electric eel, Sigma) and 80 units choline oxidase (EC 1.1.3.17 from Alcaligenes, Sigma). The mobile phase consisted of 0.4 M KH_2PO_4, adjusted to pH 8.0 with KOH. This was filtered (0.45 μm) and degassed with helium before use at a flow rate of 0.6 ml/min without recycling. Evolved hydrogen peroxide was measured using an electrochemical detector (BAS LC 4B) with a platinum electrode set at +0.5 V. In vivo values were not corrected for in vitro recovery, which under the present conditions was 15 ± 0.7% (mean ± S.E.M., n = 5).

2.4. Data analysis

Results were expressed as a percentage of the mean of six pre-injection acetylcholine values. Data were subjected to square root transformation and analysis of variance (ANOVA). Post-hoc analysis was performed by Students t-test for unpaired data. Probability levels ≤ 5% were considered statistically significant.

2.5. Drugs

MK-801 hydrogen maleate, (±)CPP and CGS19755 were obtained from RBI. PCP hydrogen chloride was obtained from Ultrafine Chemicals, Salford, UK. R(+)-HA-966 was synthesised by Merck Sharp and Dohme Research Laboratories. All drugs were dissolved in 0.9% NaCl and administered in a volume of 1 ml/kg.

3. Results

3.1. Effects of MK-801, PCP and R(+)-HA-966 on hippocampal acetylcholine efflux

Hippocampal acetylcholine efflux under basal conditions was 0.95 ± 0.04 pmol/20 μl (mean ± S.E.M., n = 81) and remained relatively stable over the period of the experiment. Administration of vehicle (0.9% NaCl, 1 ml/kg i.p.) had no significant effect on the extracellular acetylcholine concentration when compared with pre-injection values (Fig. 1). In contrast, MK-801 (0.25 and 0.5 mg/kg i.p.) increased hippocampal acetylcholine efflux to a maximum of 179 ± 19% (n = 6) and 368 ± 34% (n = 6) of basal efflux respectively. This increase was maximal 80 min after injection and values had returned to basal levels 180 min after injection at the lower dose (0.25 mg/kg i.p.), but not the higher dose (0.5 mg/kg i.p.) (Fig. 1A). Similarly, PCP (5 and 10 mg/kg i.p.) also markedly increased hippocampal acetylcholine efflux. At the lower dose (5 mg/kg i.p.), the increase was maximal (314 ± 51% of basal efflux, n = 5) 40 min after injection with values returning to basal levels by 120 min (Fig. 1B). A more prolonged increase was observed at the higher dose (10 mg/kg i.p.) which was also maximal (450 ± 29% of basal efflux, n = 4) 40 min after injection but had not returned to basal values by 180 min (Fig. 1B). In contrast to the marked increase of hippocampal acetylcholine efflux produced by the ion-channel blockers MK-801 and PCP, the NMDA/glycine site antagonist R(+)-HA-966 did not significantly affect hippocampal acetylcholine efflux when compared with vehicle-treated animals (Fig. 1C). Interestingly, and in contrast to the effects of systemic injection, local administration of MK-801 (100 μM, 10 μM) via the dialysis probe directly into the hippocampus did not increase acetylcholine efflux when compared with pre-infusion values (Fig. 2).

3.2. Effects of MK-801, PCP and R(+)-HA-966 on striatal acetylcholine efflux

Basal striatal acetylcholine efflux increased slowly over the first 2 h from initial values of 2.07 ± 0.25 pmol/20 μl to 3.64 ± 0.37 pmol/20 μl at time = 0 (mean ± S.E.M., n = 17). The injection of vehicle (0.9% NaCl 1 ml/kg i.p.) did not significantly affect striatal acetylcholine efflux compared with pre-injection values (Fig. 3). In contrast to their effects on hippocampal acetylcholine efflux, MK-801 (0.5 mg/kg i.p.) slightly

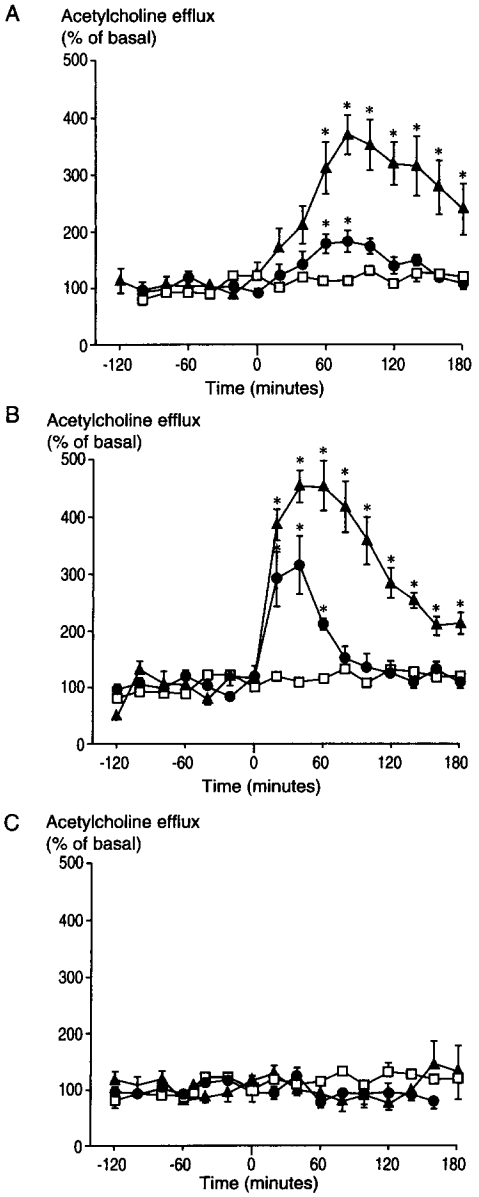

Fig. 1. The effects of NMDA receptor antagonists on hippocampal acetylcholine efflux in vivo. Values are means ± S.E.M. as a percentage of pre-injection basal acetylcholine efflux. The effects of vehicle (0.9% NaCl, 1 ml/kg i.p., □, basal 1.01 ± 0.18 pmol/20 μl, n = 6); (A) MK-801 (0.25 mg/kg i.p., ●, basal 0.75 ± 0.11 pmol/20 μl, n = 6; 0.5 mg/kg i.p., ▲, basal 1.01 ± 0.07 pmol/20 μl, n = 6); (B) PCP (5 mg/kg i.p., ●, basal 1.04 ± 0.15 pmol/20 μl, n = 5; 10 mg/kg i.p., ▲, basal 1.04 ± 0.09 pmol/20 μl, n = 4) or (C) R(+)-HA-966 (30 mg/kg i.p., ●, basal 0.88 ± 0.16 pmol/20 μl, n = 5; 60 mg/kg i.p., ▲, basal 0.55 ± 0.06 pmol/20 μl, n = 6). $^*P < 0.05$ compared with the same time point in vehicle-treated rats using Student's t-test following ANOVA.

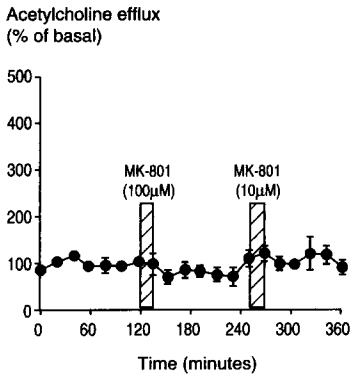

Fig. 2. The effect of MK-801 administered via the dialysis probe on hippocampal acetylcholine efflux in vivo. MK-801 was included in the Ringer solution for two 20 min periods (hatched bars) at concentrations of 100 μM and 10 μM respectively, separated by a 2 h period of perfusion with Ringer solution containing neostigmine, as described in Methods. Values are means ± S.E.M. as a percentage of pre-injection basal acetylcholine efflux (1.27 ± 0.24 pmol/20 μl, n = 4).

decreased and PCP (10 mg/kg i.p.) had no significant effect on striatal acetylcholine efflux when compared with saline-injected rats (Fig. 3). A similar lack of effect on striatal acetylcholine efflux was also observed following R(+)-HA-966 (30 mg/kg i.p.) (Fig. 3).

3.3. Effect of R(+)-HA-966 on the increase of hippocampal acetylcholine efflux by MK-801

Pretreatment of rats with R(+)-HA-966 (60 mg/kg i.p.) 20 min before administration of MK-801 (0.5 mg/kg i.p.) attenuated the increase of hippocampal

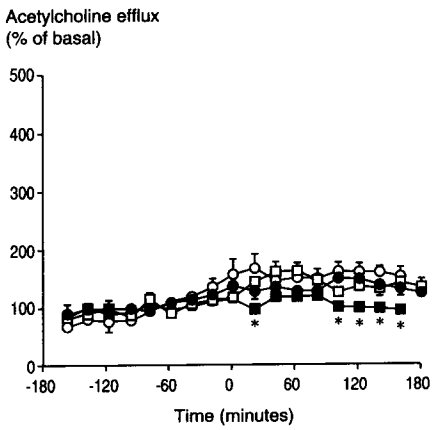

Fig. 3. Effect of NMDA receptor antagonists on striatal acetylcholine efflux in vivo. Values are means ± S.E.M. as a percentage of pre-injection acetylcholine efflux. The effects of vehicle (as described in legend to Fig. 1) (1 ml/kg i.p., ○, basal 2.35 ± 0.35 pmol/20 μl, n = 11); MK-801 (0.5 mg/kg i.p., ■, basal 2.79 ± 0.11 pmol/20 μl, n = 4); PCP (10 mg/kg i.p., □, basal 1.95 ± 0.61 pmol/20 μl, n = 4) or R(+)-HA-966 (30 mg/kg i.p., ●, basal 2.69 ± 0.55 pmol/20 μl, n = 4).

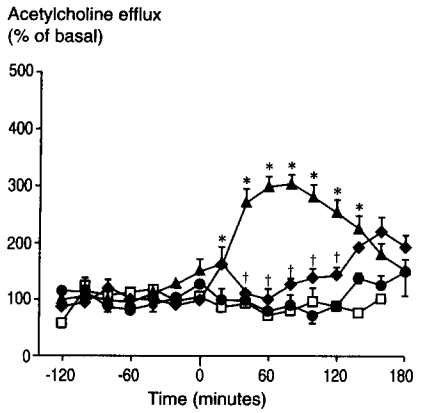

Fig. 4. Effect of R(+)-HA-966 on the increase of hippocampal acetylcholine efflux by MK-801. Values are means ± S.E.M. as a percentage of pre-injection acetylcholine efflux. Rats were pretreated with either vehicle (as described in the legend to Fig. 1) (1 ml/kg i.p., ▲, basal 0.67 ± 0.03 pmol/20 μl, n = 4) or R(+)-HA-966 (60 mg/kg i.p., ◆, basal 1.10 ± 0.17 pmol/20 μl, n = 6) 20 min before MK-801 (0.5 mg/kg i.p.) at time zero. Rats pretreated with R(+)-HA-966 (60 mg/kg i.p., ●, basal 0.55 ± 0.06 pmol/20 μl, n = 6) or 0.9% NaCl (1 ml/kg i.p., □, basal 1.18 ± 0.17 pmol/20 μl, n = 6), 20 min before 0.9% NaCl (1 ml/kg i.p.) at time zero. $^{*}P < 0.05$, $^{\dagger}P < 0.05$ compared with the same time point in saline-pretreated rats given saline and in saline-pretreated rats given MK-801 respectively using Student's t-test following two way ANOVA.

acetylcholine efflux previously observed with this dose of MK-801. The blockade by R(+)-HA-966 was most prominent over the first 2 h, after which hippocampal acetylcholine efflux increased to approximately similar values to those observed in MK-801-treated rats (Fig. 4).

3.4. Effects of CGS19755 and (±)CPP on basal and MK-801-enhanced hippocampal acetylcholine efflux

Administration of the competitive glutamate receptor antagonist CGS19755 (5 mg/kg i.p.) 20 min before vehicle injection (0.9% NaCl, 1 ml/kg i.p.) caused a small increase (approximately 50% above basal values) of hippocampal efflux, which at some time points achieved statistical significance when compared with vehicle-pretreated animals. Pretreatment of rats with CGS19755 (5 mg/kg i.p.) 20 min before MK-801 (0.5 mg/kg i.p.) did not however attenuate the increase of acetylcholine efflux but in fact prolonged the effects of MK-801. Thus, rats given the combination of CGS19755 and MK-801 had significantly higher acetylcholine efflux levels at 180 min than rats given saline and MK-801 (Fig. 5).

Pretreatment of rats with (±)CPP (10 mg/kg i.p.) also increased hippocampal acetylcholine efflux by approximately 50% of basal values and, as with

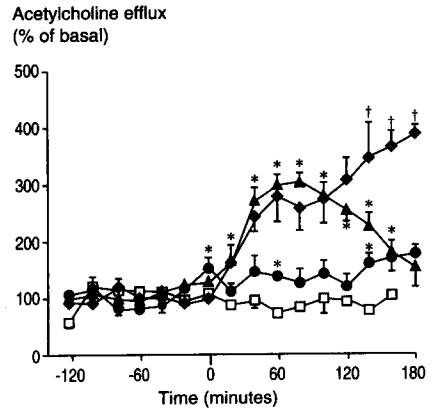

Fig. 5. Effect of CGS19755 on the increase of hippocampal acetylcholine efflux by MK-801. Values are means ± S.E.M., as a percentage of pre-injection acetylcholine efflux. Rats were pretreated with either vehicle (as described in legend to Fig. 1) (1 ml/kg i.p., ▲, basal 0.67 ± 0.03 pmol/20 μl, $n = 4$) or CGS19755 (5 mg/kg i.p., ◆, basal 0.59 ± 0.12 pmol/20 μl, $n = 4$), 20 min before MK-801 (0.5 mg/kg i.p.). Rats pretreated with CGS19755 (5 mg/kg i.p., ●, basal 1.09 ± 0.21 pmol/20 μl, $n = 6$) or 0.9% NaCl (1 ml/kg i.p., □, basal 1.18 ± 0.17 pmol/20 μl, $n = 6$) 20 min before 0.9% NaCl (1 ml/kg i.p.) at time zero. $^{*}P < 0.05$, $^{\dagger}P < 0.05$ compared with the same time point in saline-pretreated rats given saline and in saline-pretreated rats given MK-801 respectively using Student's t-test following two way ANOVA.

CGS19755, some individual time points were significantly greater than vehicle controls. This small increase appeared to be sustained for 180 min. In contrast to the potentiating effects of CGS19755 on MK-801-enhanced acetylcholine efflux, 20 min pretreatment with (±)CPP (10 mg/kg i.p.) significantly attenuated the increase of acetylcholine efflux by MK-801 (0.5 mg/kg i.p.) (Fig. 6).

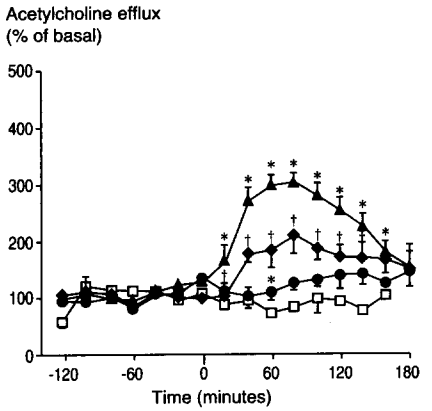

Fig. 6. Effect of (±)CPP on the increase of hippocampal acetylcholine efflux by MK-801. Values are means ± S.E.M. as a percentage of pre-injection acetylcholine efflux. Rats were pretreated with either vehicle (0.9% NaCl 1 ml/kg i.p., ▲, basal 0.67 ± 0.03 pmol/20 μl, $n = 4$) or (±)CPP (10 mg/kg i.p., ◆, basal 0.97 ± 0.14 pmol/20 μl, $n = 6$) 20 min before MK-801 (0.5 mg/kg i.p.). Rats pretreated with (±)CPP (10 mg/kg i.p., ●, basal 0.97 ± 0.17 pmol/20 μl, $n = 7$) or 0.9% NaCl (1 ml/kg i.p., □, basal 1.18 ± 0.17 pmol/20 μl, $n = 6$), 20 min before 0.9% NaCl (1 ml/kg i.p.) at time zero. $^{*}P < 0.05$, $^{\dagger}P < 0.05$ compared with the same time point in saline-pretreated rats given saline and in saline-pretreated rats given MK-801 respectively using Student's t-test following two way ANOVA.

4. Discussion

Results in the present study demonstrate marked differences in the ability of antagonists at different modulatory sites of the NMDA receptor complex to enhance hippocampal acetylcholine efflux. Thus, compared with vehicle, systemic administration of the non-competitive NMDA receptor antagonists MK-801 and PCP markedly increased hippocampal acetylcholine efflux. These findings confirm and extend results in a previous study (Hasegawa et al., 1993), which showed that MK-801 over a similar dose range increased cortical acetylcholine efflux to a similar magnitude and with a comparable time course. In contrast to the effects observed with non-competitive NMDA receptor antagonists, administration of the low efficacy partial agonist at the glycine site R(+)-HA-966 or the competitive glutamate antagonists (±)CPP and CGS-19755 caused a small (approximately 50%) increase which was maintained for 3 h without returning to control values. Results in the present study also demonstrate the lack of effect of PCP and R(+)-HA-966, and a tendency for MK-801 to decrease striatal acetylcholine efflux at doses (of MK-801 and PCP) which caused marked increases of hippocampal acetylcholine release. It is unlikely that a lack of effect of NMDA receptor antagonists in the striatum is due to the slowly increasing baseline which we (Hutson et al., 1990), but not others (Damsma et al., 1988), have previously observed as scopolamine, a muscarinic receptor antagonist, increased striatal acetylcholine efflux under similar conditions to those in the present study (Hutson et al., 1990). The inability of MK-801 and PCP to increase striatal acetylcholine efflux also suggests that the increase observed with these compounds in the hippocampus is unlikely to be due to an interaction with presynaptic muscarinic or nicotinic autoreceptors on cholinergic neurones.

The excitatory effects of MK-801 and PCP contrast markedly with the notable lack of intrinsic activity of these compounds on [^{3}H]acetylcholine release in vitro. Thus, NMDA or glutamate have been shown to enhance [^{3}H]acetylcholine release from slices of cortex (Ulus et al., 1992; Lodge and Johnston, 1985), striatum (Ulus et al., 1992; Lehmann and Scatton, 1982) and nucleus accumbens (Jones et al., 1987) and in each case, NMDA antagonists including MK-801 and/or PCP are able to block these effects without affecting the release of [^{3}H]acetylcholine per se. One exception

to this is the lack of effect of NMDA or glutamate on [^{3}H]acetylcholine release on hippocampal slices in vitro where, possibly because of the absence of cholinergic interneurones, it is thought that the presence of NMDA receptors on cholinergic cell bodies/dendrites is responsible for regulating [^{3}H]acetylcholine release (Lehmann and Scatton, 1982).

The anatomical site at which non-competitive NMDA receptor antagonists act to enhance hippocampal acetylcholine efflux is unknown at present. Radioligand binding (Maragos et al., 1988; McDonald et al., 1990), electrophysiological (Lamour et al., 1984), neurotoxic lesioning (Malthe-Sorenssen et al., 1980) and in vitro neurotransmitter release (Nishimura and Boegman, 1990) studies indicate the presence of NMDA receptors in the hippocampus and septal nuclei. However, in contrast to its effects on hippocampal acetylcholine efflux when given systemically, infusion of MK-801 via the probe directly into the hippocampus at either 10 or 100 μM did not affect hippocampal acetylcholine efflux. This suggests either that the concentration of MK-801 used was not high enough to block local NMDA receptors or that the increase of hippocampal acetylcholine efflux by systemically administered MK-801 is not mediated within the hippocampus. Given the high affinity (IC_{50} ca. 30 nM) of MK-801 for the NMDA receptor ion channel (Wong et al., 1986, 1988) and an estimated recovery of approximately 10% for small molecular weight compounds through the probe at this flow rate, the local tissue concentration of MK-801 (molecular weight 337.4) should be in the region of 1–10 μM. It seems unlikely therefore that a concentration of MK-801 inadequate to block the NMDA receptor was achieved and the lack of effect is more likely that its site of action is somewhere other than in the hippocampus. This result would also exclude an action of MK-801 at presynaptic muscarinic M_2 or nicotinic receptors which are located on cholinergic terminals and are known to regulate acetylcholine release.

It is conceivable that changes of hippocampal acetylcholine efflux following MK-801 and PCP is a secondary consequence of activating mesocorticolimbic dopamine pathways originating in the A10 ventral tegmental area (VTA). Excitatory amino acid receptors within the VTA have been demonstrated electrophysiologically (Seutin et al., 1990) and Kalivas et al. (1989) have shown that infusion of glutamate into the VTA enhances dopamine release in the nucleus accumbens and increases locomotor activity. Dopamine-containing cells within the VTA project to several forebrain regions including the amygdala, olfactory tubercles, nucleus accumbens and lateral septum (Simon et al., 1979; Lindvall, 1975). Dopaminergic afferents to the lateral septum make contact with, amongst others, a population of GABA-containing neurones (Onteniente et al., 1987) which terminate on cholinergic cell bodies in the medial septum/diagonal band of Broca which in turn provide the principle cholinergic innervation of the hippocampus.

The interaction between mesolimbic dopamine neurones and the septohippocampal cholinergic system is not clear and there is neurochemical evidence suggesting both facilitatory (Imperato et al., 1993) and inhibitory (Robinson et al., 1979) roles for dopamine in hippocampal cholinergic transmission. However, there is an interesting parallel between the effects of NMDA receptor antagonists on hippocampal acetylcholine efflux and the known effects of these compounds on brain dopamine metabolism. Thus, previous studies demonstrated the ability of MK-801 and PCP, but not R(+)-HA-966 or (±)CPP and CGS19755, to increase mesocorticolimbic dopamine metabolism and consequent locomotor activity (Hutson et al., 1991; Bristow et al., 1993, 1994). Similarly, the lack of effect of MK-801 and PCP on striatal acetylcholine efflux at doses which enhanced hippocampal acetylcholine efflux is also consistent with previous studies (Hutson et al., 1991; Bristow et al., 1993) which showed that striatal dopamine metabolism was unaffected by doses of MK-801 and PCP which significantly increased mesocorticolimbic dopamine metabolism. The lack of effect of MK-801 and PCP on striatal dopamine metabolism and acetylcholine efflux further supports the suggestion that the increase of hippocampal acetylcholine efflux is due to the activation of mesolimbic and not nigrostriatal dopamine neurones by MK-801 and PCP. Consistent with this suggestion, recent studies (Barton and Hutson, 1995) showed that the increase of hippocampal acetylcholine efflux evoked by MK-801 was blocked by pretreatment with SCH23390, a dopamine D_2 receptor antagonist.

The interactions between MK-801 and compounds acting at the NMDA/glycine or glutamate modulatory sites are also of interest. The present data show that R(+)-HA-966 (at a dose which does not affect hippocampal acetylcholine efflux per se) was able to attenuate the increase of hippocampal acetylcholine efflux by MK-801. A similar interaction was also shown between MK-801 and R(+)-HA-966 with regard to mesocorticolimbic dopamine metabolism and associated hyperlocomotion (Bristow et al., 1993), again reinforcing the possibility that hippocampal acetylcholine is influenced as a secondary consequence of manipulating VTA dopamine neurones. The interaction between MK-801 and competitive glutamate antagonists (±)CPP and CGS19755 seems to be more complicated. Thus, in the present study, pretreatment with (±)CPP attenuated the effects of MK-801 on hippocampal acetylcholine release. However, pretreatment with CGS19755 had no effect on the magnitude of the MK-801-induced increase of hippocampal acetyl-

choline efflux and, in contrast, prolonged the maximal response. In each case, these effects were observed with a dose of (±)CPP and CGS19755 which only marginally affected hippocampal acetylcholine efflux. However, as Giovannini et al. (1994) have demonstrated that intraventricular administration of (±)CPP (1–50 nmol) markedly increased hippocampal acetylcholine outflow, it is conceivable that these compounds do not penetrate the blood-brain barrier sufficiently well to achieve a high enough concentration to affect acetylcholine outflow when given systemically. Nevertheless, previous studies have demonstrated that systemic pretreatment (at comparable doses to those used in the present study) with (±)CPP and CGS19755 blocks activation of brain dopamine synthesis and locomotor activity induced by amphetamine (Bristow et al., 1994), indicating that these compounds are able to interact with dopamine neurones in a similar manner to glycine site antagonists. However, we have not examined their ability to modulate the effects of MK-801 or PCP on dopamine metabolism. Therefore, in this case we cannot draw a parallel to the effects of these drugs on dopamine metabolism. It is difficult to see why two competitive glutamate antagonists should not interact with MK-801 in a similar manner, i.e. either both attenuating the effects of MK-801 on acetylcholine efflux or both having no effect. One possibility for this apparent discrepancy may be pharmacokinetic or metabolism differences between (±)CPP and CGS-19755, although as these compounds were able to block the effects of amphetamine (Bristow et al., 1994) at similar doses and pretreatment times, this does not seem likely. It is also conceivable that either or both of these drugs differentially affect the disposition of MK-801 in brain, thereby prolonging or diminishing its neurochemical effects.

In conclusion, the present results demonstrate that NMDA receptor antagonists which act at different modulatory sites on the NMDA receptor complex do not affect striatal acetylcholine efflux and differentially influence hippocampal efflux, possibly as a consequence of modulating mesolimbic dopamine pathways. It is clear from these and other studies (Hutson et al., 1991; Bristow et al., 1993, 1994) that different classes of NMDA receptor antagonists have markedly different neurochemical and behavioural profiles.

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