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A comparison between the *in vivo* and *in vitro* activity of five potent and competitive NMDA antagonists

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1 Phosphonate analogues of glutamate have been tested and compared as N-methyl-D-aspartate (NMDA) antagonists in electrophysiological and binding experiments. The compounds tested were three established NMDA antagonists: D-2-amino-5-phosphonopentanoate (D-AP5), DL-2-amino-7-phosphonoheptanoate (DL-AP7), 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonate (CPP), and two novel putative NMDA antagonists: 3-(2-carboxypiperidin-4-yl)propyl-1-phosphonate (CPPP) and 3-(2-carboxy-piperidin-4-yl)methyl-1-phosphonate (CPMP).

2 When administered electrophoretically to rat spinal neurones *in vivo*, these compounds were found to be selective NMDA antagonists with little effect on excitations evoked by quisqualate and kainate. CPMP and CPPP were approximately equipotent with CPP and about 5 times more potent than D-AP5.

3 Following systemic administration, 2–5 mg kg^{-1} i.v. of CPP, CPMP and CPPP reduced NMDA-evoked excitations by 70–100% whereas 50–100 mg kg^{-1} of D-AP5 and DL-AP7 produced a similar effect. The onset of the effects required 20–30 min and lasted more than six hours.

4 On bath application to cortical wedges, the IC_{50} values (μM) for antagonism of 40 μM NMDA were: CPP, 0.64 ± 0.06 (mean ± s.e.mean; $n > 4$); CPMP, 1.65 ± 0.13; CPPP 0.89 ± 0.09; D-AP5, 3.7 ± 0.32; DL-AP7, 11.1 ± 2.1; and DL-AP4 and DL-AP6 were inactive at 100 μM.

5 In binding studies with [^{3}H]-CPP, the K_i values (nM) were: CPP, 446 ± 150 (mean ± s.e.mean; $n \geq 3$); CPMP, 183 ± 74 and CPPP, 179 ± 13 whereas against NMDA (10 μM)-stimulated [^{3}H]-TCP (thienylcyclohexylpiperidine) binding the IC_{50} values (μM) for CPMP and CPPP respectively were 5.6 ± 2.7 and 4.5 ± 2.2.

6 Systemic administration of CPPP and CPMP, at doses sufficient to antagonize NMDA, also reduced cardiovascular responses to 5-hydroxytryptamine (Bezold-Jarisch reflex). This illustrates a role for NMDA receptors in central cardiovascular control.

7 The results indicate the systemic doses of piperidine and piperazine analogues of D-AP5 which may be used for assessing the role of NMDA receptors in central synaptic function.

Introduction

Since the division of postsynaptic glutamate receptors into three major subtypes (McLennan & Lodge, 1979; Davies & Watkins, 1979), there has been a continual development of more selective and potent N-methyl-D-aspartate (NMDA) antagonists. Dicarboxylic antagonists, e.g. aminoadipic, suberic and dipimelic acids (Biscoe *et al.*, 1977; Hicks *et al.*, 1978; Watkins & Evans, 1981) have been superseded by D-2-amino-5-phosphonopentanoate (AP5) and 2-amino-7-phosphonoheptanoate (AP7) (Evans *et al.*, 1982). Although these phosphonate compounds are more potent than aminoadipate *in vitro* and after direct injection into the brain, they are still relatively inactive after systemic administration. More recently a carboxypiperazine (Figure 1; Davies *et al.*, 1986; Lehmann *et al.*, 1987b; Childs *et al.*, 1988) analogue of AP7 has been shown to be more potent than the parent compound in neurochemical, neurophysiological and behavioural studies, some of which suggest that they do cross the blood brain barrier following systemic administration. These *in vivo* tests

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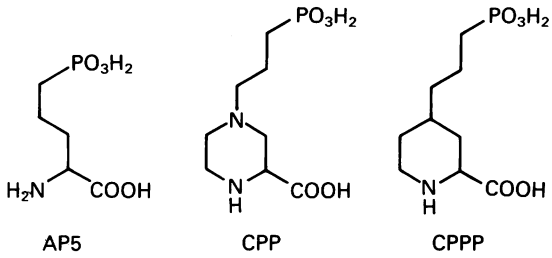

Figure 1 Structures of 2-amino-5-phosphonopentanoate (AP5), 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonate (CPP) and 3-(2-carboxypiperidin-4-yl)propyl-1-phosphonate (CPPP). 3-(2-carboxypiperadin-4-yl)methyl-1-phosphonate (CPMP) differs from CPPP by the phosphonate side chain having two less carbon atoms.

were not, however, direct measures of the ability of the drugs to antagonize NMDA. Carboxypiperidine analogues (Figure 1) are similarly putative NMDA antagonists (Lehmann *et al.*, 1987a).

In view of the therapeutic potential of systemically active NMDA antagonists (Patel *et al.*, 1988; Rothman & Olney, 1987), it seems important to assess directly their potency as antagonists of the excitatory action of NMDA *in vivo* following systemic administration, so that this can be correlated with potency in animal models of neurological disease.

We have, therefore, compared the potency of established and novel antagonists of the excitatory action of NMDA of central neurones *in vivo*, following systemic and electrophoretic administration, with their potency in binding studies and on cortical slices *in vitro*.

Because of the recent evidence supporting a role for excitatory amino acids in central pathways mediating cardiovascular reflexes (e.g. Guyenet *et al.*, 1987), we have examined the two new carboxypiperidine derivatives for their ability to reduce the Bezold-Jarisch reflex. It has been previously shown that this reflex is reduced by the non-competitive NMDA antagonist, MK-801 (Verberne *et al.*, 1987).

Methods

Spinal and brainstem neurones in vivo

The methods employed for microelectrophoretic studies on these neurones have been described previously (Anis *et al.*, 1983). In brief, rats were anaesthetized with pentobarbitone (40 mg kg^{-1} i.p. initially and supplemented i.v.) or with halothane in O_2/N_2O. After exposure of the spinal cord by lumbar laminectomy and resection of the dura mater or of the brain stem via the foramen magnum, the centre barrel (3.6 M NaCl) of seven barrel glass micropipettes (5–10 μm tip diameter) was used to record extracellular action potentials from single neurones. For ejection by electrophoresis, the outer barrels contained 5 of the following solutions (all approximately pH 8): N-methyl-DL-aspartate Na (NMDA; 200 mM), kainate Na (5 mM in 200 mM NaCl), quisqualate Na (5 mM in 200 mM NaCl), D-2-amino-5-phosphonopentanoate Na (D-AP5; 25 mM in 175 mM NaCl), 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonate Na (CPP; 25 mM in 175 mM NaCl or 5 mM in 200 mM NaCl), 3-(2-carboxypiperidin-4-yl) propyl-1-phosphonate Na (CPPP; 5 mM in 200 mM NaCl) and 3-(2-carboxypiperidin-4-yl)methyl-1-phosphonate Na (CPMP; 25 mM in 175 mM NaCl or 5 mM in 200 mM NaCl). The sixth barrel (200 mM NaCl) was used for current balancing. The firing rate of each neurone in response to cyclical ejection of the agonists was displayed continuously on a chart recorder. After establishing agonist control responses, one of the antagonists was ejected until amino acid responses had reached a new steady state. After recovery was observed, a second and on some cells a third antagonist were tested. Where possible it was the intention to compare the currents of drug that gave approximately 80% reduction in response to NMDA.

In a similar way, antagonists were also injected i.v. to compare systemic doses required to produce reduction of the response to local ejection of the amino acids.

Cortical wedges in vitro

Cortical wedges, cut from 500 μm thick slices of rat cingulate cortex, were placed in a two compartment chamber and perfused (2 ml min^{-1}) at room temperature (23°C) with magnesium-free artificial CSF (Harrison & Simmonds, 1984) containing tetrodotoxin (0.1 μM) to prevent spontaneous synaptic activity. Depolarization of neurones in response to bath application of amino acids was recorded as a d.c. potential change across a grease seal barrier placed near the junction between grey and white matter. Such potentials were amplified and displayed continuously on a pen recorder. Amino acids and other drugs were added to the superfusate. Dose-response relationships and IC_{50} values were calculated in order to estimate the potency of the antagonists.

Bezold-Jarisch reflex

These methods have been described in detail previously (Verberne *et al.*, 1987). Briefly, rats were

anaesthetized (urethane, 1.5 g kg^{-1}, i.p.), tracheotomised and allowed to breathe Carbogen (95% O_2 and 5% CO_2) – enriched room air. The right femoral artery was cannulated and connected to a pressure transducer coupled to and continuously displayed on a polygraph. Heart rate (HR) was derived from arterial blood pressure (BP) signal using a tachometer. The Bezold-Jarisch reflex was elicited by bolus i.v. injection of 5-hydroxytryptamine (5-HT) (Salmoiraghi *et al.*, 1956). Dose-dependent heart rate (HR) and diastolic arterial blood pressure (DABP) reductions were obtained to 5-hydroxytryptamine (5-HT; 0.5–8.0 μg kg^{-1}, i.v.). These responses were repeated 15 min after administration of CPMP (2.0 or 5.0 mg kg^{-1}, i.v.; both $n = 4$ rats) or CPPP (5.0 mg kg^{-1}, i.v. $n = 5$) or saline (0.9% w/v, $n = 13$). Data are presented as means ± group standard error of changes from resting HR and DABP and were subjected to an analysis of variance with repeated measures (Snedecor & Cochran, 1972). Subsequent orthogonal partitioning of the sums of squares demonstrated that the $\log_{10}$ [5-HT] dose-response curves were best described by linear partitioning. The significance of differences between slopes were determined by calculation of the t statistic with the Boneferoni adjustment for multiple comparisons. The group standard errors were determined from the square root of EMS/n, where EMS is the error mean square from the analysis of variance and n is the number of animals in the group.

Binding studies

[^{3}H]-*CPP binding* [^{3}H]-CPP is highly selective for the NMDA receptor (Olverman *et al.*, 1987; Murphy *et al.*, 1987). Cerebral cortical membranes for [^{3}H]-CPP binding assays were prepared from male Sprague-Dawley rats (200–250 g) employing procedures similar to those previously described (Olverman *et al.*, 1984; 1987). The final pellet was gently re-suspended in 50 mM Tris-HCl buffer pH 7.7, and then homogenized using a polytron homogenizer (setting 3, 3 s).

[^{3}H]-CPP binding was assayed in a final volume of 500 μl by incubating 400 μl of membrane suspension (350–450 μg protein), 50 μl [^{3}H]-CPP (final conc. 10 nM) and 50 μl of drug (33.3 μM–3.33 mM) for 30 min at 25°C. Incubations were terminated by centrifugation and after aspiration of the supernatant, the pellet was gently rinsed with 1 ml of ice-cold buffer. Non-specific binding was defined with 500 μM L-glutamate.

[^{3}H]-*TCP binding* Cerebral cortical membrane homogenates for [^{3}H]-N-(1-[2-thienyl]cyclo-hexyl)-piperidine ([^{3}H]-TCP) binding assays were prepared from male Sprague-Dawley rats (200–250 g) by a method similar to that described by Foster & Wong (1987), except that the crude membrane pellet was used and ice-cold distilled water was employed for the multiple ($n = 7$) cycles of washing. The final pellet was taken up in 5 mM Tris-HCl pH 8.0 buffer.

[^{3}H]-TCP binding was performed as described by Largent *et al.* (1986), except that the well-washed cortical membrane preparation (80–100 μg protein) described above was employed and 10 μM NMDA was included in the assay. In preliminary experiments, this concentration of NMDA was shown to produce a sub-optimal stimulation of [^{3}H]-TCP binding (c.f. Foster & Wong, 1987). The concentration of [^{3}H]-TCP was 5 nM and non-specific binding was defined with 5 μM MK-801.

Data analysis Binding data from individual displacement studies were analysed by iterative curve fitting, which provided an estimate of IC_{50} (concentration of drug inhibiting 50% of specific binding) (McPherson, 1983). More detailed analyses were performed using the iterative, non-linear curve fitting programme 'LIGAND' (Munson & Rodbard, 1980), where displacement data were analysed according to a model for the binding of the competing drug to a single site. Final estimates of inhibition constants (K_i) were obtained by analysis of pooled data files.

Sources of drugs and chemicals CPMP (CGS19755) and CPPP were synthesized by P.O. at Eli Lilly Laboratories, Indianapolis. [^{3}H]-CPP (20 Ci mmol^{-1}) was supplied by Tocris Neuramin (Buckhurst Hill, U.K.) and [^{3}H]-TCP (47 Ci mmol^{-1}) by Du Pont, NEN (Boston, U.S.A.). MK-801 ((+)-5-methyl-10,11-dihydro-5H-dibenzo-[a,d]cyclohepten-5,10-imine) was a gift from Dr G.N. Woodruff, MSD Neuroscience Research Centre, Harlow. Other compounds were obtained from Sigma and Tocris Neuramin. Structures of some of the putative NMDA antagonists are shown in Figure 1.

Results

Effects on spinal neurones in vivo

Local ejection of antagonists The results are summarized in Table 1. Following electrophoretic ejection, all the antagonists rapidly reduced excitations induced by NMDA, whereas responses to quisqualate and kainate were not significantly affected. The antagonists did not show any direct excitatory or inhibitory effects on the few spontaneously active neurones and their effects were rapidly reversed on stopping their ejection. In terms of the mean ejecting currents required to reduce the responses to NMDA

Table 1 Potency of some phosphonate analogues as antagonists of N-methyl-D-aspartate (NMDA) on cerebral cortical neurones

Compound	IC_{50} *vs 40 μM NMDA cortical wedges*	K_i *vs* [^3H]-*CCP*	IC_{50} *vs NMDA stimulated* [^3H]-*TCP binding*
D-AP5	3.70 ± 0.32	370	
DL-AP7	11.1 ± 2.1		
CPP	0.64 ± 0.06	446 ± 150	
CPMP	1.65 ± 0.13	183 ± 74	5.6 ± 2.7
CPPP	0.89 ± 0.09	179 ± 13	4.5 ± 2.2

For details of individual assays see text. DL-AP4 and DL-AP6 were ineffective as NMDA antagonists on the cortical wedge preparation at 100 and 31.6 μM respectively. Values are means ± s.e.mean.
For details of abbreviations used see text.

by approximately 80%, CPPP, CPP and CPMP were about equipotent, and 3–4 times more potent than D-AP5. Thus on 11 brain stem neurones, CPPP (range 5–20 nA; mean ± s.e.mean, 13 ± 5), CPMP (range 5–30 nA; mean 15 ± 7) and CPP (range 10–30 nA; mean 17 ± 7) reduced NMDA actions by 75 ± 24, 80 ± 26 and 72 ± 23, respectively. On 6 spinal neurones on which they reduced NMDA responses to the same extent, the mean ejecting current of D-AP5 was 5 times greater than that of CPPP. Similar relative potencies were observed from individual comparisons of up to 3 antagonists on the same cell, although in general CPMP was usually the most potent. An example of a comparison between CPP and CPMP is presented in Figure 2, from which it can be seen that 10 nA of CPP was almost equieffective with 5 nA of CPMP. In this example, responses to NMDA had returned to control values within 4 min of stopping antagonist ejection, which is typical of recovery times from local ejection of these compounds.

Intravenous injection of antagonists Following a single i.v. injection, the excitatory responses to

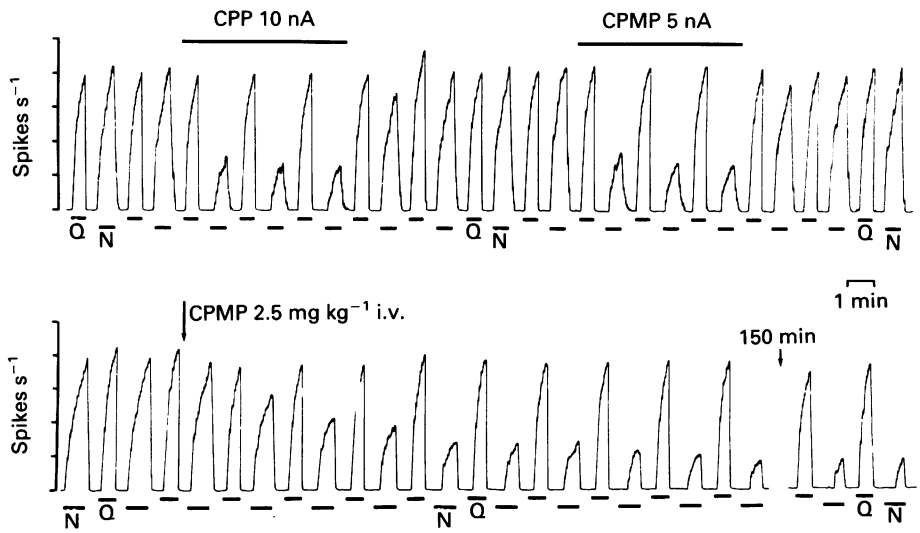

Figure 2 Effect of CPP and CPMP on excitation of a dorsal horn neurone by excitatory amino acids. Approximately equal and submaximal increases in extracellularly recorded firing rate were achieved by the cyclical electrophoretic ejection of N-methyl-DL-aspartate (NMDLA) 90 nA (N) and quisqualate 30 nA (Q). CPP 10 nA and CPMP 5 nA ejected from other barrels selectively antagonized NMDLA to approximately the same extent. Following recovery from these local ejections, 2.5 mg kg^{-1} of CPMP was injected intravenously and during the course of the next 30 min the response to NMDLA slowly declined and no recovery was seen despite stable recordings for more than 3 h. Ordinate scales: firing rate in spikes s^{-1}. For abbreviations used see Figure 1 legend.

NMDA declined slowly, usually 20–30 min being required to produce maximal antagonism, at which time responses to other agonists remained close to control values. The duration of this selective NMDA antagonism was also very long with all the compounds. The maximum recovery being only 25% return toward control. This was despite 6 h of stable recording conditions, as judged by action potential amplitude and by responses to quisqualate and kainate. Because of this slow reversibility, it was not possible to test more than one antagonist on one neurone per animal. With this limitation it appears that CPPP, CPMP and CPP are approximately equieffective as NMDA antagonists by this systemic route. Between 2 and 5 mg kg^{-1} i.v. of any of these three compounds (minimum of 3 tests for each compound) almost abolished the 60–80 Hz increase in firing rate seen in the control response to NMDA (see Figure 2). D-AP5 was at least 10 times less potent than these compounds and presumably similar to D-AP7, which on the one cell tested, required 80 mg kg^{-1} of DL-AP7 to produce an 80% reduction in the NMDA response.

Effects on cortical neurones in vitro

All antagonists tested produced a selective reduction of the NMDA-induced depolarization in cortical wedges with little or no effect on responses to quisqualate or kainate (Figure 3). For any given dose maximum effects occurred within 15–20 min of starting perfusion with the agonist and full recovery was usually achieved within 30 min of washing out. Where tested the dose-response curve for NMDA was shifted to the right in a parallel fashion with no apparent change in the maximum response. The IC_{50} values against depolarizations produced by 40 μM NMDA are given in Table 1. From these studies CPPP and CPP were the most potent compounds tested being about 10 times more potent than D-AP5. In a previous study (see Martin, 1987), the D isomer of AP5 was about twice as potent as the racemic mixture and this in turn was about twice as potent as the racemic AP7.

Bezold-Jarisch reflex

Intravenous injections of 5-HT produced dose-dependent reductions in DABP and HR, characterized by a rapid and brief fall in HR occurring immediately after injection which was associated with a marked reduction in DABP. A secondary, non-reflex mediated fall in blood pressure was always observed after each dose of 5-HT. The effects of CPMP, CPPP and saline on the initial reflex responses to 5-HT are shown in Figure 4. Administration of normal saline did not alter the slopes of the dose-response curves to 5-HT for either the hypotensive or bradycardic responses. CPMP (5 mg kg^{-1}, i.v.) administration produced a significant reduction in the slopes of the 5-HT dose-response curves for both DABP and HR (−82% and −80%, respectively; $P < 0.0125$). A lower dose of CPMP (2 mg kg^{-1}, i.v.) failed to alter significantly these parameters (data not shown). CPPP (5 mg kg^{-1}, i.v.) administration also significantly reduced the slopes of the 5-HT dose-response curves for both DABP and HR (−51% and −48%, respectively; $P < 0.0125$). CPMP (5 mg kg^{-1}, i.v.) reduced

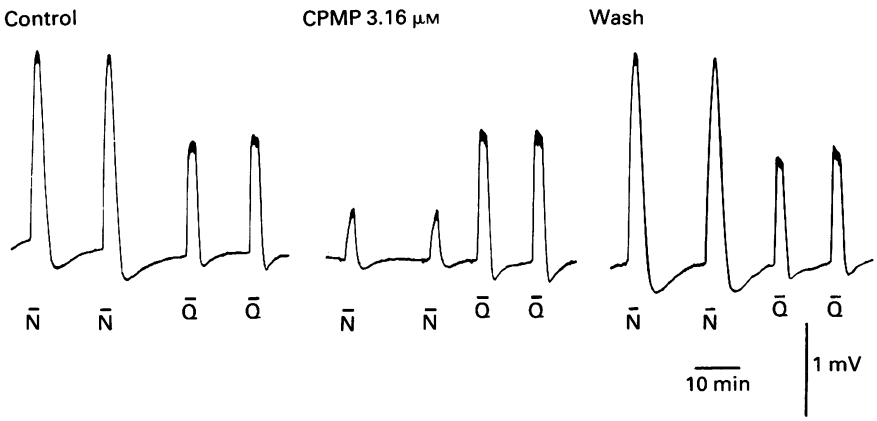

Figure 3 Selective antagonism of N-methyl-DL-aspartate (NMDLA) by CPMP on neurones in a cortical wedge preparation. Depolarizations were induced by 2 min superfusion of the cortical grey matter with 40 μM NMDA (N) or 40 μM quisqualate (Q). Introduction of 3.16 μM CPMP produced a 75% reduction in the NMDA response with no effect on that of quisqualate. The effects were reversed after 30 min wash in drug-free medium. For abbreviations used see Figure 1 legend.

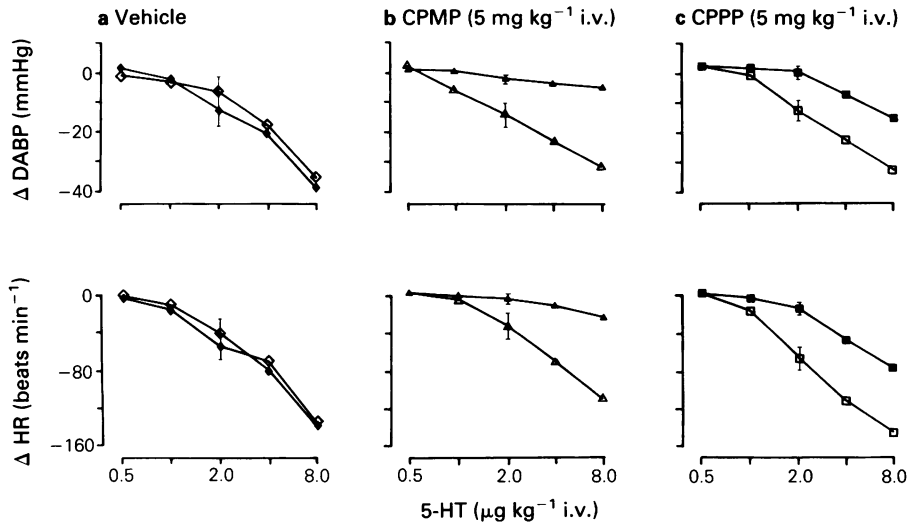

Figure 4 Effect of CPMP and CPPP on 5-hydroxytryptamine (5-HT)-induced hypotension and bradycardia. The dose-response relationship of the Bezold-Jarisch reflex was examined in urethane-anaesthetized rats before (open symbols) and after (closed symbols) the injection of (a) vehicle alone, (b) CPMP or (c) CPPP. Each point represents the mean and vertical lines show group error of changes in cardiovascular parameters. It can be seen that the N-methyl-D-aspartate (NMDA) antagonists reduced both parts of the reflex suggesting a central synapse utilizing NMDA receptors. Ordinate scales: change in diastolic blood pressure (DABP) in mmHg (upper) and change in heart rate in beats min^{-1} (lower). For abbreviations used see Figure 1 legend.

the reflex hypotensive and bradycardic responses to 8 $\mu g\,kg^{-1}$ 5-HT by 84% and 81%, respectively, whereas CPPP at the same dose reduced these responses by 52% and 46%, respectively.

Binding studies

Under the experimental conditions employed in this study [^{3}H]-CPP bound to a single site with a dissociation constant of 446 ± 150 nM and density of binding sites of 2.63 ± 0.69 (both $n = 3$) pmol mg^{-1} protein. Both CPMP and CPPP were able to displace completely the binding of [^{3}H]-CPP (Figure 5). Each compound yielded displacement curves with slope factors (pseudo Hill coefficients) close to unity. Inhibition constants (K_i) for CPMP and CPPP were 183 ± 74 and 179 ± 13 nM (both $n = 3$), respectively. Drug inhibition data for other excitatory amino acid ligands were consistent with [^{3}H]-CPP binding to an NMDA receptor site (Olverman *et al.*, 1984); single estimates of K_i values for L-glutamate and D-2-amino-5-phosphono-pentanoate (D-AP5) were 910 and 370 nM, respectively.

CPMP and CPPP completely inhibited the NMDA-stimulated binding of [^{3}H]-TCP to well washed membranes (Figure 5). The IC_{50} values determined for CPMP and CPPP were 5.6 ± 2.7 μM and 45.0 ± 21.6 μM (both $n = 3$), respectively.

Discussion

The two new piperidine compounds, CPPP and CPMP, were approximately as potent as the piperazine, CPP, as NMDA antagonists. This was the case in binding and electrophysiological studies *in vitro* and *in vivo* following microelectrophoresis or systemic injection. CPP has become accepted as one of the most potent and selective NMDA antagonists available. It is clear from these studies that a piperidine moiety will adequately replace the piperazine of CPP, but this appears to have little benefit in terms of permeation into the brain. But when *in vitro* and microelectrophoretic potencies are compared with those following intravenous injection, it appears that both piperidine and piperazine derived compounds penetrate the blood brain barrier better than AP5 and AP7. Difficulties of quantifying the systemic effects of excitatory amino acid antagonists were compounded by only being able to test one substance per animal. Hence it is not possible to be definite about the relative permeabilities of CPP, CPMP and CPPP into the brain but there appear to be no major differences between these three compounds.

[^{3}H]-CPP is an effective radioligand for studying the potency of novel excitatory amino acid agents at the NMDA receptor (Murphy *et al.*, 1987; Olverman *et al.*, 1987). CPP and CPPP differ only in the ring

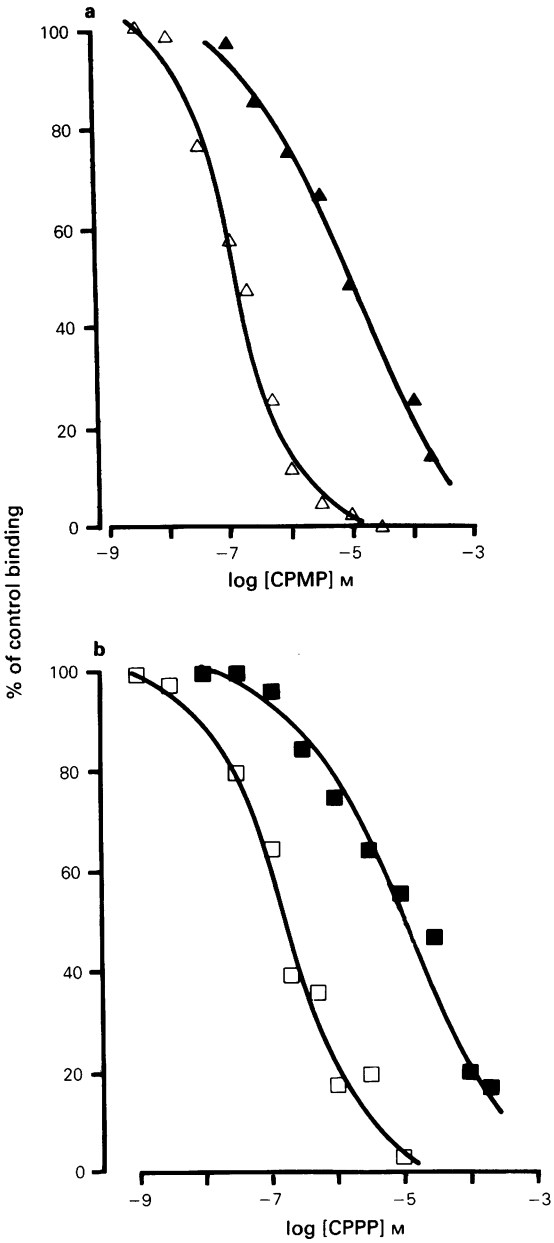

Figure 5 Displacement of the binding of [^{3}H]-CPP (open symbols) and of N-methyl-D-aspartate (NMDA)-stimulated [^{3}H]-thienylcyclohexylpiperidine (closed symbols) to cortical membranes by (a) CPMP and (b) CPPP. Each point represents the mean of two or three determinations from a single experiment showing the binding isotherm for a single site (see text for details). Ordinate scales: percentage of specific binding. For abbreviations used see Figure 1 legend.

moiety of the molecule, piperazine versus piperidine, respectively. This difference, however, does not alter the potency of the two molecules. Presumably this region on the molecule does not play a significant role in the interaction with the receptor. CPMP is also equipotent with CPP and this molecule differs from CPPP by having the propyl-phosphonate side chain reduced to methyl-phosphonate. Results with another series of NMDA antagonists based on phosphonic acid derivatives demonstrated that the most potent in the series were D-AP5 and D-2-amino-7-phosphonoheptanoate (D-AP7) (Olverman *et al.*, 1984). Whilst CPPP can be considered to be a partially conformationally restricted analogue of D-AP7, it is reasonable to conclude that CPMP is a restricted analogue of D-AP5. Therefore, it is not surprising to observe similar potencies for CPMP and CPPP at the [^{3}H]-CPP binding site.

Inhibition of NMDA-stimulated [^{3}H]-TCP binding represents a functional assay for interactions of excitatory amino acid antagonists at the NMDA receptor-ionophore complex. The method we employed is similar to that used by Foster & Wong (1987) who studied the inhibition of [^{3}H]-MK-801 binding by D-AP5. Our results indicate that CPPP and CPMP do not differ significantly in potency at the NMDA site stimulating [^{3}H]-TCP binding.

The binding studies and the *in vitro* cortical studies are consistent with all the antagonists acting competitively at the NMDA receptor. With some minor variations, the relative potencies of the compounds are similar in all three *in vitro* assays. But the absolute values of the IC_{50} vary about 100 fold between the [^{3}H]-CPP displacement protocols and antagonism of NMDA-stimulated TCP binding, with those from the cortical wedges being intermediate. Presumably these variations are in part due to the differences in agonist concentration in the various assays. The ability of CPMP and CPPP to attenuate NMDA-stimulated [^{3}H]-TCP binding was not studied in detail, but the experiments demonstrated that both compounds were effective displacers in this procedure which reflects the functional coupling of the ionophore and the receptor recognition site (Javitt *et al.*, 1987; Foster & Wong, 1987). Our studies were performed using a concentration of NMDA (10 μM) which produces approximately 80% stimulation of [^{3}H]-TCP binding (unpublished observations), and both CPMP and CPPP totally displaced binding. Foster & Wong (1987) recently studied the inhibition of glutamate-stimulated [^{3}H]-MK-801 binding by the competitive antagonist, D-AP5, using full dose-response curves and Schild analysis, and the resultant pA_2 agreed well with the affinity of D-AP5 determined by saturation experiments. Thus K_i values for CPMP and CPPP versus [^{3}H]-CCP

binding are probably a better index of the true affinities of the two carboxypiperidine-phosphonates for the NMDA receptor than are the IC_{50} values versus NMDA-stimulated [^{3}H]-TCP binding.

The present study shows that both CPMP and CPPP are able to inhibit the Bezold-Jarisch reflex. The systemically active doses of CPMP and CPPP were similar to those that we found previously in this study to reduce responses to NMDA selectively by about 80%. Previously we had observed that MK-801, a non-competitive NMDA antagonist which probably acts as a channel blocker (Foster & Fagg, 1987), also reduced the Bezold-Jarisch reflex (Verberne *et al.*, 1987), at doses equivalent to those that blocked NMDA responses of central neurones (Davies *et al.*, 1988). It may thus be concluded that central NMDA receptors mediate this cardiovascular reflex. Although there are receptors for NMDA on vagal afferents in the periphery (Lewis *et al.*, 1987), the doses of CPPP and CPMP suggest that access to the CNS has to be achieved in order to block the response. Evidence from previous studies suggests that glutamate may be a transmitter of a population of vagal afferents (Talman *et al.*, 1984). These afferents project directly to the nucleus of the solitary tract where microinjected glutamate receptor antagonists are able to block vagal baroreflex responses (Granata & Reis, 1983; Guyenet *et al.*, 1987). In addition, a region within the caudal ventrolateral medulla (CVLM) must also be considered as a possible central locus of action for CPMP and CPPP, since kynurenate (Guyenet *et al.*, 1987) and D-AP5 (Gordon, 1987) microinjected into the CVLM were able to block vagal baroreflexes. Together these observations suggest that medullary NMDA receptors mediate important cardiovascular reflexes and that they may provide novel targets for antihypertensive drugs.

In summary, our results show that two novel carboxypiperidine derivatives of CPP are potent and selective NMDA antagonists, with effects on NMDA receptor-mediated responses following systemic injection in the 2–5 mg kg^{-1} range. Along with CPP they will be useful tools for assessing the importance of NMDA receptors in physiological and pathological events in the mammalian CNS.

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