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CGP 37849 and CGP 39551: novel and potent competitive N-methyl-D-aspartate receptor antagonists with oral activity

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1 The pharmacological properties of CGP 37849 (DL-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid; 4-methyl-APPA) and its carboxyethylester, CGP 39551, novel unsaturated analogues of the N-methyl-D-aspartate (NMDA) receptor antagonist, 2-amino-5-phosphonopentanoate (AP5), were evaluated in rodent brain *in vitro* and *in vivo*.

2 Radioligand binding experiments demonstrated that CGP 37849 potently (K_i 220 nM) and competitively inhibited NMDA-sensitive L-[^{3}H]-glutamate binding to postsynaptic density (PSD) fractions from rat brain. It inhibited the binding of the selective NMDA receptor antagonist, [^{3}H]-(±)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonate (CPP), with a K_i of 35 nM, and was 4, 5 and 7 fold more potent than the antagonists ((±)-*cis*-4-phosphonomethylpiperidine-2-carboxylic acid) (CGS 19755), CPP and D-AP5, respectively. Inhibitory activity was associated exclusively with the *trans* configuration of the APPA molecule and with the D-stereoisomer. CGP 39551 showed weaker activity at NMDA receptor recognition sites and both compounds were weak or inactive at 18 other receptor binding sites.

3 CGP 37849 and CGP 39551 were inactive as inhibitors of L-[^{3}H]-glutamate uptake into rat brain synaptosomes and had no effect on the release of endogenous glutamate from rat hippocampal slices evoked by electrical field stimulation.

4 In the hippocampal slice *in vitro*, CGP 37849 selectively and reversibly antagonized NMDA-evoked increases in CA1 pyramidal cell firing rate. In slices bathed in medium containing low Mg^{2+} levels, concentrations of CGP 37849 up to 10 μM suppressed burst firing evoked in CA1 neurones by stimulation of Schaffer collateral-commissural fibres without affecting the magnitude of the initial population spike; CGP 39551 exerted the same effect but was weaker. *In vivo*, oral administration to rats of either CGP 37849 or CGP 39551 selectively blocked firing in hippocampal neurones induced by ionophoretically-applied NMDA, without affecting the responses to quisqualate or kainate.

5 CGP 37849 and CGP 39551 suppressed maximal electroshock-induced seizures in mice with ED_{50}s of 21 and 4 mg kg^{-1} p.o., respectively.

6 CGP 37849 and CGP 39551 are potent and competitive NMDA receptor antagonists which show significant central effects following oral administration to animals. As such, they may find value as tools to elucidate the roles of NMDA receptors in brain function, and potentially as therapeutic agents for the treatment of neurological disorders such as epilepsy and ischaemic brain damage in man.

Introduction

N-methyl-D-aspartate (NMDA) receptor mechanisms have been implicated in the pathophysiology of a number of neurological disorders and a great deal of interest has centred on the therapeutic potential of agents which block the actions of this excitatory amino acid receptor sub-type (see collection of articles edited by Cavalheiro *et al.*, 1988). Many studies have demonstrated that activation of the NMDA receptor is involved in the generation of epileptiform activity and in hypoxic-ischaemic neuronal damage, and conversely that NMDA receptor antagonists are anticonvulsant and cerebroprotective in animal models of epilepsy and stroke (see reviews by Meldrum, 1985; Rothman & Olney, 1986; Choi, 1988; Patel *et al.*, 1988; Iversen *et al.*, 1989; Albers *et al.*, 1989). Such findings have led to the prediction that NMDA receptor antagonists with appropriate potency, selectivity and bioavailability may form novel agents for the treatment of seizure disorders and ischaemic neuropathologies in man (Meldrum, 1985; Rothman & Olney, 1986; Albers *et al.*, 1989; Iversen *et al.*, 1989).

Antagonism of NMDA receptor mechanisms potentially may be achieved by a number of different approaches. In addition to the transmitter recognition site, the NMDA receptor complex comprises an allosteric regulatory site and a channel binding domain (Foster & Fagg, 1987b), and possibly also sites defined by the actions of Zn^{2+}, polyamines, tricyclic antidepressants, ifenprodil and CGP 31358 (see Lodge, 1989; Baud *et al.*, 1989). At present, however, the two most well characterized sites are (1) the transmitter recognition site, at which substances such as D-2-amino-5-phosphonopentanoate (D-AP5), (±)-3-(2-carboxypiperazin-4-yl) propyl-1-phosphonate (CPP) (±)-*cis*-4-phosphonomelthylpiperidine-2-carboxylic acid (CGS 19755) competitively antagonize the actions of excitatory amino acids (Evans *et al.*, 1982; Davies *et al.*, 1986; Lehmann *et al.*, 1987; 1988), and (2) a channel site at which non-competitive antagonists such as phencyclidine (PCP) and (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d] cyclohepten-5,10-imine maleate (MK 801, Wong *et al.*, 1986; Lodge *et al.*, 1988) bind to function as open-channel blockers.

The potential therapeutic advantage of competitive antagonists is that, following systemic administration, their beneficial effects may be discriminated from their side-effects (Meldrum, 1985; Lehmann *et al.*, 1987; 1988; Ferkany *et al.*, 1988; France *et al.*, 1989; Tricklebank *et al.*, 1989), and therefore they may prove to be suitable for the treatment of disorders in man requiring either acute or chronic drug administration. The disadvantage of currently-available substances, however, is that they are not active following oral administration. Here we describe a novel analogue of AP5 which exhibits high potency and selectivity for the NMDA receptor *in vitro* and is centrally-active following oral administration *in vivo*. As

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Figure 1 Chemical structures of (left) CGP 37849 (DL-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid; or 4-methyl-APPA) and (right) CGP 39551.

detailed elsewhere (Fagg *et al.*, 1989a; Schmutz, Portet, Jekes, Klebs, Vassout, Allgeier, Heckendorn, Fagg, Olpe & van Riezen, unpublished observations), this compound (CGP 37849; DL-(E)-2- amino-4-methyl-5-phosphono-3-pentenoic acid, or 4-methyl-APPA) and its carboxy-ethylester (CGP 39551) (for structures, see Figure 1) show potent oral anticonvulsant properties which can be separated from their behavioural side-effects and hence are potential candidates for novel anticonvulsant therapy in man. Preliminary results of this study have been presented in abstract form (Fagg *et al.*, 1988; 1989; Schmutz *et al.*, 1989).

Methods

Radioligand binding assays

NMDA receptor assays Subcellular fractions enriched in postsynaptic densities (PSDs) were isolated from the brains of adult male Tif:RAIf(SPF) rats (180–250 g) as previously described (Foster & Fagg, 1987a). In brief, synaptic plasma membranes were isolated by sucrose density gradient centrifugation, treated with 0.4% Triton X-100, subjected to one freeze-thaw cycle, and washed 4 times by resuspension and centrifugation in 0.5 mM HEPES-KOH buffer (pH 7.0). The resultant crude PSDs were stored as a suspension at −80°C until assay, at which time they were thawed, washed once more and resuspended in assay buffer.

The binding of L-[3,4-^{3}H]-glutamic acid (44.1 Ci mmol^{-1}; Dupont-New England Nuclear) (Foster & Fagg, 1987a), [1,2-^{3}H]-CPP (20 Ci mmol^{-1}; Tocris Neuramin) (Olverman *et al.*, 1986; Murphy *et al.*, 1987) and [2-^{3}H]-glycine (43.5 Ci mmol^{-1}; Dupont-New England Nuclear) (Bristow *et al.*, 1986; Kessler *et al.*, 1989) was determined essentially according to previously published procedures. Aliquots (40–50 μg protein) of crude PSDs were incubated in triplicate with radioligand (50 nM L-[^{3}H]-glutamate; 25 nM [^{3}H]-CPP; 20 nM [^{3}H]-glycine) in a final volume of 0.5 ml 50 mM Tris-acetate buffer (pH 6.9 for L-[^{3}H]-glutamate; pH 7.8 for [^{3}H]-CPP; pH 7.5 for [^{3}H]-glycine), and membrane-bound radioactivity was measured after centrifugation and aspiration of the supernatant.

Incubations were 30 min at 32°C for L-[^{3}H]-glutamate and [^{3}H]-CPP, and 20 min at 4°C for [^{3}H]-glycine. Non-specific binding was determined in the presence of 0.5 mM L-glutamate, 0.1 mM CPP or 0.5 mM glycine, and was routinely about 15%, 30% and 25% of total binding, respectively. Potential inhibitors were added to the incubation tubes prior to the addition of radioligand. IC_{50} values were determined by fitting data to the logistic equation using an iterative curve fitting procedure (Research System 1; Bolt, Beranek & Newman Inc.; see Foster & Fagg, 1987a) operated on a VAX 8600 computer, and K_i values were calculated from the relationship: $K_i = IC_{50}/(1 + F/K_d)$, where F is the free concentration of radioligand and K_d its equilibrium dissociation constant (K_d values were determined by saturation analyses of the type illustrated in Figure 2; mean values used here were 250 nM for L-[^{3}H]-glutamate, and 130 nM for [^{3}H]-CPP).

Other receptors Radioreceptor assays were conducted by use of procedures which have been established and documented previously (Foster *et al.*, 1981; Honore & Nielsen, 1985; Lehmann *et al.*, 1988). Assays (and radioligands) used were as follows: quisqualate (AMPA), kainate (kainate), $GABA_A$ (muscimol), benzodiazepine (flunitrazepam), $GABA_B$ (CGP 27492), adenosine (cyclohexyladenosine), muscarinic cholinoceptor (diozalane and QNB), α_1-adrenoceptor (prazosin), α_2-adrenoceptor (clonidine), β-adrenoceptor (dihydroalprenolol), 5-HT_1 (5-HT), 5-HT_2 (ketanserin), histamine H_1 (doxepine), histamine H_2 (thiotidine), substance P (substance P) and neurotensin (neurotensin). All assays were validated using standard reference compounds.

Transport studies

The Na^+-dependent uptake of L-[3,4-^{3}H]-glutamate (52.6 Ci mmol^{-1}; Dupont-New England Nuclear) into crude synaptosomal (P_2) fractions isolated from rat brain was investigated by the method described by Yunger *et al.*, (1984) for [^{3}H]-GABA uptake with minor modifications. In brief, the P_2 fraction (recovered as a pellet in 0.32 M sucrose) was suspended in 0.32 M glucose and diluted with Krebs-Ringer bicarbonate buffer (composition in mM: NaCl 118, KCl 4.8, $CaCl_2$ 2.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, final glucose concentration 8, equilibrated with 5% CO_2 in O_2) containing 0.1 mM isoniazid and L-methionine sulphoximine to inhibit the metabolism of L-[^{3}H]-glutamate. Aliquots of this P_2 suspension were preincubated in duplicate (with test compounds as appropriate) at 37°C for 15 min before addition of L-[^{3}H]-glutamate (final concentration 1 μM, except for K_m determinations). 'Blank' tubes were incubated on ice. Incubations were continued for a further 3 min, and were terminated by rapid filtration (Millipore, 0.45 μm pore size) and washing twice with ice-cold buffer. Radioactivity retained on the filter was determined by liquid scintillation counting.

Transmitter release experiments

The electrically-evoked release of endogenous glutamate from rat hippocampal slices was determined by a slight modification of the method described by Waldmeier *et al.* (1988) for the release of endogenous GABA. Cross-chopped slices (about 0.36 × 0.36 × 1 mm) were suspended in a Krebs-Ringer bicarbonate buffer (composition as for Transport studies above, except that the final concentrations of $CaCl_2$ and D-glucose were 1.8 mM and 10 mM, respectively) equilibrated with 5% CO_2 in O_2, and aliquots were transferred to superfusion chambers with platinum ring electrodes above and below the tissue slices. Slices were superfused with buffer (0.25 ml min^{-1}, 37°C) containing 0.5 mM dihydrokainate (to inhibit reuptake) for 1 h before collecting 1.25 ml fractions for amino acid analysis. The glutamate content of the superfusate and of the slices (collected at the end of each experiment) were determined by high performance liquid chromatography (h.p.l.c.) after pre-column derivatization with *o*-phthalaldehyde, and the glutamate released in each fraction was expressed as a percentage of the tissue content at the time of collection (% fractional release).

Previous studies have shown that, in this system, the evoked release of endogenous glutamate is frequency-dependent over the range 2–40 Hz and is entirely Ca^{2+}-dependent (Thedinga *et al.*, 1989). For the experiments described here, two trains of monophasic pulses (10 Hz, 20 mA, 2 ms, 2 min duration) were delivered during fractions 3 (S_1) and 9 (S_2); the first stimulation was always in superfusion buffer alone, and the second was either in the absence or the presence of test compounds (added to the buffer 20 min previously). Drug effects on evoked release were determined by comparing the ratio S_2/S_1 in drug-treated and control slices (Waldmeier *et al.*, 1988). All experiments were conducted in triplicate.

Electrophysiological investigations

In vitro Experiments were performed on transverse hippocampal slices (450 μm) prepared from male Tif:RAIf(SPF) rats (150–200 g weight) as previously described (Olpe & Lynch, 1982). Slices were allowed to stabilize in a humidified interface chamber, and were subsequently perfused (6 ml min^{-1}; equivalent to 2 chamber volumes per min) with a physiological salt solution (composition in mM: NaCl 124,

KCl 2.5, $CaCl_2$ 2.5, $MgSO_4$ 2, KH_2PO_4 1.25, $NaHCO_3$ 26, D-glucose 10; gassed with 5% CO_2 in O_2) at 32°C. Extracellular recordings were made from the stratum pyramidale region of area CA1 by use of glass microelectrodes filled with 4 M NaCl.

Two series of experiments were performed: (1) Agonist-antagonist interactions were investigated by applying submaximal concentrations (10 μM) of NMDA, quisqualate or kainate at intervals of 5–10 min in the presence and absence of test blockers, and by plotting the firing rate of single neurones (action potentials in 5–10 s bins) on a chart recorder. All substances were applied via the perfusion system to submerged hippocampal slices.

(2) The ability of test drugs to block synaptically-evoked epileptiform activity was evaluated using an approach employed by Coan & Collingridge (1985). Stable single population spikes were first elicited in CA1 neurones by stimulation of Schaffer collateral-commissural fibres (submerged slices), and the Mg^{2+} concentration of the perfusion medium was then decreased from 2 mM to nominally 0.1 mM (conditions which reduce the voltage-dependent blockade of the NMDA receptor ion channel). Subsequent sub-maximal stimulation (at a frequency of 0.125 Hz) evoked bursts of population spikes, which previously have been shown to be sensitive to blockade by NMDA receptor antagonists (Coan & Collingridge, 1985; Lester *et al.*, 1988).

In vivo Male rats (Tif:RAIf(SPF), 280–320 g weight) were anaesthetized with chloral hydrate (400 mg kg^{-1}, i.p.) and the tips of four-barrelled micropipettes were stereotaxically positioned in the region of the hippocampal CA1 pyramidal neurones. Three barrels of the pipette contained NMDA, quisqualate and kainate (0.01 M, pH 10) and the fourth recording barrel 4 M NaCl. Excitatory amino acids were applied ionophoretically (currents 20–30 nA; adjusted to yield similar response amplitudes) for periods of 20–60 s at constant intervals of 2–3 min (retaining currents of 20 nA were employed between drug applications). The number of action potentials in 5–10 s bins was plotted on a chart recorder, and responses to ionophoretically-applied agonists were monitored for up to 8 h. Antagonists were dissolved in physiological saline and were administered cumulatively (3 h intervals) via a canula in the oesophagus.

Anticonvulsant evaluation

Convulsions in mice were elicited by maximal electroshock (Bernasconi *et al.*, 1986). Brief current pulses (16 mA, 50 Hz, 0.2 s) were applied via corneal electrodes and the number of animals displaying tonic hind limb extension seizures after saline or drug administration (5 animals per dose group) was determined. Test anticonvulsants were administered orally (via an oesophageal cannula) 2–4 h before electroshock. ED_{50} values (dose which prevented seizures in 50% of animals) were determined by the method of Spearman-Kaerber.

Results

Receptor binding profiles of CGP 37849 and CGP 39551

The high affinity of CGP 37849 for the NMDA receptor was identified on the basis of its activity at NMDA-sensitive L-[^{3}H]-glutamate binding sites in crude PSDs (Foster & Fagg, 1987a; Fagg & Baud, 1988) and subsequently was confirmed using the selective radioligand, [^{3}H]-CPP (Olverman *et al.*, 1986; Murphy *et al.*, 1987). Systematic analyses of a series of ω-phosphono amino acid analogues revealed that CGP 37849 was a highly potent inhibitor of L-[^{3}H]-glutamate binding, with a K_i similar to that of L-glutamate itself. Inhibitory activity was found to be associated with the *trans* configuration of the APPA molecule (not with the naturally-occurring *cis*-isomer) and, as in the case of AP5 (Table 1; Evans *et al.*, 1982) and other NMDA receptor antagonists (Watkins *et al.*, 1990), with the D-stereoisomer (CGP 40116). Saturation analyses indicated that the interaction of CGP 37849 with the L-[^{3}H]-glutamate binding site was of a competitive nature; increasing concentrations of the blocker did not alter the maximum number of binding sites labelled by the radioligand, but progressively decreased the apparent affinity of L-[^{3}H]-glutamate for the receptor (Figure 2).

CGP 37849 inhibited the binding of the selective NMDA receptor antagonist, [^{3}H]-CPP, to rat brain PSDs with a K_i

Table 1 Comparative potencies of CGP 37849, CGP 39551 and related substances as inhibitors of NMDA receptor binding and of maximal electroshock-induced seizures in mice

Substance	K_i (μM), and radioligand: L-Glutamate	CPP	ED_{50} (mg kg^{-1} p.o.) Electroshock
CGP 37849	0.22 ± 0.04	0.035 ± 0.020	21
CGP 40116	0.11 ± 0.04	0.019 ± 0.007	7
CGP 40117	>10	3.2 ± 0.6	>60
trans-APPA	0.39 ± 0.03	NT	107
cis-APPA	32 ± 9	NT	>25
CGP 39551	8.4 ± 3.4	0.31 ± 0.03	4
DL-AP5	1.5 ± 0.2	NT	>600
D-AP5	0.88 ± 0.45	0.26 ± 0.05	NT
L-AP5	29 ± 9	4.9 ± 0.7	NT
DL-AP7	8.9 ± 1.5	NT	>300
CGS 19755	0.84 ± 0.39	0.13 ± 0.02	140[a]
CPP	0.82 ± 0.05	0.17 ± 0.02	73[b]
L-Glutamate	0.20 ± 0.03	0.64 ± 0.05	NT
NMDA	5.3 ± 0.9	7.7 ± 0.4	NT

K_i values for the inhibition of NMDA-sensitive L-[^{3}H]-glutamate and [^{3}H]-CPP binding are means ± s.e.mean of data from 3–5 separate experiments, each conducted in triplicate. ED_{50}s for the suppression of maximal electroshock-induced seizures in mice were determined 2 or 4 h after oral drug administration (5 animals per group; method of Spearman-Kaerber). Data from [a]Lehmann *et al.* (1988) and [b]Lehmann *et al.* (1987; NMDA-evoked seizures in mice). CGP 40116 and CGP 40117 are respectively the D- and L-isomers of CGP 37849; APPA is DL-(E)-2-amino-5-phosphono-3-pentenoic acid; AP7 is 2-amino-7-phosphonoheptanoate; see text for other abbreviations. NT, not tested.

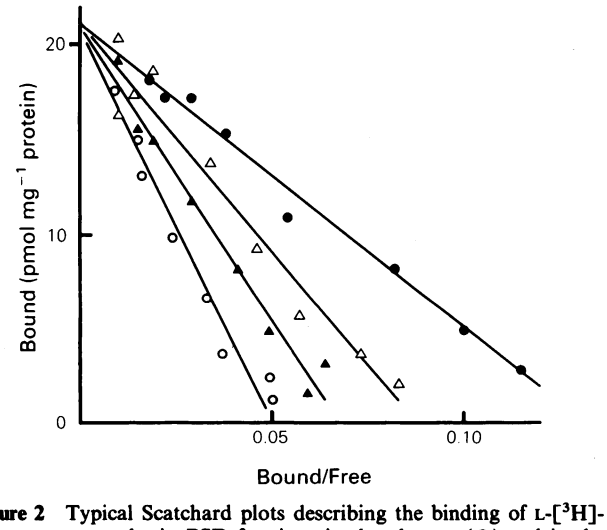

Figure 2 Typical Scatchard plots describing the binding of L-[^{3}H]-glutamate to rat brain PSD fractions in the absence (●) and in the presence of 50 nM (△), 200 nM (▲) and 500 nM (○) CGP 37849. Ordinate scale, pmol L-[^{3}H]-glutamate bound/mg PSD protein; abscissa scale, amount bound/concentration of L-[^{3}H]-glutamate (25–2000 nM). Values are means of triplicate observations.

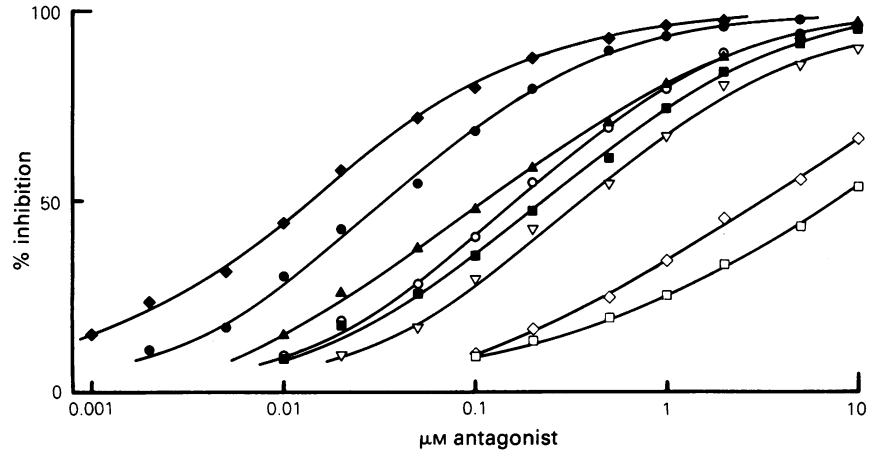

Figure 3 Composite log concentration-response curves illustrating the inhibition of [^{3}H]-CPP binding to rat brain PSD fractions by CGP 40116 (◆), CGP 37849 (●), CGS 19755 (▲), CPP (○), D-AP5 (■), CGP 39551 (▽), CGP 40117(◇) and L-AP5 (□). Values are means of data from 3–5 independent experiments. Mean K_i values (±s.e.mean) determined from these data are shown in Table 1.

of 35 nM (Figure 3 and Table 1), and was thus some 4, 5 and 7 fold more potent than the antagonists CGS 19755, CPP and D-AP5, respectively. As observed when using L-[^{3}H]-glutamate as radioligand, activity was found to reside essentially exclusively in the D-isomer (CGP 40116, K_i 19 nM; Table 1), which showed an affinity roughly 150 fold greater than that of the L-enantiomer (CGP 40117). The ethylester, CGP 39551, showed measurable inhibitory activity at both L-[^{3}H]-glutamate (K_i 8.4 μM) and [^{3}H]-CPP binding sites (K_i 0.31 μM), but was weaker than the free acid, CGP 37849 (Table 1).

Both CGP 37849 and CGP 39551 were weak or inactive in receptor binding assays for 18 other neurotransmitters or modulators. At a concentration of 50 μM, CGP 37849 inhibited strychnine-insensitive [^{3}H]-glycine binding by 26 ± 2% and CGP 39551 by 22 ± 2% (means ± s.e.mean, n = 3). In the remaining 17 receptor assays used (see Methods), both compounds showed 20% inhibition of binding or less at the test concentration of 10 μM. CGP 37849 thus is minimally 300 fold more selective for the NMDA receptor recognition site than for any of the other sites examined.

Effects of CGP 37849 and CGP 39551 on the uptake and release of L-glutamate

Alterations of synaptic function elicited by CGP 37849 and CGP 39551 (see below) potentially might result not simply from their interaction with postsynaptic NMDA receptors, but also from modifications of glutamate uptake or release. In experiments to evaluate these possibilities, no effects of the compounds were observed. As shown by other investigators (Davies & Johnston, 1976; Johnston *et al.*, 1979), L-[^{3}H]-glutamate was accumulated with high affinity (K_m 11 μM) by rat brain synaptosomes and its uptake was inhibited by the reference compounds, D-aspartate and dihydrokainate (IC_{50}s 7 μM and 315 μM, respectively; mean values, 2 separate experiments). However, neither CGP 37849 nor CGP 39551, at concentrations of 1–1000 μM, impaired L-[^{3}H]-glutamate uptake. For example, in the presence of 10 μM CGP 37849 or CGP 39551, L-[^{3}H]-glutamate uptake was respectively 104% and 100% of control values, and in the presence of 1 mM drug, 102% and 106%, respectively (means, 2 separate experiments, each in duplicate).

Similarly, neither CGP 37849 nor CGP 39551 modified the electrically-evoked release of endogenous glutamate from rat hippocampal slices. In these experiments, basal glutamate release routinely was in the range 0.2–0.4% of the tissue content per fraction, and this was increased 2.5 ± 0.2 times by 10 Hz electrical field stimulation. Under control conditions, the ratio S_2/S_1 (release evoked by the second stimulation/release evoked by the first stimulation) was close to unity (1.2), and this ratio was not altered by inclusion of 10 μM CGP 37849 (1.1) or CGP 39551 (1.0) in the superfusion medium (mean values, 2 separate experiments, each in triplicate).

Neuropharmacological actions of CGP 37849 and CGP 39551 in vitro

Agonist-antagonist interaction Electrophysiological studies using the hippocampal slice preparation indicated that CGP 37849 showed no excitatory activity, but at concentrations as low as 0.3 μM antagonized the increases in CA1 pyramidal cell firing rate induced by sub-maximal concentrations of NMDA (Figure 4a). This effect was rapid in onset and in offset, and was agonist-selective, in that responses evoked by quisqualate and kainate were unaffected. Thus, the potency and selectivity of CGP 37849 as an antagonist of NMDA receptor responses is in good agreement with its mechanism of action as determined from radioligand binding studies.

Epileptiform activity Stimulation of Schaffer collateral-commissural fibres in hippocampal slices bathed in low Mg^{2+}-containing medium previously has been shown to evoke multiple population spikes in CA1 pyramidal neurones that are reminiscent of an epileptiform burst (Coan & Collingridge, 1985; Lester *et al.*, 1988; see Figure 4b,i). Bath application of CGP 37849 at concentrations up to 10 μM markedly suppressed this type of repetitive firing, while having no effect on the first population spike of the burst (Figure 4b,ii). This effect was rapidly reversible, with the control pattern of bursting being restored within 10 min following drug wash-out (Figure 4b,iii). CGP 39551 also suppressed stimulus-induced burst firing in this experimental paradigm, but was weaker than CGP 37849 (not shown).

Anticonvulsant properties of CGP 37849 and CGP 39551

In contrast to AP5, AP7, CPP and CGS 19755, which were ineffective anticonvulsants when given orally, CGP 37849 suppressed maximal electroshock-induced seizures in mice following oral administration with an ED_{50} of 21 mg kg^{-1} (Table 1). In agreement with radioligand binding studies at the NMDA receptor (see above), anticonvulsant activity was found to reside in the D-stereoisomer (CGP 40116; ED_{50} 7 mg kg^{-1}). Unlike the investigations *in vitro*, however, the ethylester of CGP 37849 (CGP 39551; ED_{50} 4 mg kg^{-1}) was more potent than the free acid. Details of the anticonvulsant properties of CGP 37849 and CGP 39551 will be published elsewhere.

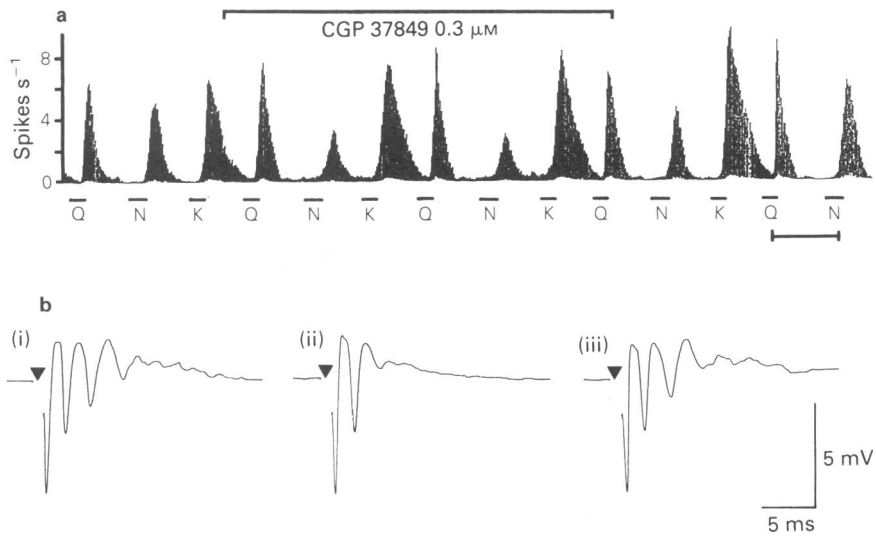

Figure 4 (a) Ratemeter record showing the effect of 0.3 μM CGP 37849 on the firing of CA1 pyramidal neurones in the hippocampal slice in response to 10 μM NMDA (N), quisqualate (Q) and kainate (K). Time bar corresponds to 10 min. (b) Multiple population spikes recorded extracellularly from CA1 neurones in the hippocampal slice (low Mg^{2+}-containing medium) in response to low-frequency stimulation of Schaffer collateral-commissural fibres: (i) control; (ii) 10 min after addition of 2 μM CGP 37849 to the bathing medium; (iii) after drug wash-out. Arrow-heads indicate the position of the stimulus artefact, which has been removed for clarity. Both experiments were repeated 5 times with similar results.

Antagonism of NMDA-evoked responses by CGP 37849 and CGP 39951 in vivo

Since CGP 37849 and CGP 39551 exhibited anticonvulsant activity following oral administration, it was of interest, in terms of understanding their mechanism of action *in vivo*, to determine whether they antagonized NMDA-evoked responses after such treatments. Figure 5 shows that this is indeed the case. At doses some 5–7 times greater than their oral ED_{50}s as anticonvulsants, both drugs selectively blocked the responses of hippocampal neurones to ionophoretically-applied NMDA, without affecting responses to quisqualate or kainate (or acetylcholine, not shown). For CGP 37849, the onset of this effect was 30–40 min after administration, with almost complete blockade of NMDA-evoked responses after 75–85 min. CGP 39551 showed a longer latency to onset;

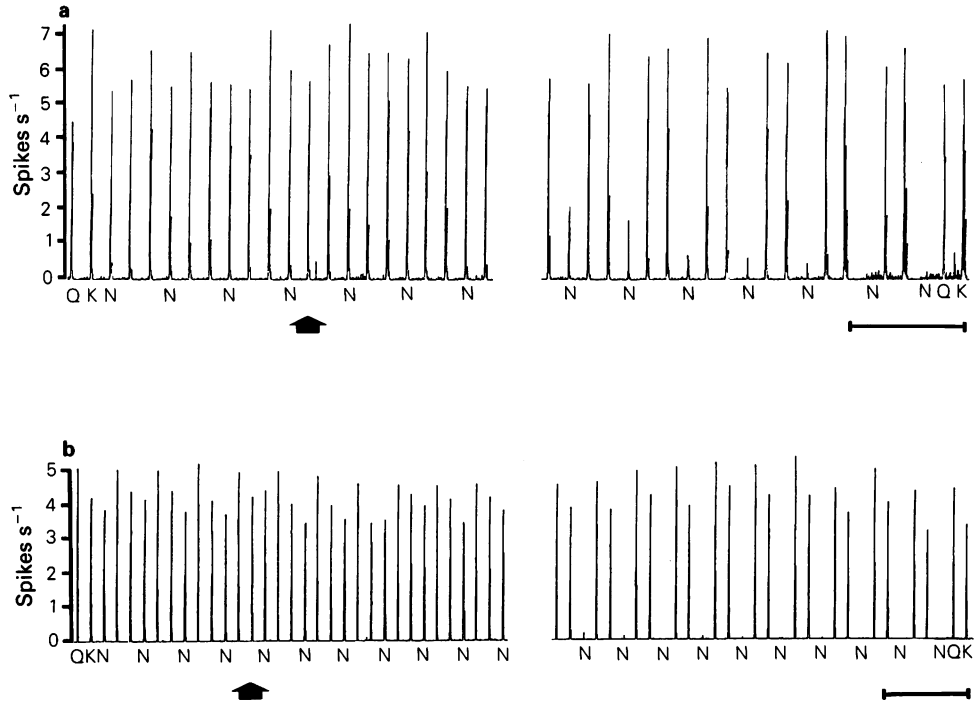

Figure 5 Ratemeter records showing the effects of (a) CGP 37849 100 mg kg^{-1} and (b) CGP 39551 30 mg kg^{-1} on the firing of rat hippocampal neurones *in vivo* in response to cycles of ionophoretically-applied NMDA (N), quisqualate (Q) and kainate (K). For clarity, only the application of NMDA (N) is indicated after the first cycle. Drugs were administered via an oesophagoeal cannula at the arrows. The breaks in the traces correspond approximately to (a) 30 min and (b) 2.5 h. Time bars represent 10 min. The experiments were repeated (a) 4 times and (b) 5 times with similar results.

clear antagonism of NMDA responses was first observed 80–90 min after oral dosage, and responses were essentially abolished after 110–120 min.

Discussion

CGP 37849 (or 4-methyl-APPA) is a novel competitive antagonist at the NMDA-preferring sub-type of excitatory amino acid receptor and, together with its carboxyethylester (CGP 39551), is the first agent of this pharmacological class reported to show significant central effects following oral administration in animals. The NMDA receptor-active properties of CGP 37849 were identified on the basis of its high affinity for L-[^{3}H]-glutamate binding sites in rat brain PSDs (predominantly NMDA-sensitive sites, see Foster & Fagg, 1987a; Fagg & Baud, 1988) and were confirmed using the selective NMDA receptor radioligand, [^{3}H]-CPP (Olverman *et al.*, 1986; Murphy *et al.*, 1987). In the hippocampal slice preparation, it showed no agonist activity, but antagonized NMDA-evoked increases in neuronal firing at concentrations within the range shown to inhibit radioligand binding to the NMDA receptor recognition site; in addition, its ability to suppress burst firing in CA1 pyramidal neurones without affecting the fast excitatory postsynaptic potential (e.p.s.p.) or population spike is in accordance with the properties of NMDA receptor antagonists as established by other investigators (Collingridge *et al.*, 1983; Coan & Collingridge, 1985; Lester *et al.*, 1988). The selectivity of CGP 37849 was indicated by its negligible interactions with binding sites for a range of other neurotransmitter receptors, including those for the quisqualate and kainate excitatory receptor sub-types, while its weak activity at strychnine-insensitive glycine binding sites provided further evidence that its primary locus of action on the NMDA receptor was indeed the transmitter recognition site. Additional demonstrations of its selectivity were obtained from electrophysiological studies in the hippocampus *in vitro* and *in vivo*, in which it failed to affect neuronal responses to applied quisqualate or kainate at doses which reduced or abolished responses to NMDA. With a K_i of 35 nM at [^{3}H]-CPP binding sites, CGP 37849 thus shows a higher affinity and selectivity for the NMDA receptor recognition site than any compound described to date.

NMDA receptor antagonists have been shown to exhibit anticonvulsant activity following central or systemic (i.p. or i.v.) administration to a variety of animal species (see Meldrum, 1985; Lehmann *et al.*, 1987; 1988; Patel *et al.*, 1988), and the oral efficacy of CGP 37849 was initially discerned from its ability to prevent maximal electroshock-induced seizures in mice. Its potency in this respect (ED_{50} 21 mg kg^{-1}, p.o.) is within a therapeutically-useful dose-range as compared with established anticonvulsant drugs (see Meldrum & Porter, 1986), and was further augmented by esterification of the carboxyl moiety (yielding CGP 39551, ED_{50} 4 mg kg^{-1}, p.o.) (detailed anticonvulsant profiles for CGP 37849 and CGP 39551 will be published elsewhere). That these compounds are indeed acting centrally as NMDA receptor antagonists following oral administration was suggested by their observed enantiomeric selectivity (D-isomer active, as for all known NMDA receptor antagonists; see Watkins *et al.*, 1990), and was more directly demonstrated by microionophoretic experiments in the rat hippocampus *in vivo*. At doses some 5–7 fold higher than their oral ED_{50}s as anticonvulsants, both compounds were shown to abolish neuronal responses to NMDA, without affecting those to quisqualate, kainate or acetylcholine. The onset of action of CGP 39551 in this regard was somewhat slower than that of CGP 37489. This observation, coupled with the weaker action of CGP 39551 *in vitro* and current understanding of structure-activity relationships at the NMDA receptor (Fagg & Baud, 1988; Watkins *et al.*, 1990), may indicate that the esterified compound functions as a more-readily absorbed pro-drug of CGP 37849 *in vivo*. However, definitive proof of the precursor-product relationship of these two substances *in vivo* is required to verify this hypothesis.

CGP 37849 and CGP 39551 are the first competitive NMDA receptor antagonists reported to show significant central effects following oral administration in animals, and hence are candidates for the treatment of neurological disorders in man which require either acute or chronic drug administration. Whilst extensive discussion is outside the realms of this paper, it is appropriate to consider some of the issues which must be resolved before this goal can be realized. Major questions have centred on the therapeutic window of NMDA receptor antagonists (loss of muscle tone occurs at sufficient dosage; see Turski *et al.*, 1988), their possible psychotomimetic effects (based on experience in man with non-competitive blockers such as PCP and ketamine; see Domino & Luby, 1981; Lodge *et al.*, 1988), and their potentially detrimental effects on learning (Morris *et al.*, 1986; Collingridge & Bliss, 1987). In this respect, recent evidence indicates that competitive NMDA receptor antagonists may have significant advantages over non-competitive blockers (Meldrum, 1985; Ferkany *et al.*, 1988; Leander *et al.*, 1988; France *et al.*, 1989; Tricklebank *et al.*, 1989). Studies on the novel competitive NMDA receptor antagonists described here indicate, especially in the case of CGP 39551, a therapeutic window at least as great as clinically-used anticonvulsant agents (Fagg *et al.*, 1989a; Schmutz *et al.*, unpublished observations), no direct impairments of learning performance in the anticonvulsant dose-range (Mondadori, Buerki & Petschke, unpublished), and no generalization to the discriminative stimulus effects of ketamine or MK801 in rhesus monkeys (France & Woods, personal communication). Pending favourable toxicological profiles, they are thus leading candidates for clinical testing in man.

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