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Short communication

Structure-activity relationships of competitive NMDA receptor antagonists

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Abstract

The interaction of structurally constrained competitive NMDA receptor antagonists, (±)-*cis*-4-phosphonomethyl-2-piperidine carboxylic acid (CGS 19755), (2-amino-4,5-(1,2-cyclohexyl))-7-phosphonoheptanoic acid (NPC 12626), (±)-6-phosphonomethyl-decahydroisoquinoline-3-carboxylic acid (LY 274614), (*S*)-α-amino-5-phosphonomethyl[1,1′-biphenyl]-3-propanoic acid (SDZ EAB-515) and (S)-α-amino-5-phosphonomethyl[1,1′:4′,1″-terphenyl]-3-propanoic acid (SDZ 215-439), with their receptor was assessed using radioligand binding, protection against neurotoxicity in cortical neuronal cultures and computerised molecular modelling. All compounds inhibited the specific binding of [^{3}H]CGS 19755 and/or [^{3}H]CGP 39653 (inhibition constants 40–2000 nM), and protected neuronal cultures from NMDA-mediated injury (IC_{50} values 1.3–5.6 μM). Quantitative conformational analyses indicated that the molecules fitted well to a NMDA receptor model. Our results draw attention to a deep hydrophobic pocket, defined by the bi- and terphenyl containing antagonists (SDZ EAB-515, SDZ 215-439), which may influence potency and selectivity.

Keywords: NMDA receptor; Competitive antagonist; Radioligand binding; Neuroprotection; Molecular modelling

1. Introduction

The NMDA subtype of ionotropic receptor for the major excitatory transmitter of the central nervous system, L-glutamate, is now well recognised to be a multi-domained receptor-ionophore complex possessing a number of rather different pharmacotherapeutic targets (McBain and Mayer, 1994). Given that the NMDA receptor appears to mediate a cascade of biochemical events that ultimately can produce neuronal death (excitotoxicity) in ischaemia, hypoxia and in head and spinal cord injury, and that NMDA receptor antagonists are neuroprotective in relevant experimental models (Meldrum and Garthwaite, 1990), the development of, and improvements in, the potency of NMDA receptor antagonists have been high priorities.

In this context, competitive NMDA receptor antagonists have received considerable attention. Indeed, there have been remarkable achievements in medicinal chemistry that have led to increases in our understanding of the structure-activity relationships governing the pharmacological activity of competitive NMDA receptor antagonists (Jane et al., 1994). Structural modifications and the restriction of conformation have produced considerable increases in potency, with the retention of pharmacological specificity, as molecular complexity has advanced from the initial aminoalkylphosphonates (2-amino-5-phosphonopentanoate and 2-amino-7-phosphonoheptanoate, DAP5 and DAP7), to single ring structures (e.g. CGS 19755; Fig. 1) and to even more complex molecules (Fig. 1) (Jane et al., 1994). Despite advances in our knowledge of the pharmacological profiles of these molecules, there have been only a few attempts to analyse their structure-activity relationships using computerized molecular modelling. Recently we reported some observations on the structural requirements of NMDA receptor agonists (O'Callaghan et

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al., 1992) and now describe pharmacological and conformational analyses of a number of structurally constrained competitive NMDA receptor antagonists.

2. Materials and methods

2.1. Materials

Radioisotopes were purchased from New England Nuclear: (±)-[^{3}H]*cis*-4-phosphonomethyl-2-piperidine carboxylic acid ([^{3}H]CGS19755; 65 Ci/mmol) and (±)-(*E*)-[^{3}H]-2-amino-4-propyl-5-phosphono-3-pentenoic acid ([^{3}H]CGP39653; 32 Ci/mmol). The following drugs were gifts: (2-amino-4,5-(1,2-cyclohexyl))-7-phosphonoheptanoic acid (NPC 12626) and (2*R*,4*R*,5*S*)-(2-amino-4,5-(1,2)-cyclohexyl))-7-phosphonoheptanoic acid (NPC 17742; Guilford, Baltimore, MD, USA), (*E*)-2-amino-4-methyl-5-phosphonopent-3-enoic acid (CGP 37849; Ciba-Geigy, Basel, Switzerland), (*S*)-α-amino-5-phosphonomethyl[1,1′-biphenyl]-3-propanoic acid (SDZ EAB-515), (*S*)-α-amino-5-phosphonomethyl[1,1′:4′,1″-terphenyl]-3-propanoic acid (SDZ 215-439) and (*R*,*E*)-4-(phosphonoprop-2-enyl)piperazine-2-carboxylic acid (SDZ EAA 494, D-CPPene; Sandoz, Berne, Switzerland), (±)-*cis*-4-phosphonomethyl-2-piperidine carboxylic acid (CGS 19755) and (±)-6-phosphonomethyldecahydroisoquinoline-3-carboxylic acid (LY 274614; Eli Lilly, Indianapolis, IN, USA). All other materials were purchased commercially and were of the highest grade available.

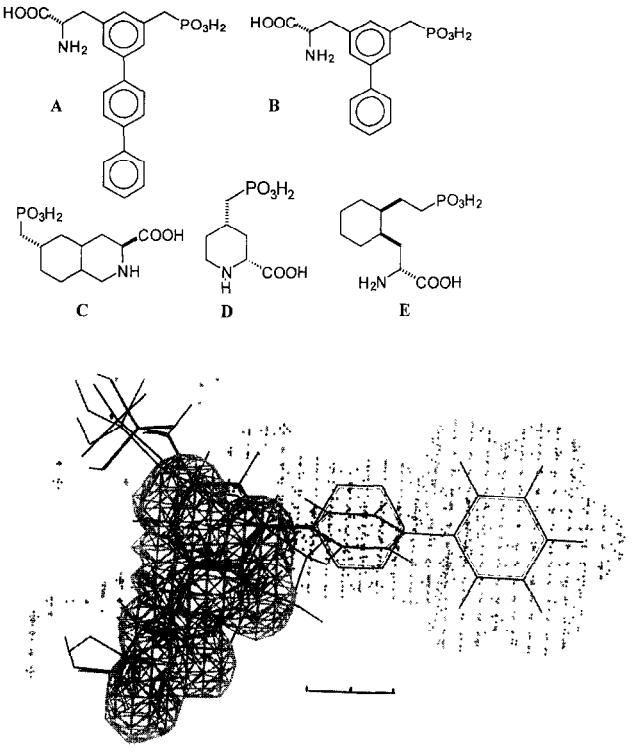

Fig. 1. Structures of competitive NMDA receptor antagonists showing the absolute stereochemistry employed in the conformational analyses: (A) SDZ 215-439, (B) SDZ EAB-515, (C) LY 274614, (D) CGS 19755 and (E) NPC 17742. Illustration of the common molecular volume occupied by SDZ 215-439, SDZ EAB-515, LY 274614, NPC 17742 and CGS 19755. The receptor essential volume depicted as the contour and the volume exclusively occupied by SDZ 215-439 shown as the 'dot' surface. Scale bar = 2 Å.

2.2. Pharmacological procedures

Binding experiments with [^{3}H]CGS 19755 or [^{3}H]CGP 39653 were performed as described previously (Ryan et al., 1993) using crude synaptic membranes. Binding data were analysed by computer-assisted curve-fitting (Beart et al., 1994). Neuroprotective activity was assessed in a procedure employing cultured murine neocortical neurones. Briefly, cortices from 15 day mouse embryos were digested and cells (1.25×10^6/well) were cultured as described elsewhere (Westergaard et al., 1993). At 8 days experiments were performed on washed cortical cultures (> 90% neurones as shown by staining with the neuronal

Table 1
Pharmacological and conformational data for competitive NMDA receptor antagonists

Drug	Affinity for NMDA receptor K_i (nM)	Neuroprotective activity IC_{50} (M)	Global minimum kcal/mol	RMS (Å)	ΔE kcal/mol
(±)-CGS 19755	180 (140–270)	5.3 (3.3–9.2)	−22.4	0.7	0.0
(±)-NPC 12626	2000 (1400–2800)	1.6 (1.2–2.1)	−13.4	0.2	0.0
(±)-LY 274614	140 (100–200)	1.3 (0.92–1.9)	−17.1	0.3	0.2
SDZ 215-439	38 (22–65) [a]	1.4 (0.84–2.3)	−3.8	0.2	0.8
SDZ EAB-515	140 (100–200)	3.4 (2.1–5.5)	0.2	0.0	1.3
	230 (190–280) [a]				

K_i (dissociation constant) and IC_{50} (concentration producing 50% inhibition of NMDA cytotoxicity) were determined as described. Estimates are computer-derived values (95% confidence intervals in parentheses). K_i values are from analyses of pooled data files from two independent experiments employing 12 drug concentrations in triplicate determinations (SDZ EAB-515 single experiment) with [^{3}H](±)-CGS 19755 (10 nM) or [a] with [^{3}H](±)-CGP 37849 (2 nM). Neuroprotective activity (IC_{50} values) against cytotoxicity induced by exposure of 8 day murine cortical cultures to NMDA (100 μM, 5 h) was determined from experiments with 3–6 individual cultures at each of 7 drug concentrations. Minimum energy conformations, RMS (root mean square, minimum three points) fit to SDZ EAB-515 and the energy difference (ΔE) between the global minimum and the conformation of best fit are given for the NMDA receptor antagonists: specific enantiomers used in these analyses are given in the text and Fig. 1.

marker, enolase) with NMDA (100 μM, 5 h)-mediated neurotoxicity assessed using a procedure whereby the formazan generated by the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) is directly proportional to the number of viable cells (Manev et al., 1990). The percentage of surviving cells relative to control was determined and IC_{50} values were calculated by computerized curve-fitting.

2.3. Conformational analyses

The following enantiomers exhibit preferential activity and were used in conformational analyses: (2*R*)-CGS 19755; (3*S*,4a*R*,6*S*,8a*R*)-LY274614 (LY235959) and *cis*-(2*R*,4*R*,5*S*)-NPC 12626 (NPC 17742) (Jane et al., 1994; Ornstein et al., 1993; Ferkany et al., 1993). Each compound was analysed using procedures described previously (O'Callaghan et al., 1992; Beart et al., 1994). The molecules were built up and optimised within SYBYL 6.1 (Tripos) using the Maximim 2 force field. The minimised structures were then optimised with the self-consistent molecular orbital package MOPAC using the AM1 method; charges obtained were used in all subsequent calculations. Low energy conformations were ascertained by systematically rotating all torsion angles with a maximum step size of 20°. A conformation from each local minimum was taken and re-optimised. All low energy conformations were superimposed, primarily by overlap of heteroatoms on to each of the viable conformations of SDZ EAB-515.

3. Results

All of the competitive NMDA receptor antagonists examined were able to fully inhibit specific binding. Hill coefficients were in the range of 0.73–1.02, consistent with the molecules interacting with a single site model of binding. Rank potency order: SDZ 215-439 > SDZ EAB-515 = (±)-LY 274614 = (±)-CGS 19755 > (±)-NPC 12626 (Table 1). SDZ 215-439, a terphenyl containing antagonist (Müller et al., 1992), was approximately 5-fold significantly more potent than its biphenyl analogue SDZ EAB-515 (Table 1, Fig. 1). The preferentially active enantiomer of NPC 12626, NPC 17742 (Ferkany et al., 1993), was some 10-fold significantly more effective at displacing the binding of [^{3}H]CGS 19755 than (±)-NPC 12626 and possessed a K_i value of 210 (170–260) nM. Other molecules considered to be benchmark NMDA receptor antagonists, D-CPPene and (±)-CGP 37849, were equipotent with SDZ 215-439: K_i values 24 (15–37) and 22 (17–28) nM, respectively.

Since all of the molecules were active in the radioligand binding assay for the NMDA receptor, their ability to protect cortical cultures from NMDA-induced neurotoxicity was determined to provide a functional index of pharmacological activity. The competitive NMDA receptor antagonists fully protected the neuronal cultures from NMDA-mediated injury (Table 1). (±)-CGS 19755 was significantly less potent than (±)-LY 274614, SDZ 215-439 and (±)-NPC 12626, which were equipotent in their ability to protect neuronal cultures from NMDA-induced cytotoxicity. SDZ EAB-515 possessed intermediate potency and was less effective as a neuroprotective agent than (±)-LY 274614 under these conditions (Table 1).

In the studies of structure-activity relationships, all low energy conformations of the NMDA receptor antagonists were superimposed, primarily by overlap of heteroatoms on to each of the viable conformations of SDZ EAB-515. A model was derived which showed a good correlation between potency and fit for all compunds except CGS 19755, which fitted somewhat less well to the model than expected with a RMS of 0.7. The NMDA receptor model is shown in Fig. 1 and selected results are included in Table 1. Two other benchmark NMDA receptor antagonists, D-CPPene and CGP 37849, exhibiting high affinity also fitted to the model; parameters derived from the fitting of these compounds to the NMDA receptor model were 3.4 kcal/mol, 0.3 Å, 0.0 kcal and 2.44 kcal/mol, 0.62 Å and 0.0 kcal, respectively, for global minimum energy, root mean square fit, and energy difference between global minimum energy and conformation of best fit (see Table 1 for details). The model was further refined with two other conformationally restrained NMDA receptor antagonists, SC 48981 and CGP 39653 (Jane et al., 1994), which fitted well to our NMDA receptor model (data not shown). The NMDA receptor competitive antagonist site contained the following features: (1) interaction points to accept the amino and acidic functional groups of the primary amino end; (2) a region to accommodate the ω-phosphonate group; and (3) a deep hydrophobic pocket. The Cartesian coordinates of the NMDA receptor site model were: α-carboxyl C 1.7, 3.2, 1.5 Å, N 1.8, 1.4, 2.3 Å, and ω-P 3.9, 3.3, 2.4 Å. The overall pharmacophore is T-shaped and approximately 10 Å across the 'top' from heteratom to heteroatom, and possesses a hydrophobic pocket at least 14.5 Å long. Volume comparisons were performed to determine the volume occupied by all ligands and that occupied exclusively by the more potent ligands, especially SDZ 215-439 is shown in Fig. 1.

4. Discussion

Our multidisciplinary investigation has provided a number of interesting insights into the characteristics and interactions of competitive NMDA receptor antagonists and their structure-activity relationships. Whilst there was considerable variation in the affinity the conformationally restrained antagonists displayed for the NMDA receptor, the drugs were quite similar in their abilities to attenuate

NMDA-induced neurotoxicity. For example, SDZ 215-439 despite being some 5-fold more potent than SDZ EAB-515 in binding experiments was equi-effective with the latter antagonist in terms of neuroprotective activity against excitotoxicity (see Table 1). Overall neuroprotection in this well-established functional model appeared not to correlate with binding data indicating that potency alone does not determine the effectiveness of competitive NMDA receptor antagonists as neuroprotective agents.

The receptor model described here is a more refined version of the recognition site proposed by Hutchinson et al. (1989) and further extended by Ortwine et al. (1992) and Bigge (1993). However, our model differs from these models in that a common model is possible for both agonists and antagonists. This model was developed using a large number of competitive antagonists and agonists by employing a combination of conformational analyses, superimposition and an in-house program INTERVOL (O'Callaghan et al., 1992), which calculates possible interaction sites of a set of compounds by examining the spatial volumes which functional groups can access. There are two possible interaction sites for the phosphono group, such as that in CGS 19755. One of these positions was similar to that predicted by Bigge (1993), whilst the other more likely potential interaction site would indicate a somewhat less folded conformation. However, the deep cleft (approximately 14.5 Å) identified by our study of the bi- and terphenyl-substituted antagonists (SDZ EAB-515 and SDZ 215-439, respectively) (Müller et al., 1992) has not been incorporated into these earlier models. This hydrophobic pocket is in the same region as the *n*-propyl side chain of CGP 39653 and the fused ring of SC 48981. Structurally constrained competitive NMDA receptor antagonists may also show some selectivity for the various heteromeric NMDA receptors that are now known to exist. NMDA receptor antagonists like LY 274614 have previously been suggested to be selective for receptors containing the NR2D subunit (Buller et al., 1993). Recently, an analogue of CGP 39653 with an extended side chain containing a phenyl moiety (CGP 55802A; Marti et al., 1993) directed to the pocket defined by SDZ EAB-515 and SDZ 215-439, has been demonstrated to have high affinity for an heteromeric receptor assembly containing the NR2A subunit (Marti et al., 1993). Conformationally restrained, competitive NMDA receptor antagonists may offer the possibility of targeting drugs to specific heteromeric receptors, discretely localised in brain, such that untoward side effects might be minimised.

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References

Beart, P.M., M.C. Ryan, L.D. Mercer, B. Jarrott and M.G. Wong, 1994, Heterocyclic aminoalcohols related to ifenprodil as σ receptor ligands: binding and conformational analyses, Eur. J. Pharmacol. 269, 193.

Bigge, C.F., 1993, Structural requirements for the development of potent *N*-methyl-D-aspartic acid (NMDA) receptor antagonists, Biochem. Pharmacol. 45, 1547.

Buller, A.L., R.A. Morrisett and D.T. Monaghan, 1993, The NR2 subunit contributes to the pharmacological diversity of native NMDA receptors, Soc. Neurosci. Abstr. 19, 1356.

Ferkany, J.W., G.S. Hamilton, R.J. Patch, Z. Huang, S.A. Borosky, D.L. Bednar, B.E. Jones, J. Willetts and E.W. Karbon, 1993, Pharmacological profile of NPC 17742 [2*R*,4*R*,5*S*-(2-amino-4,5-(1,2-cyclohexyl))-7-phosphonoheptanoic acid], a potent, selective and competitive *N*-methyl-D-aspartate receptor antagonist, J. Pharmacol. Exp. Ther. 264, 1993.

Hutchinson, A.J., M. Williams, C. Angst, R. De Jesus, L. Blanchard, R.H. Jackson, E.J. Wilusz, D.E. Murphy, P.S. Bernard, J. Schneider, T. Campbell, W. Guida and M.A. Sills, 1989, 4-(Phosphonoaklyl)- and 4-(phosphonoalkenyl)-2-piperidinecarboxylic acids: synthesis, activity at *N*-methyl-D-aspartic acid receptors, and anticonvulsant activity, J. Med. Chem. 32, 2171.

Jane, D.E., H.J. Olverman and J.C. Watkins, 1994, Agonists and competitive antagonists: structure-activity and molecular modelling studies, in: The NMDA Receptor, 2nd edn., eds. G.L. Collingridge and J.C. Watkins, p. 31.

Manev, H., M. Bertolino and G. De Erausquin, 1990, Amiloride blocks glutamate-operated cationic channels and protects neurones in culture from glutamate-induced death, Neuropharmacology 29, 1103.

Marti, T., D. Benke, S. Mertens, R. Heckendorn, M. Pozza, H. Allgeier, C. Angst, D. Laurie, P. Seeburg and H. Mohler, 1993, Molecular distinction of three *N*-methyl-D-aspartate-receptor subtypes in situ and developmental receptor maturation demonstrated with the photoaffinity ligand ^{125}I-labeled CGP 55802A, Proc. Natl. Acad. Sci. USA 90, 8434.

McBain, C.J. and M.L. Mayer, 1994, *N*-Methyl-D-aspartic acid receptor structure and function, Physiol. Rev. 74, 723.

Meldrum, B. and J. Garthwaite, 1990, Excitatory amino acid neurotoxicity and neurodegenerative disease, Trends Pharmacol. Sci. 11, 379.

Müller, W., D.A. Lowe, H. Neijt, S. Urwyler, P. Herrling, D. Blaser and D. Seebach, 1992, Synthesis and *N*-methyl-D-aspartate (NMDA) antagonist properties of the enantiomers of α-amino-5(phosphonomethyl)[1,1'-biphenyl]-3-propanoic acid. Use of a new chiral glycine derivative, Helv. Chim. Acta 75, 855.

O'Callaghan, D., M.G. Wong and P.M. Beart, 1992, Molecular modelling of *N*-methyl-D-aspartate receptor agonists, Mol. Neuropharmacol. 2, 89.

Ornstein, P.L., M.B. Arnold, N.K. Augenstein, J.B. Deeter, J.D. Leander, D. Lodge, D.O. Calligaro and D. Schoepp, 1993, Tetrazole amino acids as competitive NMDA antagonists, Biorg. Med. Chem. Lett. 3, 2067.

Ortwine, D.F., T.C. Malone, C.F. Bigge, J.T. Drummond, C. Humblet, G. Johnson and G.W. Pinter, 1992, Generation of *N*-methyl-D-aspartate agonist and competitive antagonist pharmacophone models. Design and synthesis of phosphonoalkyl-substituted tetrahydroisoquinolines as novel antagonists, J. Med. Chem. 35, 1345.

Ryan, M.C., P.M. Beart and D.J. De Vries, 1993, Extracts of marine biota with bioactivity at glutamate receptor subtypes, Pharmacol. Commun. 3, 225.

Westergaard, N., P.M. Beart and A. Schousboe, 1993, Transport of L-[^{3}H]arginine in cultured neurons: characteristics and inhibition by nitric oxide synthase inhibitors, J. Neurochem. 61, 364.