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PHARMACOLOGY OF (2*R*, 4*R*, 5*S*)-2-AMINO-4,5-(1,2-CYCLOHEXYL)-7-PHOSPHONOHEPTANOIC ACID (NPC 17742); A SELECTIVE, SYSTEMICALLY ACTIVE, COMPETITIVE NMDA ANTAGONIST

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Abstract: NPC 17742, the most potent isomer of the mixture, NPC 12626, was evaluated in tests predictive of NMDA antagonism. The compound was shown to be a selective and systemically active NMDA antagonist.

Excitatory amino acids (EAAs) are the most prevalent neurotransmitters in the mammalian central nervous system (CNS)[1]. Current understanding defines at least five receptor families for EAAs which are broadly grouped according to effector mechanisms; metabotropic receptors directly activate second messenger systems whereas ionotropic receptors are coupled to membrane ion channels[2]. Included in the latter category are receptors for the isoxazole AMPA [2*RS*-2-amino-3-(3-hydroxy)-5-methylisoxazol-4-yl)propionic acid], the pyrrolidine neurotoxin, kainic acid and the synthetic aspartate analog, *N*-methyl-D-aspartate (NMDA). Given the diversity of EAA receptor subunits which have been cloned and expressed, it is apparent that multiple receptor subtypes exist within each receptor family[3]. For this reason, EAA receptors are thought to offer a rich and varied opportunity to identify compounds useful to explore normal CNS function as well as to develop new therapeutics for pathological conditions affecting the brain.

By virtue of the early identification of prototypical antagonists[4], significant progress has been made toward identifying competitive blockers of the NMDA site. Included among these are D(-)CPP [2*R*(-)-3-(2-carboxypiperazine-4-yl)propyl-1-phosphonic acid[5]], CGS 19755 [(±)*cis*-4-phosphonomethyl-2-piperidine carboxylic acid[6]], CGP 37849 [(*E*)-2-amino-4-methyl-5-phospho-3-pentenoic acid[7]], D(-)CPPene [(*R,E*)-4-phosphonoprop-2-enyl)piperazine-2-carboxylic acid[8]] and LY 274614 [3*SR*, 4a*RS*, 6*SR*, 8a*RS*-6-(phosphonomethyl-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic acid[9]] (Figure 1). Each of these agents is undergoing extensive evaluation for conditions as varied as epilepsy, stroke, post-traumatic brain injury and anxiety.

Previously, NPC 12626 [2-amino-4,5-(1,2-cyclohexyl)-7-phosphonoheptanoic acid] was introduced as a systemically active competitive NMDA antagonist[10]. While the compound was useful as a probe of NMDA receptor function, the presence of 3 chiral centers made the agent unsuitable for further development. Using a combination of high performance liquid chromatography, enantiospecific chemical synthesis and x-ray crystallography, the 2*R*, 4*R*, 5*S* configuration of NPC 12626 was identified as the most potent of the 8 isomers in the mixture. This compound, the synthesis of which is described in an accompanying chapter[11], was designated NPC 17742. The present manuscript provides an overview of the pharmacology of NPC 17742. The data are consistent with the notion that NPC 17742 is a competitive NMDA antagonist having good systemic activity.

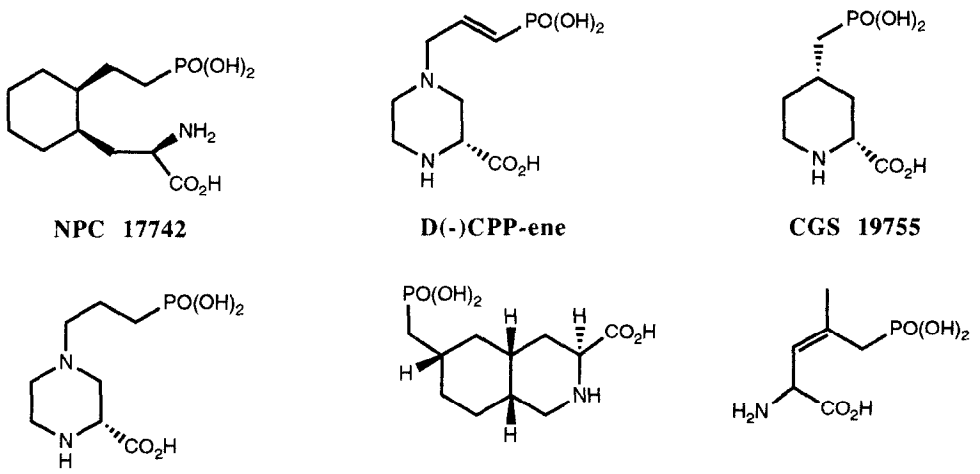

Figure 1. Structures of systemically active competitive NMDA receptor antagonists[5-9].

The separation, isolation and acquisition of quantities of the 4 *cis* isomers and 2 pairs of *trans* isomers of NPC 12626 have been described by Hamilton et al.[11]. As reported therein, the 2*R*, 4*R*, 5*S* conformation of NPC 12626 was found to be the most potent isomer to inhibit NMDA-sensitive [^{3}H]L-glutamate binding to rat brain membranes. Similarly, the 2*R*, 4*R*, 5*S* compound was the most potent inhibitor of the specific binding of [^{3}H]CGS 19755 to rat brain membranes (Table 1). Only the 2*R*, 4*R*, 5*S* and 2*S*, 4*R*, 5*S* compounds partially inhibited the binding of [^{3}H]glycine to strychnine-insensitive sites with affinities similar to those inhibiting the specific binding of [^{3}H]CGS 19755 to NMDA recognition sites (Table 1). These results confirm prior reports on the differential sensitivity of the NMDA-linked glycine receptor to competitive NMDA antagonists and extend the observation to a defined series of isomers[12]. Like other NMDA antagonists[10], NPC 17742 (10 μM) antagonized NMDA-induced stimulation of [^{3}H]TCP [1-(2-(thienyl)cyclohexyl]piperidine] binding to the NMDA receptor ionophore producing a 13-fold decrease in the potency of NMDA. Likewise, in the *Xenopus* oocyte expression system[13], NPC 17742 (pK_B = 6.9) antagonized glycine-dependent NMDA-induced inward currents but had no effect on AMPA- or kainic acid-mediated responses at concentrations ≤ 100 uM.

In agreement with results of receptor binding assays, NPC 17742 was found to be the most potent isomer to antagonize NMDA-induced convulsions[14] when administered either intracerebroventricularly (ICV) or intraperitoneally (IP) to mice (Table 1).

Unambiguous synthesis of NPC 17742 in kilogram quantities provided the opportunity to more thoroughly study its pharmacological properties. Consistent with the notion that NMDA antagonists are broad spectrum anticonvulsants, 30 minute pretreatment of mice with NPC 17742 prevented NMDA- (Table 1), pentylenetetrazol- (ED_{50} = 4.5 mg/kg; i.p.) and maximal electroshock-induced (ED_{50} = 13 mg/kg) seizures. These

results have been supported by other findings indicating NPC 17742 also prevents cocaine-induced seizures in rodents[15] and, like CGP 37849, at doses as low as 0.1 mg/kg (IM) once daily, controls seizures in animals having dilantin-sensitive idiopathic epilepsy[16].

TABLE I
NMDA RECEPTOR AFFINITIES AND ANTAGONIST POTENCIES OF THE ISOMERS OF NPC 12626

COMPOUND		IC_{50} (uM)	ED_{50} VERSUS NMDA	
			ICV (nmoles)	IP (mg/kg)
2*R*, 4*R*, 5*S*	NPC 12626	0.27	0.28	1.05
2*S*,4*S*, 5*R*	NPC 12626	2.4	8.5	32
2*S*, 4*R*, 5*S*	NPC 12626	2.6	44.6	40
2*R*, 4*S*, 5*R*	NPC 12626	5.6	8.5	33
(-) *trans*	NPC 12626	4.05	20	> 100
(+) *trans*	NPC 12626	28	49	>100
DL(±)CPP		0.19	1.2	2.2
CGS 19755		0.087	0.19	2.6

Shown are the IC_{50}s or ED_{50}s for the isomers of NPC 12626 to inhibit the specific binding of [^{3}H]CGS 19755 to NMDA recognition sites on rat brain membranes or to inhibit NMDA-induced (250 mg/kg;IP) seizures in male CF-1 mice when given 15 min (ICV) or 30 min (IP) prior to NMDA. The values were generated from highly purified samples obtained using preparative HPLC methods. Numbers for CPP are taken from ref 10. Binding assays were performed on at least 3 separate occasions.

Using the well-established paradigm of drug-discrimination[17] and rats trained to discriminate NPC 12626 (20 mg/kg; i.p.), several NMDA antagonists including NPC 12626 (ED_{50}= 18.1 mg/kg; i.p.), NPC 17742 (ED_{50} = 2.1 mg/kg; i.p.), CGS 19755 (ED_{50} = 2.3 mg/kg; i.p.), CGP 37849 (ED_{50} = 0.8 mg/kg; i.p.) and D-CPPene (Ed_{50} = 0.8 mg/kg; i.p.) substituted for NPC 12626[18]. As expected, NPC 17742 was some 9-fold more potent than the mixture of isomers and equipotent to CGS 19755. Preliminary results in our laboratory indicate this potency separation is maintained in rats trained to discriminate NPC 17742 (4 mg/kg; i.p.) from saline; NPC 17742 (ED_{50} = 1.9 mg/kg; i.p.) appeared approximately 4 - 5 times more potent than NPC 12626 (ED_{50} = 8.9 mg/kg; i.p.).

Preliminary studies have demonstrated that NPC 17742 prevents damage to hippocampal CA_1 neurons in a gerbil model of transient global ischemia[10] when administered both prior to and after the ischemic insult (50

mg/kg, i.p.; 30 and 10 min prior to, and 60 min after ischemia), or when given immediately after the initiation of reperfusion (50 mg/kg, i.v.; 10 min post ischemia followed by 50 mg/kg delivered by constant infusion over 120 min with a 50 mg/kg bolus i.v. injection at 121 min). Whether neuroprotective effects will be found following longer delays between reperfusion and initiation of compound administration is under investigation. Likewise, ongoing studies are exploring the utility of NPC 17742 in other models of stroke and physical trauma to the CNS.

Similar to other competitive NMDA antagonists, NPC 17742 appears to have a wide safety index in rodents. Indeed, when considered as a class, the competitive NMDA antagonists are remarkable among psychoactive compounds and appear to approach the benzodiazepines with respect to the ratio of doses producing pharmacological responses and morbidity. For example, in mice, preliminary evidence suggests the acute LD_{50} for NPC 17742 is > 1 g/kg following IP administration and is substantially higher following oral administration. Similar results were obtained with NPC 12626 following oral, IP or intravenous delivery where doses as high as 2 - 3 g/kg were nonlethal.

Whether NPC 17742 or other competitive NMDA antagonists will achieve therapeutic utility awaits the outcome of clinical trials. There is substantial concern that this class of compounds may elicit side effects of sufficient magnitude as to preclude their general use. In particular, the induction of phencyclidine-like psychotomimetic responses remains a concern[19] as does the potential for NMDA antagonists to disrupt learning or memory, or both[20]. As is the case for any psychoactive agent, the potential abuse liability of competitive NMDA antagonists also needs to be investigated. The possibility that tolerance to the desired effects may develop upon chronic administration is also a concern although experience suggests this appears infrequently with receptor antagonists. Any of these outcomes could preclude the use of competitive NMDA antagonists in conditions requiring prolonged treatment.

On the other hand, the broad spectrum of potential therapeutic activities associated with NMDA receptor blockade warrants the continued development of such agents. Even if competitive antagonist are associated with side effects which prevent chronic use, they may prove valuable in clinical settings (e.g., stroke, trauma, status epilepticus) where short term administration of medications is the rule. Finally, as structurally diverse NMDA antagonists become increasingly available, and as more information is gathered on the molecular characteristics of NMDA receptors, it seems likely that subtle distinctions will emerge for individual compounds. For example, NPC 17742 but not CGP 37849[21], prevents pentylenetetrazol-induced convulsions. From a synthetic, pharmacological and clinical perspective, it is apparent that substantial work remains to be done in describing the pharmacology of competitive NMDA receptor antagonists.

In summary, NPC 17742 has been identified as the most active isomer of the mixture, NPC 12626. As expected, NPC 17742 shares the pharmacological properties of the parent compound but is substantially more potent. As such, NPC 17742 should provide a valuable additional tool to further define the role of NMDA receptors in both normal brain function and pathophysiological conditions affecting the CNS.

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