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EXCITATORY AMINO ACID ANTAGONISTS INDUCE A PHENCYCLIDINE-LIKE CATALEPSY IN PIGEONS: STRUCTURE-ACTIVITY STUDIES*

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Summary—The excitatory amino acid antagonists D,L-2-amino-5-phosphonovalerate (D,L-AP5), its isomers D-(−)-AP5 and L-(+)-AP5, D,L-2-amino-4-phosphonobutyrate (AP4), D,L-2-amino-7-phosphonoheptanoate (AP7), β-D-aspartylaminomethylphosphonic acid (ASP-AMP), *cis*-2,3-piperidinedicarboxylic acid (*cis*-PDA), and γ-D-glutamylaminomethylsulphonic acid (GAMS) were tested for their ability to produce a phencyclidine (PCP)-like catalepsy in pigeons when administered intracerebroventricularly. Each of the antagonists produced catalepsy, although L-AP5, and the non-selective antagonists GAMS and *cis*-PDA, produced the effect only at toxic doses. The rank order of potency to produce catalepsy was AP7 > D-AP5 > D,L-AP5 > *cis*-PDA > ASP-AMP > AP4 > L-AP5 > GAMS; there was a strong positive correlation between this rank order of potency *in vivo* and the potency order of these compounds *in vitro* as NMDA antagonists. The antagonists did not displace significant amounts of [^{3}H]*N*-[1-(2-thienyl)cyclohexyl]piperidine (a congener of phencyclidine) from its recognition site in the brain of pigeon. Thus, the PCP-like catalepsy that is produced by the excitatory neurotransmission at NMDA-preferring receptors that are distinct from, but related to, PCP receptors. The results strongly support the hypothesis that a reduction of neurotransmission at excitatory synapses, utilizing NMDA-preferring receptors, may underlie catalepsy in pigeons induced by PCP.

Key words: excitatory amino acid antagonists, phencyclidine, catalepsy, pigeons.

Receptors for excitatory amino acids are classified according to their selective sensitivity to activation by *N*-methyl-D-aspartate (NMDA), kainate, or quisqualate (Watkins and Evans, 1981). The NMDA-preferring receptor is the most extensively characterized of the three types of receptor, because of the availability of potent and highly selective NMDA antagonists, such as D,L-2-amino-5-phosphonovalerate (D,L-AP5).

Phencyclidine (PCP) and PCP-like drugs selectively antagonize excitation of neurons induced by NMDA with little effect on the equivalent excitation induced by kainate or quisqualate (Anis, Berry, Burton and Lodge, 1983). Therefore, certain behavioral effects of PCP may be mediated by a reduction of neurotransmission at excitatory synapses utilizing NMDA-preferring receptors. If this hypothesis is correct, drugs that are known to antagonize the effects of NMDA should produce PCP-like behavioral effects. Because of its pharmacological specificity and stereoselectivity, the catalepsy test in pigeons provides a suitable procedure for studying PCP-like behavioral activity of compounds (Koek, Woods, Rice, Jacobson, Huguenin and Burke, 1984).

Recently, the selective NMDA antagonist D,L-2-amino-5-phosphonovalerate (D,L-AP5), was found to produce PCP-like catalepsy in pigeons, lending support to this hypothesis of mechanism of action for PCP (Koek, Kleer, Mudar and Woods, 1986a). The aims of the present study were: (1) to investigate whether other excitatory amino acid antagonists produced PCP-like catalepsy in pigeons; (2) to investigate whether their ability to induce PCP-like catalepsy was positively correlated with their rank order of potency as NMDA antagonists; and (3) to study whether the excitatory amino acid antagonists interacted with PCP-receptors in the brain of the pigeon.

METHODS

Catalepsy assay

White Carneaux pigeons were used. They were housed individually with food, grit and water freely available.

A chronic, indwelling guide cannula was implanted in each pigeon with the tip inserted directly into the lateral ventricle; the patency of the cannula was

*Portions of these data were presented by W.K. at the meeting of the Federation of American Societies for Experimental Biology, St Louis, MO. (Mudar, Koek, Jacobson and Woods, 1986).

assessed by a radiographic method (France, Adams and Woods, 1985), following surgery and once every month afterwards.

Drugs were injected intracerebroventricularly (i.c.v.) in a volume of 10 μl/pigeon, during a 1-min period. The injection cannula was removed from the guide cannula 30 sec after the end of the injection period. The presence or absence of catalespy (defined as loss of righting, without head-drop and without eye closure; Koek *et al.*, 1984; Koek, Woods, Jacobson, Rice and Lessor, 1986b) was assessed at successive time intervals after the injection. Each dose was tested in 5–6 pigeons that were selected at random from the group of cannulated pigeons ($n = 40$). Only data obtained from tests that were both preceded and followed by a positive X-ray were included in the analysis. Tests with drugs were separated by at least 48 hr.

Binding assay for TCP

Whole brain (minus cerebellum) from White Carneaux pigeons was removed and frozen immediately. Frozen brains was thawed and homogenized in 70 vol of 5 mM Tris–HCl buffer (pH 7.4) with a Brinkman polytron (setting 6, 20 sec). The homogenates were centrifuged three times at 20,000 *g* for 20 min at 5°C, with intervening resuspensions of the pellet in fresh buffer. The final pellet was resuspended in 70 vol of fresh Tris buffer and stored at 0°C.

Binding of [^{3}H]*N*-1[1-(2-thienyl)cyclohexyl]piperidine (TCP) to membranes from brain was determined by incubating 900 μl of tissue (containing 0.5 mg of protein), 50 μl of 5 μM [^{3}H]TCP (55.3 Ci/mmol; New England Nuclear) and 50 μl buffer (or a final concentration of 10 μM TCP for a determination of nonspecific binding) for 1 hr at 5°C. The reaction was terminated by rapid filtration (Brandel Cell Harvester, Gaithersburg, Maryland) through Schleicher & Schuell No. 32 filters which had been presoaked in 0.03% polylysine (Sigma, M_r 150,000–300,000) for 2 hr at 5°C. The filters were washed with two 5.0 ml aliquots of ice-cold Tris buffer and placed in counting vials containing 10 ml of Hydrofluor scintillation cocktail (National Diagnostics).

The AP4, D,L-AP5, AP7, GAMS, and *cis*-PDA, in concentrations of 1, 10 and 100 nM, and 1, 10, and 100 μM, were examined for their ability to inhibit the binding of [^{3}H]TCP to recognition sites in the membranes from the brain of pigeons.

Drugs

The following drugs were used: D,L-2-amino-5-phosphovalerate (D,L-AP5), isomers D-(−)-AP5 and L-(+)-AP5, D,L-2-amino-4-phosphonobutyrate (AP4), D,L-2-amino7-phosphonoheptanoate (AP7), β-D-aspartylaminomethylphosphonic acid (ASP-AMP), *cis*-2,3-piperidinedicarboxylic acid (*cis*-PDA) and γ-D-glutamylaminomethylsulphonic acid (GAMS). Each was dissolved in 1.0 N NaOH, to which sterile water was added: pH 6–8. Each of the above was purchased from Tocris Chemicals (Buckhurst Hill, England), except for TCP and PCP (National Institute on Drug Abuse, Rockville, Maryland).

RESULTS

Catalepsy assay

Each of the excitatory amino acid antagonists induced PCP-like catalepsy in pigeons at 15 min after intracerebroventricular administration (Fig. 1). For all drugs, except L-AP5, the straight lines shown in Figure 1 provided a sufficient fit (Chi-square test; $P > 0.05$) to the data. Further, these lines did not deviate significantly from parallel (slope-ratio test; $P > 0.05$). The D-(−)-AP5 was about 24 times more potent than L-(+)-AP5 in producing catalepsy (ED_{50}: 0.21 and 5 μmol, respectively) and was nearly equipotent to the racemate, D,L-AP5 (ED_{50}: 0.22 μmol). Unlike the racemate or the D-isomer of AP5, L-(+)-AP5 (top panel, Fig. 1) failed to induce catalepsy in all pigeons at the lowest dose tested (i.e. 5.6 μmol; a dose that was lethal in 40% of the pigeons). As a result, the ED_{50} dose of L-(+)-AP5 could not be calculated by means of the method of Litchfield and Wilcoxon (1949), but was calculated by interpolation.

The congeners of D,L-AP5, AP4 and AP7, also induced catalepsy, as did the dipeptide ASP-AMP (Fig. 1, middle panel); the potency order of these compounds to induce catalespy was AP7 > AP5 > ASP-AMP > AP4. In addition, catalepsy was induced by intracerebroventricular administration of the non-selective antagonists *cis*-PDA and GAMS. However, this effect was produced only at lethal doses; 1.0 μmol of *cis*-PDA was lethal in 40% of the animals tested, as was 18.0 μmol of GAMS. Compared to D,L-AP5, the potency order of these compounds to induce catalespy was D,L-AP5 > *cis*-PDA > GAMS (Fig. 1, lower panel).

The mean duration of catalepsy that was induced by the excitatory amino acid antagonists at the largest doses was not significantly different ($F[6,27] = 1.19$, $P > 0.2$; one-way analysis of variance, Keppel, 1973). The mean duration of catalepsy (± 1 SEM), averaged for the drugs, was 79.4 $\pm$ 12.9 min.

Table 1 shows the ED_{50} values of the excitatory amino acid antagonists to produce PCP-like catalepsy. A statistically significant and positive correlation of 0.81 (Spearman rank correlation coefficient; Siegel, 1956) was found between the order of potency of the antagonists to produce catalepsy and their order of potency in blocking the excitatory effects of NMDA on motor neurons of the frog. Further, a significant positive correlation of 0.79 was found between the order of potency of the antagonists to produce PCP-like catalepsy and the order of potency of these compounds to inhibit the binding of [^{3}H]D-AP5 in brain of the rat. Finally, the order of

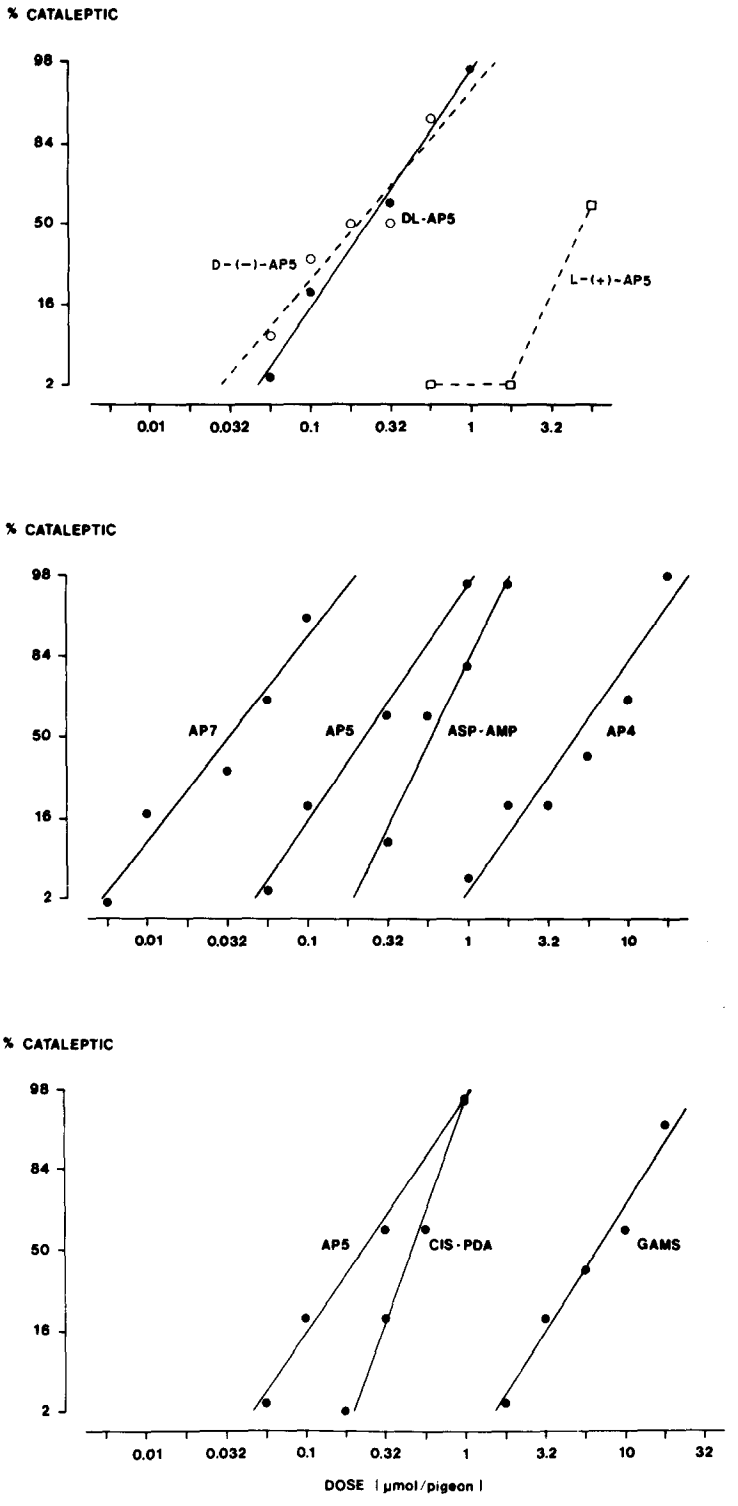

Fig. 1. Catalepsy in pigeons, assessed for 15 min after intracerebroventricular administration of excitatory amino acid antagonists (upper panel: the selective NMDA antagonist AP5 and its isomers; middle panel: congeners of AP5 and the selective NMDA antagonist ASP-AMP; lower panel: the non-selective antagonists *cis*-PDA and GAMS; for purposes of comparisons, the same dose–effect curve of D,L-AP5 is shown in all three panels). Ordinates: percentage of pigeons showing PCP like catalepsy, plotted on a probit scale. Abscissae: dose administered intracerebroventricularly in μmol/pigeon. ○ D-(−)-AP5; ● D,L-AP5; □ L-(+)-AP5.

Table 1. Correlations between the rank order of excitatory amino acid antagonists to produce PCP-like catalepsy in pigeons and their potency order to inhibit effects of NMDA, kainate and quisqualate on motor neurons of the frog (from Jones *et al.*, 1984) and to inhibit binding of [^{3}H]D-AP5 in the brain of the rat (from Olverman *et al.*, 1984). Correlations were measured by means of Spearman's rank correlation coefficient r_s; a two-tailed test was used to assess statistical significance

	PCP-like catalepsy in pigeons (ED_{50} in μmol)	Relative potency in blocking effects excitatory amino acids on motor neurons of the frogs [D-(−)-AP5 at NMDA site = 1.0]			Relative potency in inhibiting binding of [^{3}H]D,L-AP5 in brain of the rat [D-(−)-AP5 = 1.5]
		NMDA	Kainate	Quisqualate	
D,L-AP7	0.032	0.61	0.00071	0.0014	0.26
D-AP5	0.21	1.0	0.0022	0.0042	1.0
D,L-AP5	0.22	0.52	0.00072	0.0016	0.36
cis-PDA	0.47	0.022	0.013	0.011	0.0079
ASP-AMP	0.60	0.48	0.0042	0.0073	—
AP4	4.73	0.001*	—	—	0.0024
L-AP5	5.0	0.032*	—	—	0.011
GAMS	6.68	0.0015	0.11	0.014	0.00081
r_s		0.81	−0.77	−0.89	0.79
$P <$		0.05	NS	0.05	0.05

*From Evans, Francis, Jones, Smith and Watkins (1982).
NS—not statistically significant.

potency of the antagonists to produce catalepsy and their order of potency in blocking the effects of kainate and of quisqualate on motor neurons of the frog were correlated negatively.

Binding assay for TCP

In the binding assay, AP4, D,L-AP5, AP7, GAMS, and *cis*-PDA did not displace significant amounts of [^{3}H]TCP from its binding sites in membranes from the brain of the pigeon, neither when tested in the effective concentration range of TCP (EC_{50} = 38 nM) and PCP (EC_{50} = 129 nM), nor when tested in the micromolar range (data not shown). Even in a concentration of 100 μM, AP4, AP5, AP7, *cis*-PDA and GAMS displaced less than 50% [^{3}H]TCP.

DISCUSSION

Catalepsy in pigeons, an effect that is produced exclusively by PCP-like agents (e.g. Koek *et al.*, 1984), was shown to be also produced by intracerebroventricular administration of excitatory amino acid antagonists (i.e. D,L-AP5, D-(−) and L-(+)-AP5, AP4, AP7, ASP-AMP, *cis*-PDA and GAMS).

Likewise, catalepsy is produced by PCP-like compounds that stereoselectively displace PCP from recognition sites (e.g. Koek *et al.*, 1984). The AP5-induced catalepsy was stereoselectively produced, as shown by the observation that D-(−)-AP5 was more potent than L-(+)-AP5 in producing catalepsy. Further, whereas D-(−)-AP5 produced catalepsy in all the pigeons tested, L-(+)-AP5 failed to do so when tested in up to lethal doses. If the ability to induce catalepsy in pigeons resides almost exclusively in the D-isomer of AP5, it would be expected that D-AP5 would be twice as potent as the racemate. The absence of a potency difference between D-(−)-AP5 and D,L-AP5, that was observed in the present study, may perhaps be related to a degree of variability in the behavioral data that precluded the detection of small differences in potency.

On the basis of the relative potencies of the excitatory amino acid antagonists in blocking the effects of NMDA on motor neurons of the frog (Jones, Smith and Watkins, 1984) and in inhibiting the binding of the highly selective NMDA antagonist [^{3}H]D-(−)-AP5 in brain of the rat (Olverman, Jones and Watkins, 1984), a potency order of D-(−)-AP5 > AP7 > D,L-AP5 > ASP-AMP > *cis*-PDA > GAMS > AP4 in producing PCP-like catalepsy can be predicted for these compounds if their catalepsy-inducing effects are dependent upon antagonism at NMDA-preferring receptors. Indeed, the rank order of potency of these compounds to produce catalepsy was found to be in good agreement with the rank order of potency as predicted from electrophysiological and binding data; given this strong positive correlation, a negative relationship between the rank order of potency to induce catalepsy and the rank order of potency to antagonize the electrophysiological effects of kainate/quisqualate may be expected, because the compounds selected for study resulted from attempts to develop selective NMDA antagonists and selective non-NMDA antagonists.

The significant correlation between the potencies of the excitatory amino acid antagonists to produce catalepsy and their potencies as NMDA antagonists suggests that the catalepsy produced by these compounds may result from reduced excitatory neurotransmission at NMDA-preferring receptors. These receptors may be distinct from PCP receptors, because the antagonists failed to displace significant amounts of labelled TCP from binding sites for PCP in brain of pigeon (this study) and because the congener of PCP ketamine has been reported to be inactive at displacing AP5 (Olverman and Watkins, cited in Martin and Lodge, 1985). However, the NMDA-preferring receptor and the PCP receptor may form a receptor complex similar to that demon-

strated for the benzodiazepines and γ-aminobutyric acid-(GABA) (Loo, Braunwalder, Lehmann and Williams, 1986a). Since the experiments described here were conducted, Loo, Braunwalder, Lehmann and Williams (1986b) reported that the binding of [^{3}H]TCP is dependent upon the presence of endogenous glutamate in membrane preparations of the rat; as the concentration of endogenous glutamate was reduced by repeated washing of the membrane preparations, there was a parallel reduction in the binding of [^{3}H]TCP. Further, AP7 attenuated stimulation by glutamate of the binding of TCP. Thus, the effects of NMDA antagonists on the binding of TCP may be dependent on the concentration of endogenous amino acid receptor agonists; in the present study, the concentration of these endogenous agonists was not controlled.

The results presented in this study support the hypothesis that a reduction of neurotransmission at excitatory synapses utilizing NMDA receptors may underlie certain behavioral effects of PCP (Koek *et al.*, 1986a).

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