Stereoselective Antagonism of Phencyclidine's Discriminative Properties by Adenosine Receptor Agonists

Abstract. *Rats trained to discriminate between phencyclidine and saline vehicle were used to test various agents for their ability to mimic or block the phencyclidine cue. Ketamine, dexoxadrol, tiletamine, and phencyclidine analogs were found to mimic phencyclidine's behavioral effects. Treatment with the adenosine receptor agonists N^6-cyclohexyladenosine and L-phenylisopropyladenosine blocked the discriminative properties of phencyclidine. These results suggest that adenosine receptor agonists might be useful in treating phencyclidine-induced psychosis.*

Phencyclidine (PCP) was developed in the late 1950's as an anesthetic agent, but was eliminated from clinical trials due to the severe side effects it produced (*1*). During the last 10 years, however, PCP has been widely abused, and the number of emergency room admissions for PCP intoxication has been increasing. The behavioral effects of PCP often resemble schizophrenia (*2*), although the effects of acute PCP intoxication can be distinguished from psychosis on the basis of patient history and neurological signs (*3*). Frequently, however, manifestations of psychopathology remain after the acute effects of PCP have diminished. The psychosis resulting from other hallucinogenic drugs, such as lysergic acid diethylamide (LSD), generally last only 8 to 24 hours, but PCP psychosis can last for 2 weeks. With PCP, subjects may display autistic and delusional thinking typical of schizophrenics (*4*). A more striking link between PCP and schizophrenia was provided by a study in which PCP and other hallucinogens were given to hospitalized schizophrenics (*2*). The effects of LSD were no more severe in the schizophrenics than in normal subjects. By contrast, schizophrenics given PCP became extremely disorganized and the reactions persisted for up to 6 weeks.

The similarity between PCP-induced psychosis and schizophrenia suggests that antagonists of PCP might be useful as antipsychotic agents. However, there has been no known specific antidote for PCP, although phenothiazines, haloperidol, and diazepam have been used for their antipsychotic and sedating effects (*3*, *5*).

In searching for an antagonist of PCP, we required an animal subject that would be sensitive to alterations in PCP-induced subjective effects. PCP can serve as a discriminative stimulus in rats (*6*). That is, rats can learn to make one response when under the influence of PCP (pressing the left lever in a two-lever operant chamber for a food reward) and another response when given saline (pressing the right lever for reward). The rats can then be tested with various drugs to determine their similarity to PCP.

Twenty-four rats were trained to discriminate PCP (3.2 mg/kg) from saline vehicle (*7*). The ability of various doses of several compounds to mimic or block PCP's actions was then tested. When given low doses of PCP, few rats chose the lever associated with the 3.2 mg/kg dose (Table 1). As the test dose was increased, more animals responded by pressing the PCP-appropriate lever, and, at the training dose, all the animals chose the appropriate lever. Higher doses of PCP disrupted responding and the animals exhibited pronounced ataxia.

Ketamine, tiletamine, and dexoxadrol mimicked PCP's subjective effects in a dose-related manner (Table 1). This finding is consistent with clinical data indicative of a PCP-like action following administration of these drugs in humans (*8*). Compound GK-5, a *cis*-4-methyl derivative of PCP, competes with [^{3}H]PCP for binding to brain membrane sites, disrupts rotorod performance (*9*), and (as seen in Table 1) substitutes for PCP in our discrimination paradigm. Compound GK-4, the *trans* enantiomer of GK-5, also displaces [^{3}H]PCP but does not disrupt rotorod performance (*9*), nor does it mimic PCP (Table 1). In contrast, 1-(1-phenylcyclohexyl)-3-methylpiperidine [PCMP(+)] but not the optical isomer [PCMP(−)] mimics PCP's discriminative properties (Table 1) in a manner consist-

Table 1. Dose-response profile for compounds mimicking PCP in rats trained to discriminate PCP (3.2 mg/kg) from saline vehicle. All compounds were administered subcutaneously 30 minutes before a 15-minute test. The percentage of animals completing ten presses of the lever previously paired with PCP before making ten or fewer responses on the vehicle lever was calculated. The mean number (± standard error) of total responses per test are indicated. At the highest doses of PCMP(+) and ketamine only one and two animals, respectively, of the eight tested made more than ten responses during the test session.

Treatment	Dose (mg/kg)	Number of animals	Number of animals choosing PCP lever	Mean number of responses per test
Vehicle		24	0	553 ± 21*
PCP	0.1	8	1	536 ± 47
	0.178	16	2	588 ± 36*
	0.32	16	3	694 ± 48*†
	0.76	16	5	663 ± 50*
	1.0	16	11	643 ± 36*
	1.78	16	13	612 ± 34*
	2.4	8	7	639 ± 74*
	3.2	24	24	471 ± 23†
	5.6	6	5	155 ± 60*†
Ketamine	3.2	8	2	563 ± 43
	5.6	8	5	369 ± 85†
	10.0	8	6	516 ± 38
	17.8	8	7	208 ± 26*†
	32.0	2	2	12 ± 5
Dexoxadrol	0.32	8	0	555 ± 63
	1.0	16	0	662 ± 44*
	3.2	24	17	621 ± 24*
	5.6	24	21	574 ± 27*
Tiletamine	0.1	8	0	678 ± 46*†
	0.32	8	0	789 ± 65*†
	0.56	8	3	692 ± 48*
	1.0	8	7	506 ± 48†
	3.2	8	7	152 ± 40†
GK-5	3.2	8	2	647 ± 55*†
	10.0	12	9	628 ± 48*
	17.8	8	7	390 ± 61†
GK-4	10.0	12	0	734 ± 52*†
PCMP(+)	0.32	8	0	795 ± 58*†
	1.0	8	4	767 ± 32*†
	3.2	8	6	162 ± 57*†
	10.0	1	1	19
PCMP(−)	10.0	8	0	670 ± 60*

*Significantly different from the number of PCP-associated responses by the same animals ($P < .05$, Student's two-tailed t-test). †Significantly different from the number of saline-associated responses by the same animals ($P < .05$).

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Table 2. Stereoselective antagonism of PCP cuing by adenosine analogs. All the compounds were administered 30 minutes before a 15-minute test. All were given subcutaneously except for theophylline, which was administered orally. Essentially identical results were obtained by administering CHA, L-PIA, or D-PIA up to 2 hours before testing. Theophylline alone (32 mg/kg) did not alter PCP discriminability, but prevented CHA from blocking PCP's effects.

Treatment	Dose (mg/kg)	Number of animals	Number of animals choosing PCP lever	Mean number of responses per test
PCP* + saline		24	24	471 ± 23
PCP + CHA	0.032	8	5	277 ± 58†
	0.10	8	3	331 ± 60
	0.178	16	5	182 ± 34†
	0.32	12	2	75 ± 18†
PCP + L-PIA	0.1	11	4	312 ± 56
	0.178	10	1	209 ± 42†
PCP + D-PIA	0.1	10	7	337 ± 54
	0.178	11	9	339 ± 66
	1.0	10	8	165 ± 46†
Dexoxadrol (5.6 mg/kg) + saline		24	21	574 ± 27†
Dexoxadrol (5.6 mg/kg) + L-PIA	0.1	10	4	323 ± 38†
	0.178	8	0	100 ± 23†
PCP + haloperidol	0.056	8	8	296 ± 75
	0.1	7	4	184 ± 69†
	0.178	9	5	64 ± 23†
	0.32	3	2	20 ± 11†
PCP + diazepam	1.0	8	7	383 ± 86†
	3.2	7	6	153 ± 40
PCP + chlorpromazine	0.32	10	7	196 ± 41†
	1.0	3	2	137 ± 47†
PCP + theophylline (32 mg/kg) + CHA	0.32	10	7	188 ± 37†

*The PCP dose in each case was 3.2 mg/kg. †Significantly different from the number of responses by the same animals given PCP alone ($P < .05$).

ent with reported stereoselective receptor binding (*10*). Thus the PCP cue can be elicited by various agents stereoselectively. This effect appears to be specific for PCP-like compounds, since many agents, including LSD, did not mimic PCP (*11*).

In searching for PCP antagonists, we tested a number of agents that affect neurotransmitter systems for their ability to block the PCP cue. We found that when rats were treated with potent adenosine receptor agonists the discriminative effects of PCP could be abolished (Table 2). Thus, most animals injected with N^6-cyclohexyladenosine (CHA) (0.32 mg/kg) or L-phenylisopropyladenosine (L-PIA) (0.178 mg/kg) in combination with PCP (3.2 mg/kg) chose the saline-associated lever. By contrast, the dextrorotatory isomer of PIA, which is 5 to 100 times weaker in binding to the adenosine receptor than L-PIA, failed to alter PCP discrimination at doses up to 1.0 mg/kg (*12*).

Since dexoxadrol mimicked PCP's discriminative properties (Table 1) and is structurally different from the other compounds that mimicked PCP (*8*), we investigated the ability of L-PIA to block dexoxadrol's PCP-like effects. All animals given dexoxadrol plus L-PIA pressed the saline-associated lever (Table 2), indicating that the blocking effects of L-PIA are not limited to structural variants of PCP.

The combination of PCP and adenosine receptor agonists often significantly reduced the number of responses made during the 15-minute test (*13*). While adenosine analogs are potent sedative agents (*14*), generalized sedation alone cannot account for the observed antagonism of the PCP cue, since haloperidol, diazepam, and chlorpromazine also disrupted responding without significantly antagonizing the ability of PCP to act as a discriminative stimulus (Table 2) (*15*).

Theophylline and other methylxanthines antagonize many of adenosine's effects (*16*). Therefore, we tested such antagonists for their ability to prevent the blockade of PCP discrimination produced by adenosine analogs. Theophylline (32 mg/kg, orally) partially reversed the anti-PCP action of CHA (Table 2). This finding supports the contention that the actions of CHA and L-PIA are mediated by adenosine receptors. However, it does not appear that PCP's effects are attributable to antagonism of the effects of endogenous adenosine, because methylxanthines neither mimicked PCP's discriminative properties nor potentiated a subthreshold dose of PCP.

The mechanism by which adenosine analogs antagonize PCP's discriminative stimulus properties is unknown. PCP and adenosine may compete for a subpopulation of receptor sites, preventing PCP from exerting its effects. With claims being made for the existence of stereoselective PCP receptors (*9, 10, 17*), it will be of interest to determine whether labeled PCP can be displaced from such binding sites by potent adenosine receptor agonists (*18*). PCP is known to increase the release of neurotransmitters and to block their inactivation by reuptake (*19*). Since adenosine analogs decrease catecholamine release from synaptic terminals (*20, 21*), we may be observing the balancing of two opposing pharmacological effects, resulting in a normalization of catecholamine secretion and a "vehicle-like" discriminative state. PCP is also known to exert complex effects on acetylcholine-containing neurons (*22*). Thus, PCP increases the turnover of acetylcholine in various areas of the brain, has anticholinergic properties, and inhibits acetylcholinesterase. Since adenosine and related nucleotides inhibit the release of acetylcholine by a methylxanthine-sensitive mechanism (*21, 23*), a functional antagonism may exist between PCP and adenosine analogs on cholinergic neurons.

Regardless of the mechanisms involved, this study demonstrates antagonism of PCP's subjective effects and raises the possibility that adenosine receptor agonists might be useful in treating PCP-induced as well as endogenous psychosis.

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7. Saline vehicle or PCP (3.2 mg/kg, as the HCl salt) was administered subcutaneously 30 minutes before the training sessions, which were conducted as described by R. G. Browne [*Psychopharmacology* **74**, 245 (1981)]. At least eight animals were tested per treatment condition. The values in Tables 1 and 2 represent the number of animals making at least ten responses

per session. By using a sample size of, say, 12 and assuming 100 percent discrimination accuracy, we can make probability estimates for the selection of drug or vehicle levers. Thus, no more than three rats could choose the vehicle lever for P to equal .05. If nine or more rats selected the drug lever, then the treatment condition was subjectively identical to the training dose of PCP, with $P < .05$. When four to eight of the 12 rats chose the PCP lever, responding was considered different from both training conditions (that is, not significantly different from 50 percent).
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12. A higher dose of D-PIA (3.2 mg/kg) blocks PCP cuing. This agrees with the correspondingly weaker ability of D-PIA to bind to adenosine receptors [R. F. Bruns, J. W. Daly, S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5547 (1980); J. W. Daly, R. F. Bruns, S. H. Snyder, *Life Sci.* **28**, 2083 (1981)].
13. Observations of the animals given CHA or L-PIA in combination with PCP indicate that the adenosine analogs tested do not completely antagonize all the behavioral effects of PCP. Thus, doses of CHA which block PCP cuing did not antagonize the locomotor hyperactivity produced by PCP. Similarly, administration of adenosine analogs did not alter the median lethal dose of PCP.
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15. A reduction in the percentage of drug-associated responses to about 50 percent following various treatments is not uncommon in drug discrimination paradigms. We have observed a nonspecific reduction in PCP discriminability similar to that seen with haloperidol and diazepam (Table 2) when rats were treated with subtoxic doses of vasopressin, doxapram, and naloxone. In contrast, agents including physostigmine, scopolamine, tacrine, mecamylamine, diphenhydramine, methysergide, cyproheptadine, cinanserin, clonidine, prazosin, propranolol, baclofen, clozapine, sulpiride, phenytoin, Metrazole, and a number of purine derivatives all failed to reduce PCP-associated discrimination below the 75 percent level, even at doses high enough to frequently produce a significant disruption of responding.
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Female Control of Male Reproductive Function in a Mexican Snake

Abstract. *Male* Thamnophis melanogaster *court immediately when exposed to estrogen-treated, attractive females and continue courting for 6 to 8 days. Males exposed to estrogen-treated females will court both intact and ovariectomized females. These males undergo a period of testicular recrudescence, whereas males exposed only to ovariectomized females do not. Sexual attractivity can be induced in female* T. melanogaster *without estrogen treatment by heavy feeding, which results in significant increases in liver size and activity.*

The semiaquatic garter snake *Thamnophis melanogaster* is active throughout the year at Lago de Cuitzeo, Michoacan, Mexico (*1*). Several brief, synchronous breeding periods can occur each year, and the timing of these periods may vary within and between years. We report that female *T. melanogaster*, treated with estrogen, elicited a period of sexual activity in males both in the field and the laboratory. This period of sexual activity lasts 6 to 8 days, with males becoming less discriminatory and courting ovariectomized and intact untreated females as well as estrogen-treated females. Measures of testicular activity increased after sexual activity ceased in males exposed to estrogen-treated females both in the field and the laboratory. Female sexual attractivity increased after extensive feeding. The results indicate that (i) female *T. melanogaster* initiate the breeding periods and in turn stimulate testicular activity in males, and (ii) female *T. melanogaster* can become attractive and initiate the onset of a breeding period in response to nutritional condition.

Perception of a pheromone present on the skin of attractive female *Thamnophis* is necessary to elicit courtship behavior from male conspecifics (*2, 3*). This attractiveness pheromone is synthesized in the liver and is chemically related to vitellogenin, the circulating precursor of yolk (*3*). Sexual attractivity can be induced in female *Thamnophis* by treating them with exogenous estrogen (*4–7*), which stimulates the liver. Conversely, ovariectomy renders female *Thamnophis* unattractive (*4*).

Ten males, maintained in isolation cages, were offered two females daily in behavioral tests (*7*). Ovariectomized females were offered for the first 5 days, and no courtship activity was observed. Beginning on day 6, estrogen-treated intact females (*5–7*) were offered and were immediately courted. After day 13 no additional courtship activity was observed even though estrogen-treated females were changed every 5 days. However, ovariectomized females, when offered to males in daily alternation with estrogen-treated females, were courted after day 3, although not to the extent that estrogen-treated females were. We thus hypothesized that breeding periods could be initiated in nature by some females becoming attractive in a cyclic fashion. Males exposed to those females would then court other females, and a synchronous breeding period could result.

At Lago de Cuitzeo, two adjacent populations of approximately 300 snakes each were monitored for 7 days; no sexual activity was observed in either population. Twenty percent of the adult females of the test population ($N = 28$) were collected, marked, treated with estradiol (*5–7*), and released. After 1 hour and 15 minutes, three males were observed courting a treated female. Sexual activity continued for 7 days in the test population; no sexual activity was observed in the control population. Males collected after courtship activity had ceased were more reproductively active than males collected before the breeding period, as judged by measures of testicular activity (Table 1). In the laboratory, groups of males, killed at intervals during a period of controlled daily exposure to estrogen-treated females, showed increases in serum androgens and spermio-

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