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Adenosine Agonists Reduce Conditioned Avoidance Responding in the Rat

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MARTIN, G. E., D. J. ROSSI AND M. F. JARVIS. *Adenosine agonists reduce conditioned avoidance responding in the rat.* PHARMACOL BIOCHEM BEHAV 45(4) 951–958, 1993.—Because adenosine agonists may possess therapeutic potential as antipsychotic agents, we examined the activity of several prototypic agents in vivo in blocking conditioned avoidance responding (CAR) in the rat, a behavioral test predictive of antipsychotic efficacy in humans. Potency in blocking CAR is directly proportional to potency in alleviating schizophrenia. Hence, the adenosine A_1-selective agonists [cyclopentyl adenosine (CPA) and (*R*)-phenylisopropyl adenosine (*R*-PIA)], A_2-selective agonists [CV-1808 and (2-(*p*-(carboxyethyl)-phenethylamino)-5′-*N*-ethyl-carboxamido adenosine (CGS 21680)], and a nonselective agonist [5′-*N*-carboxamido adenosine (NECA)] were examined in this test. Block of CAR was first determined for standard antipsychotic agents [ED_{50} mg/kg, IP, and 95% confidence level (CL) in parentheses], such as haloperidol [0.23 (0.18, 0.39)], trifluoperazine [(0.9 (0.7, 1.0)], thioridazine [12.5 (10.5, 15.3)], metoclopramide [7.8 (6.4, 9.2)], and chlorpromazine [4.9 (4.2, 5.9)]. The paradigm consisted of a light- and tone-signaled footshock that could be avoided via a discrete lever press. Affinity for A_1 and A_2 binding sites in brain tissue from Fischer 344 rats was ascertained to be similar to that seen in other rodent strains. Each adenosine agonist blocked CAR. NECA [ED_{50} value (95% CL) = 0.07 (0.004, 0.12) mg/kg, IP] was the most potent agent, followed by: *R*-PIA [0.34 (0.23, 0.44)]; CGS 21680 [1.1 (0.8, 2.0)]; CV-1808 [1.3 (1.0, 1.8)]; and CPA [1.5 (1.3, 1.7)]. Pretreatment with caffeine (25 mg/kg, IP, −10 min) blocked the inhibition of CAR produced by the adenosine agonists, suggesting the event is mediated via purinergic receptors. As a test for extrapyramidal side effect potential, each agonist was administered at dose levels corresponding to the ED_{25}, ED_{50}, and ED_{75} values for block of CAR and catalepsy was measured. Catalepsy was prominently produced by NECA and CPA, whereas CGS 21680 and *R*-PIA produced little. Neither potency in blocking CAR nor inducing catalepsy could be highly correlated with either relative affinity or selectivity for either A_1 or A_2 binding sites. The data suggest purinergic agonists might be effective antipsychotic agents but may possess side effects that might preclude their use.

Adenosine agonists Conditioned avoidance responding Adenosine A_1 receptors Adenosine A_2 receptors

THERE is now much experimental evidence to indicate that the ubiquitous purine riboside, adenosine, serves as an important modulator of CNS function (26). This neuromodulatory action appears to be specifically mediated via different populations of adenosine receptor subtypes in the mammalian brain. These receptor subtypes, termed A_1 and A_2, have been differentiated based upon: a) agonist potencies in radioligand binding and functional assays; b) differential localization in brain determined via ligand autoradiographic studies; and c) recently described cDNAs (14). Adenosine receptors have been shown to be heterogenously distributed. The A_1 receptor is found in highest density in the hippocampus and cerebellum, with a moderate level found in the nucleus accumbens (15,20, 23). In contrast, the A_2 receptor was found in the greatest concentration in the caudate putamen, nucleus accumbens, and olfactory tubercle, areas that are also rich in dopamine receptors (12).

The most common behavioral effect seen after administration of a purine agonist is a drop in spontaneous locomotor activity mediated via CNS adenosine receptors (8,19,25). Conversely, purine antagonists, such as caffeine, enhance locomotor activity (2,5,25). Adenosine agonists have also been reported to reduce operant responding (4,5,24).

It is interesting to note that the behavioral effects produced by purinergic agents oppose those produced by dopamine D_2 agents (7,11). Specifically, adenosine agonists reduce locomotor activity whereas D_2 agonists enhance it, and adenosine antagonists increase locomotion whereas dopamine D_2 receptor antagonists reduce it. Heffner (9) reported that adenosine agonists produce behavioral effects similar to those produced by dopamine antagonists that are clinically active as antipsychotic agents. Specifically, adenosine agonists have been shown to reduce locomotor behavior in mice, decrease amphetamine-induced activity, and inhibit apomorphine-induced cage climb-

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ing in mice. Based upon their observations, Heffner et al. (9) proposed that adenosine agonists might be effective antipsychotic agents because they have a pharmacological profile similar to D_2 receptor antagonists and all antipsychotic agents have some dopamine D_2 receptor blocking properties (22).

The purpose of the present experiments was to examine the activity of several prototypic adenosine agonists on conditioned avoidance responding (CAR) in the Fischer 344 rat. Blockade of CAR is a property shared by all known antipsychotic agents. The potency in blocking CAR in the rat is highly correlated with potency in treating the symptoms of schizophrenia in man (1,13). To measure CAR, the paradigm to be employed utilizes a discrete trial lever press response to avoid an impending signaled footshock. The model has a built-in index for sedation in that it also measures loss of responding to escape the footshock. This paradigm has been used as a screen to identify potential antipsychotic agents (16–18,21). Adenosine agonists that were shown to be active in the CAR procedure were also tested for the induction of catalepsy, an indicator of extra pyramidal side effect potential.

METHOD

Radioligand Binding

Receptor binding methodology was modified from that described earlier (10). Rat striatal and cortical tissue, obtained from Fischer 344 rats, was homogenized using a Brinkman polytron (setting 6, 20 s) in 20 vol ice-cold 50 mM Tris-HCl, pH 7.4. This membrane homogenate was then centrifuged at 48,000 × g for 10 min at 4°C. The resulting pellet was resuspended in buffer containing 2 IU/ml adenosine deaminase (Type III, Boehringer Mannheim, Indianapolis, IN) to 20 mg/ml original tissue weight and incubated at 37°C for 30 min to remove endogenous adenosine. This membrane homogenate was recentrifuged and the final pellet was frozen at −70°C until the time of assay.

Routine ligand competition binding assays were conducted with 5 nM (2-(*p*-(carboxyethyl)phenethylamino)-5′-*N*-ethylcarboxamido adenosine ([^{3}H]CGS 21680) (48 Ci/mmol) to label adenosine A_2 receptors in striatal membranes and 1 nM [^{3}H]cyclohexyladenosine ([^{3}H]CHA, 25 Ci/mmol) to label adenosine A_1 receptors in cortical membranes. Binding incubations were carried out, in triplicate, in 12 × 75-mm polypropylene test tubes containing an aliquot of brain membranes (100–200 μg/ml protein/ml) in 50 mM Tris-HCl, pH 7.4 (+10 mM $MgCl_2$ for the adenosine A_2 assay) and appropriate concentrations (n = 8–10) of test compounds in a final volume of 1 ml. All assays were conducted at 23°C for 2 h and nonspecific binding was defined in the presence of 20 μM 2-chloroadenosine. Binding reactions were terminated by filtration through Whatman GF/B filters (Whatman, Clifton, NJ) under reduced pressure using a Brandel cell harvester (Brandel, Gaithersburg, MD). Filters were washed twice with ice-cold buffer and bound radioactivity was determined by conventional liquid scintillation spectroscopy at an efficiency of 50–60%. IC_{50} values were determined using nonlinear regression and K_i values determined using the methods of Cheng and Prusoff (3).

Conditioned Avoidance Responding

Female Fischer 344 rats (Taconic Farms, Germantown, NY), weighing 250–350 g, were trained to perform the CAR task, which consisted of depressing a lever to avoid an impending shock. The Fischer 344 strain was used because it has been reported that this strain learns the CAR task rapidly (16). The testing apparatus consisted of eight Lafayette Instrument Modular Testing units (Lafayette Instruments, West Lafayette, IN), each of which was contained in a sound-attenuating chamber.

To begin the 1-h test session, a rat that had previously been trained in the CAR paradigm was placed in the testing cage. The test consisted of 60 discrete trials spaced at 1 per min. The conditioned stimuli were a paired light and tone that were presented to the rat for 10 s before a 0.7-mAmp footshock was delivered via the metal grid floor of the cage. A lever press during the conditioned stimulus period turned off the stimuli and avoided the shock. In the absence of an avoidance lever press, the shock would be delivered for 5 s beginning at the end of the 10-s warning period. A lever press during the 5-s shock period terminated both the light- and tone-conditioned stimuli and the shock. The latter was termed an escape response.

To determine block of CAR, each rat served as its own control. On day 1, each animal was pretreated with 0.9% saline and tested for CAR in a 1-h test session. Only animals avoiding ≥54 of the 60 shocks were used the following test day, when test compounds were administered IP. Percent reduction in CAR was calculated as the percent fall in CAR responses observed on the test day relative to the responses seen on the control day. To determine ED_{50} values, a range of doses of each test agent was utilized (n = 6–8 per dose). Linear regression was then used to determine the value at which CAR performance was reduced by 50% (ED_{50} value) together with 95% confidence limits.

If the rat failed to respond during the warning or shock periods, it received a full 5-s shock and the absence of either an avoidance or escape response was termed a failure. If the rat suffered five consecutive failures, the session was automatically terminated. Failure to escape is a sign of general sedation. The most effective side effect-free antipsychotic agents will reduce CAR without reducing escape responding. All purinergic agonists were given IP 30 min prior to the start of the test session. Dose-response curves for standard antipsychotic agents were also determined as a reference.

Caffeine Antagonism of Purinergic Block of CAR

To determine whether block of CAR produced by purinergic agents is reversed by an adenosine antagonist (caffeine), a single dose of the purinergic agents was tested. The dose used was the ED_{60} value determined from the block of CAR experiments. A counterbalanced design was utilized with a final n = 8 animals per group. A dose of caffeine of 25 mg/kg IP was given 10 min prior to the purinergic agonist, which was given 30 min prior to the CAR session (60 trials/60 min). In pilot studies, this dose of caffeine was determined to exert no effect on CAR when given alone. Each rat was given two doses of the agonist separated by an interval of at least 1 week. Half the rats in each group were pretreated with vehicle prior to the first treatment with the purinergic agent and half given caffeine. The following week, the pretreatments were switched. Any animal in which, following vehicle treatment, the purinergic agent failed to produce at least a 20% block of CAR was eliminated from the final calculations. Assuming the purinergic agent tested is effective in all animals, the paradigm should render an n of 8. Block of CAR was compared between treated and untreated rats using a paired t-test ($p < 0.05$). Because our supply of this agent was expended, CV-1808 was examined neither in this assay nor in the catalepsy experiment.

TABLE 1

K_i VALUE (±SEM) DERIVED FOR ADENOSINE AGONISTS IN INHIBITING BINDING TO BRAIN A_1 AND A_2 RECEPTORS

	Binding Site		
	K_i (nM)		
Compound	A_1	A_2	A_1 Selectivity*
R-PIA	0.7 ± 0.02	202 ± 23	288
CPA	0.9 ± 0.05	850 ± 60	944
NECA	3.8 ± 0.2	17 ± 1.5	4.5
CV-1808	303 ± 10	85 ± 10	0.3
CGS 21680	2976 ± 75	18 ± 1.5	0.006

Values represent means (± SEM) determined from at least three separate experiments.
*A_2/A_1

Catalepsy

Catalepsy was measured in female Fischer 344 rats, weighing 150–300 g. Each animal was housed individually. The purinergic agents, dissolved in the appropriate vehicle, were given at the same pretreatment time used in the CAR test. The doses selected were the ED_{25}, ED_{50}, and ED_{75} values determined from the regression line calculated previously for block of CAR experiments.

To measure catalepsy, a 3-cm cork was used. The rat's forearm was positioned on the cork and the time to remove the forearm measured. A cut-off time of 60 s was employed. Each rat was tested three times. The sum of time spent on the cork (seconds) was divided by the maximum possible time (180 s) and multiplied by 100 to determine the percent catalepsy score (a number ranging from 0 → 100).

$$\% \text{ Catalepsy} = \frac{[(\text{sum of 3 times on cork) s}]}{(180 \text{ s})} \times 100.$$

Each dose of each drug was given to six rats. As a positive control, haloperidol (0.4 mg/kg, IP) was given in a dose that should produce ~50% catalepsy and, as a further control, three animals were given vehicle each time an adenosine agent was tested. Animals were rated using a random and blind scoring system. The data were expressed as mean percent catalepsy for each dose given. All results will be compared with the CAR data to estimate side effect liability of the purinergic agent.

Drugs

Chlorpromazine, thioridazine, trifluoperazine, haloperidol, and metoclopramide were used as standard antipsy-

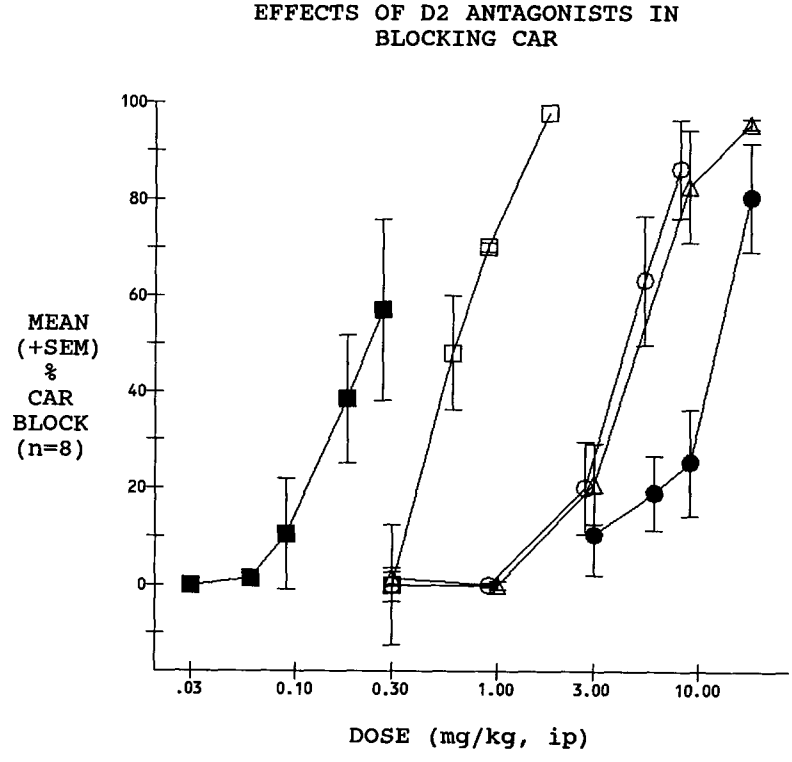

FIG. 1. Dose-response curves generated by standard antipsychotic agents that act as D_2 dopamine receptor antagonists. The graph shows mean (±SEM) percent inhibition of conditioned avoidance responding (CAR) plotted as a function of the dose administered. The following drugs are depicted by the indicated symbol: haloperidol (−■−); trifluoperazine (−□−); chlorpromazine (−○−); metoclopramide (−△−); and thioridazine (−●−). Each dose of each agent was administered to eight rats.

TABLE 2

ED_{50} VALUES DERIVED FOR BLOCK OF CAR FOR DOPAMINE RECEPTOR ANTAGONISTS AND PURINERGIC AGONISTS

Compound	Receptor	CAR (mg/kg, IP) [95% CL]	% Loss of Escape Responding at ED_{50} Value*
Chlorpromazine	D_2	4.9 [4.2, 5.9]	6.6
Thioridazine	D_2	12.5 [10.5, 15.3]	1.6
Trifluoperazine	D_2	0.9 [0.7, 1.0]	2.5
Haldol	D_2	0.23 [0.18, 0.39]	3.4
Metoclopramide	D_2	7.8 [6.4, 9.2]	8.1
R-PIA	A_1	0.34 [0.23, 0.44]	1.1
CV-1808	A_2	1.3 [1.0, 1.8]	4.0
CPA	A_1	1.5 [1.3, 1.7]	1.5
CGS 21680	A_2	1.1 [0.8, 2.0]	1.5
NECA	$A_1 + A_2$	0.07 [0.004, 0.12]	3.8

*Estimated.

chotic/antidopaminergic agents. *R*-Phenylisopropyladenosine (*R*-PIA) and cyclopentyladenosine (CPA) were used as A_1-selective adenosine agonists, whereas 2-phenylamino adenosine (CV 1808) and CGS 21680 were used as selective A_2 agonists. *N*-Ethylcarboxamido adenosine (NECA), an agonist at both A_1 and A_2 sites, was also examined. All compounds were obtained from Research Biochemicals, Inc. (Natick, MA).

RESULTS

Receptor Binding Assay

The data derived in the A_1 and A_2 binding assays are shown in Table 1.Shown in the table are K_i values for each agent at each binding site together with a selectivity index for the A_1 binding site. The selectivity index was derived by dividing the K_i values at the A_2 site by the K_i value at the A_1 site. The greater this number, the greater the selectivity for the A_1 binding site. Conversely, the smaller this number, the greater the selectivity toward the A_2 site. The agents most selective for the A_1 site were *R*-PIA and CPA, whereas the most selective agent and potent agent at the A_2 site was CGS 21680.

Dose-response curves for block of CAR in the rat following administration of standard antipsychotic compounds are shown in Fig. 1. Haldol was the most potent agent with an ED_{50} value determined to be 0.23 mg/kg IP. The order of greatest to least potency for the antipsychotic agents was (as shown in Fig. 1 and Table 2) haloperidol, trifluoperazine, chlorpromazine, metoclopramide, and thioridazine. Each agent produced dose-related falls in CAR. Shown in Table 2 are the ED_{50} values derived for each agent as well as the % loss of escape responding extrapolated from the ED_{50} value. The

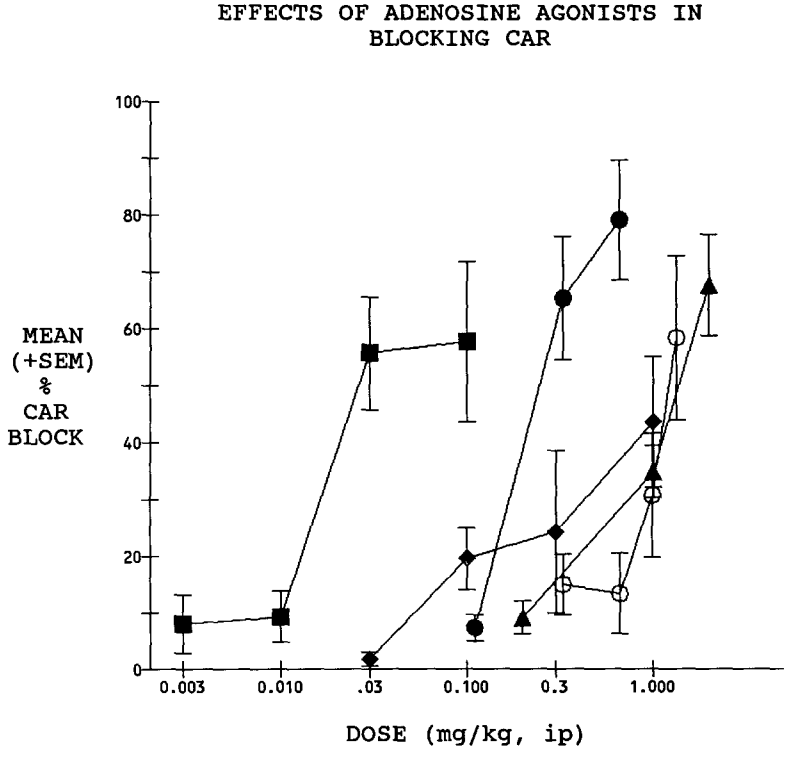

FIG. 2. Dose-response curves obtained following administration of adenosine agonists in the conditioned avoidance response (CAR) paradigm. The graph depicts mean (±SEM) % block of CAR observed during the 1-h observation period following pretreatment (−30 min) with the indicated agent. The curves are drawn for: *n*-carboxamido adenosine (NECA) (−■−); (2-(*p*-(carboxyethyl)phenethylamino)-5′-*N*-ethyl-carboxamido adenosine (CGS 21680) (−◆−); *R*)-phenylisopropyl adenosine (*R*-PIA) (−●−); cyclopentyl adenosine (CPA) (−▲−); and CV-1808 (−○−). Each agent was given to six to eight rats per dose.

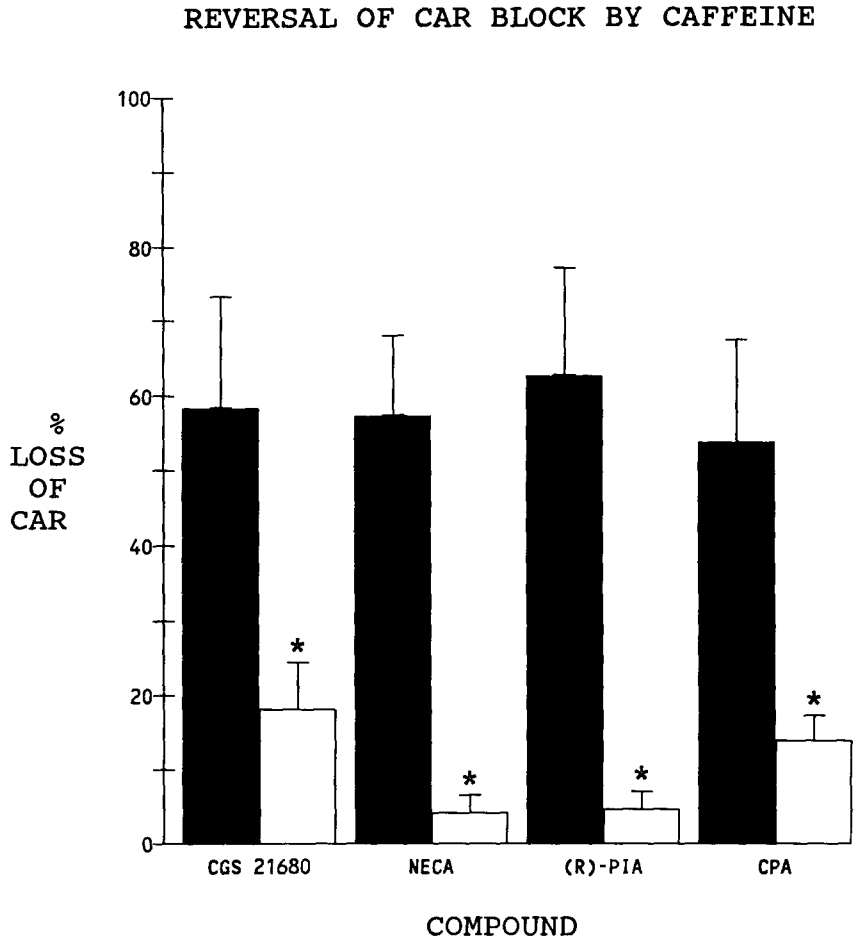

FIG. 3. Block of inhibition of conditioned avoidance response (CAR) induced by adenosine agonists by the prior treatment with caffeine (25 mg/kg, IP). The black bars indicate the mean (± SEM) % block of CAR produced by the indicated agonist alone (■), whereas the open bars indicate the mean ± SEM % of CAR produced by the agonist after treatment with caffeine. Five to seven rats were utilized per compound. Each rat was given two injections of the agonist, once alone and once following caffeine pretreatment using a counterbalanced design.

% loss of escape number in the table estimates the loss of escape responses that would be predicted to occur at the ED_{50} dose level for block of CAR.

Dose-response curves for adenosine A_1 and/or A_2 agonists are shown in Fig. 2. As can be seen in the figure, each agent produced a dose-related block of CAR. The ED_{50} values derived for each agent, together with the estimated loss of escape responding at the ED_{50} dose level, are shown in Table 2.

Each of the adenosine agonists was effective in blocking CAR, but NECA was clearly the most potent agent of the compounds tested, with an ED_{50} value determined to be 70 μg/kg IP. *R*-PIA was the next most potent, with an ED_{50} value of 0.34 mg/kg, whereas each of the other purinergic agents clustered together with ED_{50} values residing between 1.1 and 1.5 mg/kg (Table 2). Also listed in Table 2 are the estimates for loss of escape responding for the adenosine agonists.

Although NECA had the lowest ED_{50} value, the maximum % block of CAR observed was only 57%. Further, administration of NECA at the greater doses of 0.3 or 1.0 mg/kg resulted in the death of all rats treated within a 24-h period. CPA produced noticeable muscle flaccidity at all dose levels. Because some rats treated with the adenosine agonists displayed obvious flaccidity and loss of muscle tone, it was somewhat surprising to observe their loss of escape scores did not differ from those of rats treated with antidopaminergic compounds (Table 2).

CGS 21680 and CV 1808, on the other hand, produced no noticeable untoward side effects. CGS 21680, however, was the least active of all agents tested, producing only a 43.6 ± 1.1% (± SEM) inhibition of CAR at the top dose of 1.0 mg/kg IP. Pretreatment with caffeine (25 mg/kg, IP) 10 min prior to CGS 21680, NECA, *R*-PIA, or CPA, each administered at an ED_{60} dose level, produced a significant fall in the block of CAR produced by each agent. The mean % loss of avoidance produced by each agent alone or following caffeine treatment is shown in Fig. 3.

Catalepsy

Given at the calculated ED_{25}, ED_{50}, and ED_{75} values for block of CAR, each of the four adenosine agonists produced some catalepsy. The ED_{50} value for catalepsy derived for CPA

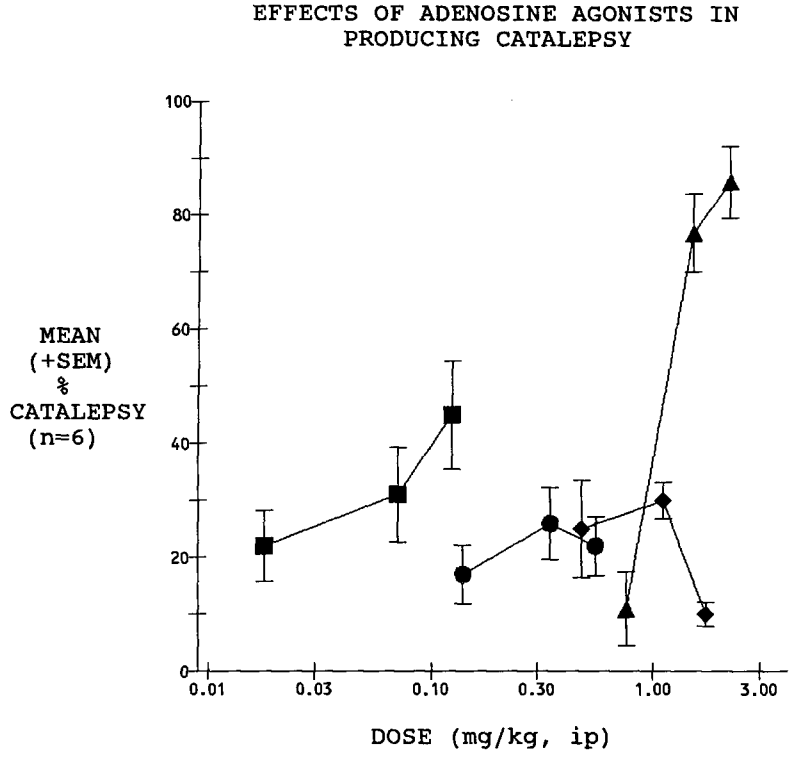

FIG. 4. Curves depicting the dose-response relationship between catalepsy and adenosine agents. Each point represents the mean ± SEM catalepsy score derived for the indicated agent at the dose level administered. Data are shown for: *n*-carboxamido adenosine (NECA) (−■−); *R*)-phenylisopropyl adenosine (*R*-PIA) (−●−); (2-(*p*-(carboxyethyl)phenethylamino)-5′-*N*-ethyl-carboxamido adenosine (CGS 21680) (−◆−); and cyclopentyl adenosine (CPA) (−▲−). Each dose of each agent was given to six rats.

was 1.3 mg/kg, which is actually less than the ED_{50} value for block of CAR (1.5 mg/kg, IP). The ED_{50} value for NECA-induced catalepsy was shifted approximately 2.1-fold greater than the ED_{50} value for block of CAR (0.07 mg/kg, IP). The 45 ± 9.4% catalepsy observed after the 0.12-mg/kg dose of NECA is a high score. The catalepsy dose-response curves derived for both *R*-PIA and CGS 21680 were relatively flat (Fig. 4) and ED_{50} values were not computed. Neither produced as much as 30% catalepsy at any of the doses tested. Haloperidol (0.4 mg/kg) was run as a positive control ($n = 3 \times 4$ experiments) for each of the dose-response curves. Distilled water was given as another control. The mean (±SEM) catalepsy score produced by haloperidol was 86.6 ± 6.6%, whereas only 12.1 ± 5.9% catalepsy was observed in water-treated rats ($n = 12$, for each group).

DISCUSSION

Adenosine agonists, regardless of their apparent selectivity or affinity for either adenosine receptor subtype, were active in vivo in blocking CAR, a behavioral measure that has been shown to be predictive of antipsychotic efficacy in humans (1,13,18). The nonselective adenosine agonist, NECA, was found to be significantly more potent in blocking CAR than either A_1- or A_2-selective agonists. In general, the activity of adenosine agonists in blocking CAR was not found to be correlated with their respective binding affinities for either the A_1 or A_2 receptor subtypes. These observations stand in contrast to other reports indicating that adenosine agonist-induced decreases in locomotor activity are mediated by activation of brain A_2 receptors (6).

The present data, however, are consistent with a recent demonstration that NECA was significantly more potent than either A_2- and A_1-selective agonists in reducing locomotor behavior in mice (19). An isobolographic analysis of the interaction of various adenosine agonists indicated that the potency of NECA in reducing motor activity may be related to an in vivo synergistic effect resulting from the activation of both adenosine receptor subtypes (19). The fact that NECA was significantly more potent than either CGS 21680 or CPA in the CAR procedure suggests the possibility of a similar in vivo synergistic action.

Relative to the standard antipsychotic agents used in this study, the adenosine agonists were not as efficacious in blocking CAR. NECA, which has the lowest ED_{50} value for block of CAR of all the compounds examined, did not produce a 100% block of the CAR response to sublethal doses. However, the D_2 receptor antagonists were found to fully block CAR responding. The nonselective adenosine antagonist, caffeine (25 mg/kg, IP), was found to markedly attenuate the ability of the adenosine agonists to disrupt CAR responding and indicates that these effects are mediated by adenosine receptors.

Animals treated with the adenosine agonists at times dis-

played the flaccidity and loss of spontaneous locomotor activity that has been previously reported for these agents (8,25). It was somewhat surprising, therefore, when animals treated with the adenosine agonists did not display greater loss of escape responding during the CAR sessions. The % loss of escape responding, in fact, was less or equal to that observed following treatment with the standard antipsychotic agents. It seems that this measure may not be sensitive in detecting behavioral deficits following treatment with adenosine agents. Clearly, loss of escape responding detects the not so subtle effects of a narcotic or anesthetic agent but did not detect the side effects of purinergic agents. The measurement of catalepsy, on the other hand, provided a measure of behavioral impairment seemingly more in line with the observed posture of the animals.

Although the results in blocking CAR show promise in terms of a therapeutic application for adenosine agonists, the catalepsy data point out the area for greatest concern. The ideal profile of a novel antipsychotic agent would be a compound active in CAR but lacking cataleptic potential. Clozapine is such an agent (21). Haloperidol, which is prone to produce extrapyramidal side effects in the clinic, has an ED_{50} for catalepsy that is only twice its ED_{50} for CAR block (unpublished observations). NECA, although potent in blocking CAR, produced catalepsy at dose levels that were not greatly different (ED_{50} for CAR = 0.07; ED_{50} for catalepsy = 0.15). The ED_{50} value determined for CPA in producing catalepsy (1.3 mg/kg) was actually less than that required to block CAR (1.5 mg/kg, IP). Of more interest were *R*-PIA and CGS 21680, which failed to produce catalepsy in a dose-related manner. This finding indicates that it may be possible to synthesize an agent that blocks CAR with little or no side effects. Perhaps A_2-selective agents would have a lower tendency for producing catalepsy or behavioral side effects. No untoward behavioral effects of CGS 21680 were noticed during the testing sessions. The relative lack of cataleptic potential for *R*-PIA is enigmatic because NECA and CPA (other A_1 active agents) did produce catalepsy. Further work will be required to determine the crucial determinants of the catalepsy.

In summary, the present experiments demonstrate that adenosine agonists, be they active at A_1, A_2, or both binding sites, are active in vivo in a preclinical test of antipsychotic efficacy. The results from the catalepsy test underscore the area of greatest liability for such agents as viable antipsychotic agents. No attempt was made in these tests to quantify the possible changes in hemodynamics that such agents should produce. Such changes could lead to undesirable side effects. The results, taken with those reported by Heffner et al. (9), are intriguing enough for further investigation of adenosine agonists as antipsychotic agents. Clearly, an adenosine agonist with no side effect liability may be a therapeutic agent of the future for schizophrenia.

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