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Adenosinergic Modulation of the EEG and Locomotor Effects of the A_2 Agonist, CGS 21680

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JANUSZ, C. A. AND R. F. BERMAN. *Adenosinergic modulation of the EEG and locomotor effects of the A_2 agonist, CGS 21680.* PHARMACOL BIOCHEM BEHAV. 45(4) 913–919, 1993.—The present study in rats was designed to identify the respective roles of A_1 and A_2 adenosine receptor activation in the anticonvulsant and behavioral actions of adenosine. Intracaudate injections of the highly selective A_2 agonist, CGS 21680, did not affect caudate seizures. However, seizure threshold was increased in the presence of CGS 21680 after blockade of the A_1 receptor with CPX, or following activation of the A_1 receptor with R-PIA or NECA. Additionally, CGS 21680 led to a dose-related inhibition of locomotor activity when injected into the caudate. These results implicate the involvement of the A_2 adenosine receptor in the locomotor depressant actions of adenosine and also suggest possible A_2 anticonvulsant effects may depend upon the activation of the A_1 receptor.

Adenosine Anticonvulsant Locomotor activity Alkylxanthine Caudate nucleus

ADENOSINE and adenosine agonists have anticonvulsant effects in a wide variety of seizure models including, electroshock seizures (8), audiogenic seizures in sensitive rats (8) and mice (24), chemically induced seizures (13,15,31), and kindled seizures (5,34). In contrast, adenosine antagonists increase seizure activity (2,3,12,25,26) and block the anticonvulsant activity of adenosine agonists at concentrations, which have no effect on seizures when given alone (5). The anticonvulsant effects of adenosine have been linked to the activation of the A_1 adenosine receptor (5,7,10). Few studies have addressed the role of A_2 receptor activation in the anticonvulsant actions of adenosine, primarily due to the lack of highly selective A_2 compounds. In a previous study, ICV injections of the recently developed, highly selective A_2 adenosine agonist, 2-[4-(2-carboxyethyl) phenethylamino]-5'-*N*-ethylcarboxamidoadenosine hydrochloride (CGS 21680) (18), did not alter the afterdischarge duration or behavioral manifestation of an amygdala kindled seizure (20). However, CGS 21680 did significantly lengthen the postictal EEG depression associated with the kindled seizure. The following study was designed to further define the respective roles of A_1 and A_2 receptor activation in the anticonvulsant actions of adenosine. In this study, we examined the EEG and behavioral effects of direct injections of CGS 21680 into the caudate nucleus, an area known to contain a high density of adenosine A_1 and A_2 receptors (22).

METHOD

Stereotaxic Surgery

Male, Long-Evans rats (Harlan Sprague-Dawley Farms; 300–350 g) were used. Rats were anesthetized with sodium pentobarbital (60 mg/kg IP) and a chronic indwelling chemitrode was stereotaxically implanted into the caudate nucleus. The chemitrode allowed for consecutive stimulation, recording, and intracranial drug injection at the same brain site. It consisted of a bipolar electrode (MS 303/2, Plastic Products One, Roanoke, VA) cemented with Insl-x (Insl-x Products Corp., Yonkers, NY) to a 22-ga stainless-steel cannula. A 28-ga obturator blocked the cannula until the time of injection. The electrode extended 0.5 mm beyond the end of the cannula. The stereotaxic coordinates for implantation were 1.0 mm anterior to bregma, ±2.5 mm lateral to midline, and 5.4 mm ventral to the skull surface (30). The electrode assembly was anchored to the skull using acrylic cement and two stainless-steel screws, with a third screw serving as ground. Animals were allowed 1 week postsurgical recovery before experiments began. Rats were housed in an environmentally

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controlled vivarium under 12 h light-dark cycles. Food and water were available ad lib.

Afterdischarge Threshold Determination

Electrical stimulation was delivered through the implanted electrode using a Grass Model S88 stimulator equipped with two PSIU6 constant current units. A Tektronix 502 dual-beam oscilloscope was used to monitor the amplitude of the electrical stimulation, while the EEGs were recorded at 50 μV/cm on a Grass Model 7 Polygraph. A switch allowed for consecutive stimulation and recording from the same implanted electrode.

One week postsurgery, rats were placed into the recording chamber and a minimum of 1 min EEG baseline was recorded before determining the afterdischarge (AD) threshold. The AD threshold current was determined by initially stimulating the rat at 67 μA and increasing the current in 33 μA increments every min until a brief 4-6 s epileptiform AD was elicited. This level of stimulation was defined as the AD threshold. The stimulation consisted of a 1-s train of 100 Hz, biphasic, symmetrical square waves 1 ms in duration.

Drug Injections

The following day, baseline EEG was again recorded and then each rat received an intracaudate injection of a 1-3 μl solution of drug or vehicle using a 28-ga stainless-steel injector attached to a Hamilton microliter syringe. The injector was fitted into the cannula and protruded 0.5 mm beyond the end of the cannula. The injection was made at a rate of 1 μl/min. The 3-μl volume was only used for the highest CGS 21680 dose (i.e., 75 μg). Upon removal of the injector, EEG and behavior were monitored for 10 min. The rat was then placed into one of eight chambers of a Digiscan Activity Monitor (Omnitech Electronics Inc., Columbus, OH) for 5 min to record locomotor activity. At the end of the 5 min period, the rat was placed back into the recording chamber where his EEG and behavior were monitored for an additional 5 min. At 20 min postinjection, the AD threshold was redetermined, beginning at 67 μA and increasing in 33 μA increments. For analysis purposes, animals that failed to have an AD when tested up to 1000 μA were assigned a maximum value of 1000 μA. Seizure stage (33), AD duration and AD amplitude were recorded. At 30 min postinjection, rats were again placed into a chamber of the Digiscan Activity Monitor and the rat's locomotor activity analyzed for an additional 5 min. The doses of CGS 21680 used were 32 μg (55.6 nmoles), 50 μg (86.8 nmoles) and 75 μg (130.3 nmoles).

To compare the effects of A_2 activation with the effects of A_1 activation, the effects of the selective A_1 agonist L-phenyisopropyladenosine (R-PIA) and the nonselective adenosine agonist 5'-*N*- ethylcarboxamidoadenosine (NECA) on seizure threshold were examined. Rats were injected into the caudate with 34 μg (86.8 nmoles) of R-PIA. This concentration was chosen to be equimolar (86.8 nmoles) to the 50 μg dose of CGS 21680. Because of the high potency of NECA, a concentration of 7-μg/rat NECA was used (0.25 $\times$ the equimolar concentration of 50 μg CGS 21680 or 21.3 nmoles). The rest of the experiment was as described above.

Highly selective A_2 antagonists are not yet available. Therefore, the effects of the highly selective A_1 antagonist, 8-cyclopentyl-1,3- dipropylxanthine (CPX) on CGS 21680, R-PIA and NECA-induced effects on seizure threshold were examined. We predicted that CPX would block behavioral and electrophysiological effects of R-PIA, but would be less effective against NECA and CGS 21680, respectively. Rats were injected via chemitrode with 27 μg (86.8 nmoles) CPX 5 min before intracaudate injection of equimolar (86.8 nmoles) concentrations of CGS 21680 (50 μg), R-PIA (34 μg) or 7-μg NECA. The rest of the experiment was as described above.

All drugs were obtained from Research Biochemicals, Inc. (Natick, MA) except CGS 21680 which was a gift from M. Jarvis, Ciba Geigy (Summit, NJ). CGS 21680 was dissolved in saline, while R-PIA, CPX, and NECA were dissolved in dimethyl sulfoxide (DMSO). Saline and DMSO were used as vehicle injections. Doses of CGS 21680 were 32 μg/μl, 50 μg/2 μl and 75 μg/3 μl. Rats received a minimum of 1 injection per week, for a total of 2-5 injections per rat. Baseline threshold was redetermined the day previous to each drug or vehicle injection. Threshold determined under drug or vehicle influence was then converted to a percentage of the previous day's (baseline) threshold.

Statistics and Histology

Data were analyzed by Student's *t*-test, one-way and repeated measures multivariate analysis of variance (MANOVA) (BMDP386, Los Angeles, CA), as appropriate. Statistical tests were two-tailed except where stated. A Dixon's type test was used to identify outliers, which were then replaced with the group mean value (9). Data are expressed as mean $\pm$ SEM. Differences were reported as significant if $p < 0.05$.

At the end of the study, all rats were heavily anesthetized with sodium pentobarbital (120 mg/kg, IP). Three of the rats were perfused transcardially with 0.9% saline and 10% buffered formalin, and the precise chemitrode placements within the caudate were verified histologically. In the remaining 30 rats, the electrode placements were determined by gross histological examination.

RESULTS

Histology

Histological examination of the brains of the caudate implanted animals revealed that the majority of chemitrodes were located within the caudate. In one rat, the electrode tip extended too deep and into the piriform cortex. Data from this rat was only used in the locomotor analysis as the cannula was located in the caudate. In another rat, although the chemitrode was in the caudate, the AD threshold was extremely variable and data from this animal was not included in the electrophysiological analysis. Last, an AD was not elicited in three rats, due to shallow placement of the chemitrode. In these rats, a longer injector (1.0 mm beyond the tip of the cannula) was used so that the data from these animals could still be included in the locomotor analysis. Injection sites within the caudate of these animals were confirmed histologically at the end of the experiment. In addition, it was observed that the lateral ventricle on the side of the implanted chemitrode was typically dilated, although the significance of this finding to the present results is unknown.

AD Thresholds

The mean threshold current necessary to elicit an AD in the caudate was 221.1 $\pm$ 17.9 μA (n = 28). The final threshold current determined on each rat following all injections was not significantly different from the initial threshold (213 $\pm$ 17.8 μA, paired *t*-test). Thus, the baseline caudate thresholds remained stable in these rats. Interestingly, in response to each stimulation, the caudate subjects immediately fell over, then

regained their equilibrium before displaying stereotyped motor convulsive behavior. The first caudate stimulation typically elicited the falling response followed by a Stage 2 motor seizure (2.1 ± 0.2).

Saline and DMSO vehicle injections did not affect seizure threshold, amplitude, or duration and were not significantly different from each other. Therefore, each drug treatment was compared to its vehicle control.

The effects of CGS 21680 on AD threshold, duration and amplitude are presented in Table 1. As shown in the table, CGS 21680 had no significant effect on caudate AD threshold or AD duration. However, the amplitude of the AD was significantly decreased by the 75-μg dose of CGS 21680 compared to saline injections ($p < 0.01$). In addition, the effects of 50- and 75-μg CGS 21680 on AD amplitude were significantly different than those of 32-μg CGS 21680 ($p < 0.05$). The effects of CPX injections are also shown in Table 1. CPX, at an equimolar concentration to 50-μg CGS 21680 did not significantly affect AD threshold, duration, or amplitude when compared to DMSO vehicle injections. The effects of single or combined equimolar injections of CGS 21680, R-PIA and CPX are shown in Fig. 1 and Table 2. At equimolar concentrations, CPX (27 μg) injected 5 min before CGS 21680 (50 μg) led to a significant increase in AD threshold compared to CGS 21680 alone and vehicle injection ($p < 0.01$). AD duration was not significantly affected, but AD amplitude was significantly decreased by the combination of CPX and CGS compared to saline ($p < 0.05$) (Table 2). The effects of the A_1 selective agonist, R-PIA, on seizure threshold, duration, and amplitude are also shown in Fig. 1 and Table 2. R-PIA (34 μg), an equimolar concentration to 50 μg CGS 21680, led to a significant increase in AD threshold compared to vehicle injections ($p < 0.05$), but did not affect AD duration or amplitude. Injection of 27 μg CPX given 5 min before equimolar (34 μg) R-PIA blocked the R-PIA-induced increase in seizure threshold. The combination of CPX with R-PIA did not significantly affect AD duration or amplitude (Table 2). Unexpectedly, the equimolar combination of the A_1 agonist R-PIA (34 μg) and the A_2 agonist CGS 21680 (50 μg) led to an even greater increase in seizure threshold than R-PIA alone ($p < 0.01$). R-PIA and CGS 21680 combined did not significantly affect AD duration or amplitude (Table 2).

The highly potent, nonselective (K_i = 6.3 nM and 10.3 nM at A_1 and A_2 receptors, respectively; 19), adenosine agonist, NECA when injected in an equimolar concentration to 50 μg CGS 21680, caused profound cardiovascular and respiratory depression. These rats required theophylline treatment (10 mg/kg, IP), to reverse the effects of NECA. Thus, an equimolar concentration of NECA could not be used to study seizure thresholds. However, a 7-μg dose of NECA was found (Fig. 1), which increased seizure threshold ($p < 0.01$) without producing profound cardiovascular and respiratory effects. This dose of 7-μg/rat NECA was one-quarter the equimolar concentration of 50-μg CGS 21680. NECA, at a concentration of 7 μg, did not affect AD duration or amplitude (Table 2). When CPX was given 5 min before administration of NECA, an increase in seizure threshold was observed compared to NECA alone ($p < 0.05$) (Fig. 1). AD duration was not signifi-

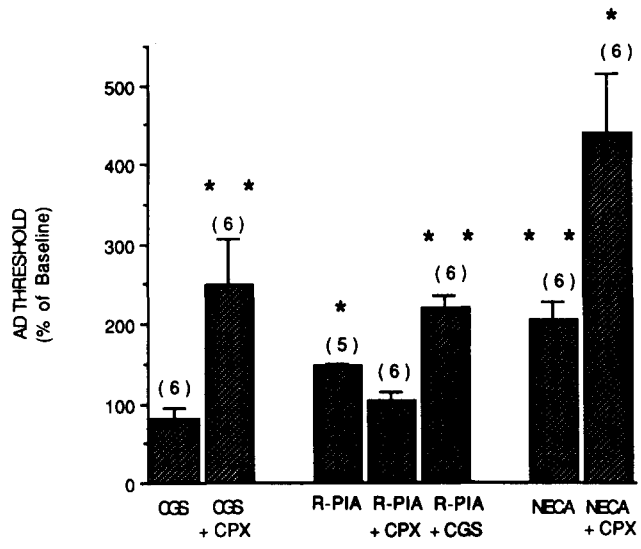

FIG. 1. The effect of CGS 21680 (CGS), CPX, R-PIA, and NECA alone and in combination on caudate seizure threshold. CGS 21680 had no effect on seizure threshold compared to vehicle injections (Data from Table 1). AD threshold was significantly increased by the combination of CGS + CPX. R-PIA also increased seizure threshold compared to vehicle injections, and these effects were reversed by CPX. Seizure threshold was again increased by the combination of R-PIA and CGS, compared to R-PIA alone and vehicle injections. NECA increased seizure threshold compared to vehicle injections and this effect was greater with the combination of CPX and NECA. Doses of the specific drugs were as follows: CGS 21680 50 μg, CPX 27 μg, R-PIA 34 μg, and NECA 7 μg/rat. The number of rats used under each condition is in parentheses. Error bars represent SEM (**$p < 0.01$, *$p < 0.05$).

TABLE 1

EFFECTS OF CGS 21680 ON CAUDATE SEIZURE THRESHOLD (MEAN ± SEM, % OF BASELINE)

Group	Dose (μg/rat)	n	AD Threshold	AD Duration	AD Amplitude
Saline	-	9	104.1 ± 14.8	134.6 ± 19.2	99.2 ± 8.1
DMSO	-	5	112.1 ± 11.4	74.6 ± 15.7	80.3 ± 11.7
CGS 21680	32	8	110.3 ± 12.6	133.0 ± 18.2	118.6 ± 6.7
	50	6	81.5 ± 12.9	145.6 ± 24.5	77.1 ± 4.6
	75	5	114.8 ± 22.5	114.7 ± 24.9	48.9 ± 13.9*
CPX	27	7	140.4 ± 23.7	112.2 ± 28.1	104.5 ± 7.2

AD = Afterdischarge.
*$p < 0.01$ compared to saline injected controls.

TABLE 2

EFFECTS OF ADENOSINE COMPOUNDS ON CAUDATE SEIZURE DURATION AND AMPLITUDE AS A PERCENT OF BASELINE (MEAN ± SEM)

Group	*n*	AD Duration	AD Amplitude
CGS + CPX	6	137 ± 19	59 ± 8*
R-PIA	5	71 ± 11	67 ± 18
R-PIA + CPX	6	80 ± 31	87 ± 29
R-PIA + CGS	6	135 ± 47	61 ± 14
NECA	6	117 ± 34	78 ± 26
NECA + CPX	6	57 ± 36	42 ± 8*

Doses of the specific drugs were as follows: CGS 21680-50 μg, CPX-27 μg, R-PIA-34 μg, and NECA-7 μg/rat.
AD = Afterdischarge.
*$p < 0.05$ compared to vehicle control injection.

cantly affected by this combination of agonist and antagonist, however, AD amplitude was significatly decreased compared to vehicle injection ($p < 0.05$) (Table 2). NECA and CPX combined appeared to decrease AD amplitude compared to NECA alone, but this effect was not significant.

Locomotor Activity

The effects of saline and DMSO intracaudate injections on locomotor behavior were not statistically different for any of the variables measured. For statistical purposes, the effects of each drug condition were compared to those of its individual vehicle control.

The effects of CGS 21680 on locomotor activity at 10 and 30 min after injections are presented in Table 3. A multivariate ANOVA showed a significant overall effect of CGS 21680 on locomotor activity ($p < 0.01$). Univariate statistics followed by individual comparisons for each locomotor variable indicated that CGS 21680 at 32, 50, and 75 μg/rat produced significant reductions in all measurements of locomotor activity, namely, total distance traveled (TOT DIST), number of movements (NO MOV), rearing activity (REAR ACT) and number of stereotypical grooming episodes (NO GRM) compared to saline injected controls at all three doses. In addition, the time spent at rest was significantly increased across all doses of CGS 21680 compared to saline injected controls. These effects were significant at 10 and 30 min postinjection, and there were no statistically significant differences in drug effects between 10 and 30 min. In fact, rats receiving the highest dose of CGS 21680 (i.e., 75 μg) remained inactive for over 3 h following drug injection.

The effects of intracaudate injections of CPX alone, R-PIA alone, and R-PIA and CGS 21680 in combination with equimolar CPX are shown in Table 4. A multivariate ANOVA indicated significant depressant effects of CPX on locomotor behavior ($p < 0.01$). Univariate statistics followed by individual comparisons showed that CPX reduced the number of movements at 10 and 30 min, and rearing and grooming were reduced 30 min postinjection.

Pretreatment with an equimolar concentration of the A_1 antagonist, CPX, did not block CGS 21680-induced locomotor depression, either at 10 or 30 min post injection (Table 4). There were no significant differences between the effects of CGS 21680 alone (data shown in Table 3), and when given following CPX pretreatment (Table 4).

R-PIA at 34 μg, a dose equimolar to 50-μg CGS 21680, also produced profound depression of locomotor activity at 10 and 30 min after injection for all locomotor variables measured. Again, pretreatment with equimolar CPX failed to reverse the depression in locomotor activity at 10 or 30 min after injection. The depressant effects of R-PIA when given alone were not significantly different from those following pretreatment with CPX.

The effects of intracaudate NECA injections on locomotor activity are shown in Table 5. NECA, at a dose of 7 μg, resulted in nearly complete suppression of all locomotor activity at 10 min after injection compared to saline controls (control data shown in Table 4). This effect persisted for several hours, and animals were not tested further. Again, pretreatment with CPX failed to reverse the locomotor depression

TABLE 3

LOCOMOTOR ACTIVITY AT 10 AND 30 MIN

Group (n)	Dose (μg/rat)	Tot. Dist.	No. Mov.	Rear Act.	g
		10 Min			
Saline (9)	-	1356.33 ± 178.7	56.9 ± 1.0	38.2 ± 4.2	42.4 ± 3.1
CGS 21680 (5)	32	497.2 ± 290.6*	23.6 ± 7.4†	10.6 ± 8.2†	17.0 ± 7.9†
(6)	50	306.5 ± 79.8†	15.0 ± 4.3†	8.2 ± 1.9†	10.8 ± 2.8†
(5)	75	242.4 ± 139.1†	11.6 ± 3.5†	4.6 ± 3.2†	7.4 ± 3.7†
		30 Min			
Saline (9)	-	1337.7 ± 236.5	50.9 ± 7.8	29.2 ± 4.9	38.0 ± 5.6
CGS 21680 (6)	32	373.5 ± 137.4†	25.0 ± 11.7*	7.8 ± 5.5†	12.5 ± 3.6†
(7)	50	74.9 ± 26.4†	8.1 ± 4.5†	0.6 ± 0.4†	4.6 ± 0.9†
(5)	75	36.2 ± 13.3†	5.2 ± 1.5†	0.8 ± 0.5†	2.6 ± 0.7†

Effects of intracaudate injection of CGS 21680 on locomotor activity at 10 and 30 min post-injection. All locomotor measures are presented per 5 min. See text for details.
*$p < 0.05$, †$p < 0.01$, compared to saline injected controls.

TABLE 4

LOCOMOTOR ACTIVITY AT 10 AND 30 MIN

Group (n)	Dose (μg/rat)	Tot. Dist.	No. Mov.	Rear Act.	g
		10 Min			
DMSO (6)	-	1027.8 ± 91.2	61.0 ± 2.6	30.7 ± 2.7	36.8 ± 3.3
CPX (5)	27	1246.0 ± 276.7	45.4 ± 4.3*	35.4 ± 3.3	36.6 ± 10.5
CPX + CGS (7)	27 + 50	166.8 ± 68.4†	13.3 ± 4.1†	6.4 ± 3.8†	6.9 ± 2.1†
R-PIA (6)	34	106.0 ± 47.4†	9.0 ± 2.6†	3.0 ± 2.6†	5.8 ± 3.0†
CPX + R-PIA (7)	27 + 34	226.9 ± 93.7†	24.0 ± 7.1†	3.6 ± 2.0†	10.1 ± 4.6†
		30 Min			
DMSO (5)	-	1046.0 ± 173.0	49.0 ± 3.8	30.2 ± 4.5	32.6 ± 3.1
CPX (6)	27	602.3 ± 190.7	28.7 ± 5.8*	12.5 ± 2.6*	19.2 ± 3.9*
CPX + CGS (7)	27 + 50	138.1 ± 69.2*	10.8 ± 1.8†	4.0 ± 1.9†	6.3 ± 1.9†
R-PIA (6)	34	52.7 ± 32.5†	7.5 ± 3.6†	2.7 ± 1.8†	2.7 ± 1.7†
CPX + R-PIA (7)	27 + 34	120.3 ± 51.0†	15.0 ± 2.9†	1.6 ± 0.9†	8.3 ± 2.7†

Effects of single or combined injections of CPX, R-PIA, and CGS 21680 on locomotor activity at 10 and 30 min post-injection. All locomotor parameters are reported per 5 min.

*$p < 0.05$, †$p < 0.01$, compared to DMSO control injections.

produced by NECA. The locomotor effects of NECA did not differ significantly from those seen following pretreatment with CPX.

Finally, the combined equimolar injections of CGS 21680 (50 μg) and R-PIA (34 μg) into the caudate resulted in nearly total suppression of locomotor activity (Table 5). These effects were greater than the effects seen with R-PIA alone or CGS 21680 alone. Also, these effects were even greater than those following 7-μg NECA, but the apparent differences did not reach statistical significance.

DISCUSSION

This study provides evidence that the afterdischarge threshold and duration are not significantly affected by the A_2-specific agonist, CGS 21680, when injected directly into the caudate, an area containing a high density of A_2 receptors. Second, this study demonstrates depressed locomotor activity following direct central activation of A_2 receptors via intracaudate injections of CGS 21680.

Adenosine has been implicated in the suppression of seizures (21). Adenosine raises motor seizure thresholds in kindled rats (10). R-PIA, cyclohexyladenosine (CHA) and NECA also increase the kindled threshold in amygdala, hippocampal, and caudate kindled rats (34). However, fully kindled seizures involve seizure spread to other areas and obscure drug effects at the site of seizure initiation. Therefore, it is important to examine the effects of adenosine and its analogs on prekindled focal seizure thresholds, as was done in the present study, without the complicating factor of seizure spread to additional brain areas.

It was previously reported that papaverine, an adenosine uptake inhibitor, decreased initial seizure threshold, while theophylline, an adenosine antagonist, did not significantly affect prekindling thresholds (11). The authors concluded that endogenous adenosine is probably not involved in controlling the process of seizure initiation, contrary to the hypothesis proposed by others (21,34). A_1 and A_2 analogs were not examined in this earlier study (11) and thus, the possibility that adenosine could be involved in seizure initiation as well as seizure termination still existed.

In the present study, the A_1 agonist, R-PIA and the nonselective A_1/A_2 agonist, NECA produced significant increases in caudate seizure thresholds, while the highly selective A_2 agonist, CGS 21680 did not affect seizure threshold. One possible explanation for the difference in the effects of NECA and CGS 21680 is that CGS 21680 is selective for the A_{2a} receptor, while NECA interacts with both A_{2a} and A_{2b} recep-

TABLE 5

LOCOMOTOR ACTIVITY AT 10 MIN

Group (n)	Dose (μg/rat)	Tot. Dist.	No. Mov.	Rear Act.	g
NECA (5)	7	51.6 ± 30.1*	11.0 ± 4.8*	0*	0*
CPX + NECA (6)	27 + 7	82.0 ± 14.2*	23.0 ± 2.4*	0.8 ± 0.4*	6.5 ± 0.6*
R-PIA + CGS (5)	34 + 50	9.2 ± 3.6*	3.8 ± 1.5*	0*	0*

Effects of single NECA or combined CPX + NECA and R-PIA + CGS injections on locomotor activity at 10 min post-injection. All parameters are presented per 5 min.

*$p < 0.01$, compared to DMSO control injections. See text for details.

tors. The A_1 antagonist, CPX, also did not significantly affect seizure threshold. Though CPX, at the dose examined, did not have any effects on its own, it was able to block the increase in seizure threshold induced by an equimolar concentration of R-PIA, indicating a significant blockade of the A_1 receptor. Unexpectedly, the A_2 agonist, CGS 21680 raised caudate seizure threshold, but only after blockade of the A_1 receptor with CPX. In addition, the increase in seizure threshold induced by NECA was even more pronounced in the presence of CPX. Therefore, blocking A_1-receptor activity appears to uncover possible A_2 effects on seizure threshold indicating the possibility of a unique interaction between the A_1 and A_2 receptors.

The mechanism of this phenomenon is unclear, but could involve an interaction between the adenosine system and that of another transmitter in the caudate. For example, there is evidence for presynaptic A_1 receptors on dopamine terminals in the caudate (27). Blockade of A_1 receptors by CPX could facilitate dopamine release and stimulate postsynaptic cAMP accumulation via activation of the dopamine D_1 receptor (1). A_2 receptor activation also enhances cAMP accumulation (23,35). Accordingly, CGS 21680 stimulates cAMP formation, although not to the same extent as NECA (16). Therefore, one possibility is that parallel activation of adenylate cyclase by dopamine and CGS 21680 may lead to greater formation of cAMP and an enhanced physiological response. Of course, such interactions may involve more than one transmitter whose release could be influenced by the A_1 receptor. At present, there is no direct evidence to support this type of interaction, but this explanation could be explored in future experiments.

The combination of the A_1 agonist, R-PIA, and the A_2 agonist, CGS 21680, produced a dramatic increase in seizure threshold, compared to administration of R-PIA alone. A similar effect was previously reported in a behavioral study, where the combination of A_1 agonists with an A_2 agonist led to an enhanced depression of locomotor activity in mice compared to the depressive effects produced by the A_1 agonists alone (29). Based on these and the current results, it appears that a synergistic interaction exists between the A_1 and the A_2 receptor. This could explain why NECA is a potent anticonvulsant, because it activates A_1 and A_2 receptors. Synergistic interactions of A_1 and A_2 receptors have not been demonstrated in vitro, and the nature of this interaction remains unknown. This effect would appear to involve a different mechanism than that described above for the combination of an A_1 antagonist and an A_2 agonist (i.e., CPX + CGS 21680). Adenosine and its pharmacologically active analogs elicit marked hypomobility in rodents (4,14,28,32). Behavioral studies suggest that conclusive identification of the specific adenosine receptor subtype involved in locomotor activity is complex (32). In mice, the rank order of potency of various adenosine agonists suggests the principal involvement of A_2 receptors, not A_1 receptors, in locomotor depression (14,32). Administration of the slightly selective A_2 agonist, 2-phenylaminoadenosine, in mice produced marked behavioral depression (32). Peripheral injections of the A_2 selective analog, CGS 21680, were reported to only weakly depress locomotor activity (28), although this could be attributed to poor penetration of CGS 21680 into brain. Indeed, a recent study demonstrated significant locomotor depression following ICV injection of CGS 21680 in rats (20).

In the present study, all of the adenosine agonists tested (CGS 21680, R-PIA and NECA) profoundly depressed locomotor activity. The greatest effects were seen with NECA, which activates A_1 and A_2 receptors, and also with the combination of the A_1 agonist R-PIA and the A_2 agonist, CGS 21680. This apparent synergism between an A_1 agonist and an A_2 agonist is similar to that reported in a previous study (29). However, the combination of CPX with NECA or CGS 21680 was not successful in producing enhanced locomotor inhibition. This may indicate that different mechanisms are involved in the modulation of locomotor behavior versus seizure activity. The data gathered in this study are consistent with a hypothesis that A_2 receptor activation, in addition to A_1 receptor activation, is involved in the modulation of locomotor activity.

Further support for the hypothesis that A_2 receptor activation can modify locomotor activity comes from the CPX data. At equimolar doses, CPX blocked the rise in seizure threshold produced by R-PIA but did not block the locomotor effects. Nor did CPX block locomotor depression induced by NECA or CGS 21680. CPX has a high affinity for the A_1 receptor (K_d = 0.42 nM) (19) and should, therefore, block a significant number of these receptors. In the present study, CPX failed to elicit locomotor stimulation, and in fact, produced some locomotor depression. Other investigators have reported similar results using CPX (6,17). The reason for the absence of locomotor stimulation by CPX is not clear. It is possible that a higher dose of CPX may have produced locomotor stimulation or reversed the R-PIA and NECA-induced locomotor depression. The fact that CPX did not increase locomotor activity in this study, is at least consistent with the hypothesis of A_2 receptor involvement in the modulation of locomotor activity.

In conclusion, the selective A_2 agonist, CGS 21680, does not alter seizure threshold when administered alone. However, manipulation of the A_1 receptor may unmask an A_2 anticonvulsant effect on seizure threshold. Also, activation of A_1 and A_2 receptors together may lead to an enhanced anticonvulsant effect. The locomotor depressant effects of CGS 21680 further support the hypothesis that activation of A_2 receptors may be important in the locomotor effects of adenosine.

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