N^6-Cyclohexyladenosine and 3-(2-Carboxypiperazine-4-yl)-1-propenyl-1-phosphonic acid enhance the effect of antiepileptic drugs against induced seizures in mice

Abdel-Azim Assi
Department of Pharmacology, Faculty of Medicine, Assiut University, Assiut, Egypt

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ABSTRACT **Purpose**: The influence of N^6-Cyclohexyladenosine (CHA), an adenosine A_1 agonist and 3-(2-Carboxypiperazine-4-yl)-1-propenyl-1-phosphonic acid (CPPene), a selective N-methyl-D-aspartate (NMDA) antagonist upon the anticonvulsant activity of diazepam (DA), sodium valproate (VP), diphenylhydantoin (DPH), phenobarbital (PB) and carbamazepine (CAZ) was investigated in mice. All agents were administered intraperitoneally. **Methods**: Convulsive seizures were induced by the use of electro shocks and pentylenetetrazole (PTZ). **Results**: CHA (2 mg/kg, i.p.) and CPPene (2.5 mg/kg, i.p.) were found to enhance the anticonvulsant activity of the tested antiepileptic drugs against both electro convulsions and PTZ-induced convulsions. Both CHA and CPPene significantly decreased the ED50 values of these drugs against both electro convulsions and PTZ-induced convulsions, and increased the convulsive threshold. CHA (2 mg/kg, i.p.) and CPPene (2.5 mg/kg, i.p.) did not affect the plasma level of any of the tested antiepileptic drugs, indicating no pharmacokinetic interactions at the systemic administration. CHA (2 mg/kg, i.p.) or CPPene (2.5 mg/kg, i.p.), alone or in combination with the tested antiepileptic drugs produced no significant changes in their effects on the heart rate, blood pressure, body temperature, gross behavior or on the locomotor activity of experimental animals. Combinations of the antiepileptic drugs with CHA (2 mg/kg, i.p.) or CPPene (2.5 mg/kg, i.p.) were also devoid of significant effects on the motor performance and long-term memory in mice demonstrated by the Chimney test and passive avoidance task. CHA (5 mg/kg, i.p.) alone or in combination with the tested antiepileptic drugs produced inhibition of locomotor activity and motor coordination, sedation and hypothermia as well as impairing of long-term memory. **Conclusion**: Adenosine A1 agonists and NMDA antagonists enhance the efficacy of common antiepileptic drugs, indicating the involvement of adenosine and NMDA receptors in the convulsive pathway. The potential therapeutic benefits of such interactions may be taken into consideration and merit further investigations in animals and humans.

Corresponding Author: Abdel-Azim Assi, Department of Pharmacology, Faculty of Medicine, Assiut University, Assiut, Egypt, assi@wpo.nccu.edu

INTRODUCTION

Epilepsies are common disorder with an overall point prevalence of 4-10 per 1000 individuals (1). Therapy is symptomatic in that available drugs inhibit seizures but neither effective prophylaxis nor cure is available. Compliance with medication is a problem because of the need for long-term therapy together with many unwanted effects. Many patients remain seizure-free on prophylactic drug therapy, however, existing antiepileptic drugs have considerable potential for concentration-dependent and idiosyncratic toxicity (2). Their use in combination is plagued by pharmacokinetic and pharmacodynamic interactions, which result in a decrease of their therapeutic benefit. Thus new compounds and combinations are urgently needed.

During the past decade, the nucleoside, adenosine, has become widely accepted as having a neuromodulatory role in both the peripheral and central nervous system primarily by actions resulting in reduction of neuronal excitability (3,4). Early studies suggest that the extracellular adenosine, derived in part from the breakdown of adenosine triphosphate (ATP), may play an important role throughout the brain as an endogenous anticonvulsant, serving to reduce the severity of seizures or to prevent their recurrences (5-7). Systemic administration or intracranial injections (8-15) of adenosine analogs have been shown to inhibit the expression of seizures in animals. Adenosine antagonists, such as theophylline or caffeine produce the opposite effect

(16-20). Moreover, rapid elevations in brain levels of adenosine have been documented after experimental seizures (21) and post-seizures in epileptic patients (4). In order to explore any possible interaction between the commonly used antiepileptic drugs and adenosine analogues, the present investigation was carried out. N6-Cyclohexyladenosine, (CHA) an adenosine A1 agonist, was selected because many studies have reported that the anticonvulsant action of adenosine and its analogues is predominantly mediated via an interaction with adenosine receptors of the A1 subtype (5, 9, 10).

The role of glutamate receptors in the modulation of seizure activity has been intensively investigated and NMDA and AMPA receptor antagonists have been reported to be powerful anticonvulsants in a wide range of animal models of epilepsy (22-29). Because NMDA receptors are involved in both seizure initiation and propagation in the CNS, it is also of interest to investigate the role of such agents in epilepsy and their influence on the anticonvulsant effect of antiepileptic drugs.

In the present study, the influence of co-administration of CHA and 3-(2-Carboxypiperazine-4-yl)-1-propenyl-1-phosphonic acid (CPPene) with each of sodium valproate, diazepam, diphenylhydantoin, phenobarbital and carbamazepine on their effectiveness against seizures induced by electric shock and pentylenetetrazole as well as on their blood concentrations were investigated. Moreover, the effect of CHA, CPPene and their combinations on some physiological and behavioral parameters was evaluated.

METHODS

Drugs & Reagents

CHA, Diphenylhydantoin, carbamazepine and pentylenetetrazole were purchased from Sigma Chemical Co. (Saint Louis, MO, USA), 3-(2-Carboxypiperazine-4-yl)-1-propenyl-1-phosphonic acid, phenobarbitone sodium and diazepam were obtained from the National Institute on Drug Abuse, Maryland, USA). Sodium valproate was obtained from Labaz-Sanofi, Switzerland.

Animals

Male albino mice (C57BL/6J), 9 weeks age, and weighing 18-22 g were used (n=10/group). Albino rats (9 weeks, 200-250 g) were also used for measurement of the effect of various agents on blood pressure. The animals were housed in cages with food and water. The experiments were conducted between 9 and 12 am, and the room temperature was kept at 25 ± 3°C. The ethical guidelines for the investigation of experimental pain in conscious animals were followed in all tests. All experiments are carried out in accordance with the U.K. Animals Act and the U.S. National Institutes of Health guide for the care and use of Laboratory animals. All efforts were made to minimize animal suffering, and to reduce the number of animals used

Convulsive seizures

Electroconvulsions

The procedure was carried out as described by Toman (30) and Swinyard et al. (31). Electroshock was applied via ear-clip electrodes and generated by a stimulator (deliver an alternating 50 Hz current, the stimulus duration being 0.3 s). The end point was tonic hind limb extension. Both electroconvulsive threshold and maximal electro-shock were determined. The convulsive threshold was determined in four groups of animals (10 mice each), using electroshocks of various intensities. The percentage of mice displaying seizures was recorded and an intensity-response curve was calculated. The convulsive threshold was calculated as CS50, which represents the current strength (in mA) required to induce tonic hind limb extension in 50% of the animals tested. The protective effect of the tested antiepileptic drugs was assessed as ED50, which represent the dose of the drug (in mg/kg) required to protect 50% of animals against maximal electroshock (50 mA)-induced convulsive seizures (Table 5). The drugs were administered intraperitoneally: CHA and CPPene 60 min; diazepam 60 min; sodium valproate 30 min; diphenylhydantoin 90 min; phenobarbitone 90 min; and carbamazepine 60 min before the test. The control animals were administered the antiepileptic drug + vehicle, i.p. ED50 values with 95% confidence limits were calculated by the method of Litchfield and Wilcoxon (32). The effect of CHA (2mg/kg) or CPPene (2.5 mg/kg), alone or in combination with antiepilep-

tic drugs on the convulsive threshold was also investigated.

Pentylenetetrazole (PTZ)-induced convulsions

PTZ was dissolved in isotonic saline (10 mg/mL) and slowly injected (n0.3mL/min) via the tail vein. The convulsive threshold (CS50) of PTZ, which represent the dose of PTZ (mg/kg) required induce clonic and tonic convulsions in 50% of animals, was calculated from a dose-effect curve, using five groups of mice (10/group).

The mice were allocated in groups (10/group) and intraperitoneally dosed with one of a range of doses of test drugs or vehicle. The effect of CHA, CPPene and antiepileptic drugs on the threshold for different seizure types induced by i.v. infusion of PTZ in mice was investigated. In addition, the ED50 values of the tested antiepileptic drugs required to protect mice against PTZ-induced convulsions were determined after their administration alone and after their co-administration with CHA (2 mg/kg) or CPPene (2.5 mg/kg). The drugs were administered by the same manner as in electroshock-induced seizure method. The effect of CHA or CPPene alone on the convulsive threshold was also investigated. The ED50 values were calculated using the probit method of Litchfield and Wilcoxon (32).

Gross Behavior

Mice were individually placed in boxes (13 × 13 × 16 cm) to observe their behavioral changes before and after intraperitoneal injection of CHA (2 mg/kg), CPPene (2.5 mg/kg), their drug combinations or vehicle. Behavior (hyperlocomotion, head weaving, biting, licking or grooming, hyperexcitability, ataxia) was observed during the first 6 h and next day at 24 h after the treatment, and the incidence of each behavioral symptom was calculated.

Passive Avoidance Acquisition and Retention Testing

Passive avoidance learning was used to investigate the effect of the tested drug combinations on memory processing (33). Mice were placed in an illuminated box (12 × 9 × 13) connected to a large dark box (26 × 9 × 13) equipped with an electric grid floor. Entrance into the dark box was punished by an electric foot-shock (0.5 mA, for 2 s). On the next day (24 h later), the same mice were placed in the illuminated box. Mice that avoided the dark compartment for more than 60 s were considered to remember the task. The drugs were administered intraperitoneally: CHA, CPPene, carbamazepine 60 min and diazepam 60 min, sodium valproate 30 min, diphenylhydantoin 90 min; phenobarbitone 90 min and before training. Retention was quantified as the percentage of animals avoiding the dark compartment for over 60 s.

Chimney Test

The effects of antiepileptic drugs alone or in combination with CHA (2 mg/kg) or CPPene (2.5 mg/kg) on motor impairment were investigated by the use of Chimney test (34). In this test, animals (n=10) have to climb backwards up a glass tube (3 cm i.d., 20 cm length, 20° angle). Motor impairment was indicated by the inability of the animals to climb backward up the tube within 60 s. Control animals were able to pass the test (normal mice climb backward through the tube within less than 3-9 s). Results were expressed as a percentage of animals failed to perform the test.

Locomotor Activity

The locomotor activity of test animals was quantitatively estimated with an activity cage provided with a counter (Model 7401, Ugo basile, Comerio, Italy). Locomotor activity was measured for 1 h under the same conditions starting from 2 h after the i.p. injection of CHA (2 mg/kg), CPPene (2.5 mg/kg), their drug combinations or vehicle.

Effect on Body Temperature

The effect of i.p. Injection of CHA (2 mg/kg), CPPene (2.5 mg/kg), their drug combinations or vehicle on the body temperature of mice was measured using a rectal thermistor probe inserted 3 cm.

Effects on blood pressure and heart rate

In this test Albino rats (160-220 g) were used as experimental animals because of the convenience of conducting the experimental procedure. In addition, rats can withstand long periods of experimentation under anesthesia. Rats were anaesthetized with urethane (1.4 mg/g). The jugular vein, trachea and carotid artery were exposed and canulated with polyethylene cannulae. The carotid canula was connected to a transducer of a two channel Oscillograph (BioScience, C.F. Palmer &

George, Washington) for continuous monitoring of blood pressure and heart rate. The effects of antiepileptic drugs alone or in combination with CHA (2 mg/kg) or CPPene (2.5 mg/kg) on blood pressure was evaluated.

Determination of the blood concentrations of antiepileptic drugs

The animals were administered with either an antiepileptic drug and vehicle, or the antiepileptic drug in combination with CHA or CPPene. The animals were killed by decapitation at times scheduled for electroshocks and blood samples of about 1 ml were collected in Eppendorf tubes. Blood samples were centrifuged at 10,000 rpm for 3 min and 100 µl of plasma were administered to Abbott System (Abbott, Irving, Texas, USA) cartridges. The plasma levels of antiepileptic drugs were estimated by immunofluorescence, using an Abbott TDx analyzer (Abbott, Irving, Texas, USA). Control drug solutions were put before and after the carousel containing the experimental samples. Eight mice were used per one experimental group and the plasma levels were expressed as mean ± SD.

Statistical analysis

Both CS50 and ED50 values and analysis of the results obtained in the convulsive tests were calculated by fitting the data by linear regression analysis as described by Litchfield and Wilcoxon (32). The effective doses (with 95% confidence limits) and their statistical significance were calculated according to this method. Results from behavioral tests were analyzed statistically with Fisher's exact probability test and Mann-Whitney's test. Student's t-test for unpaired data was used for analysis of the effect of CHA and CPPene plasma levels of antiepileptic drugs.

RESULTS

Electro convulsions

CHA (2 mg/kg, i.p.) and CPPene (2.5 mg/kg, i.p.) given alone did not produce any significant change in the electro-convulsive threshold (Table 1). Both agents, however, significantly ($P < 0.05$) increased that threshold when given with sodium valproate, diazepam, diphenylhydantoin, phenobarbital and carbamazepine as compared to control animals administered the antiepileptic drugs only (Table 1). .

Table 1: The Influence of co-administration with CHA (2 mg/kg, i.p.) and CPPene (2.5) upon the effect of antiepileptic drugs on the threshold for tonic (hind limb extension) electroshock seizures in mice

Treatment (mg/kg, i.p.)	Electro convulsive threshold (CS50 in mA; 95% confidence limits)
Control	10.6 (9.6-12.0)
CHA (2)	10.8 (9.8-12.0)
CHA (5)	12.6 (11.4-13.9)*
CPPene (2.5)	11.3 (10.2-13.7)
CPPene (5)	12.9 (11.2-14.4)
Diazepam (20.5)	13.1 (11.7-14.9)*
Diazepam (20.5) + CHA (2)	14.6@(12.9-15.4)**
Diazepam (20.5) + CPPene (2.5)	15.1@ (12.7-16.4)**
Sodium valproate (300)	13.8 (13.2-14.1)*
Sodium valproate (300) + CHA (2)	15.7@ (14.6-16.9)**
Sodium valproate (300) + CPPene (2.5)	16.9@ (14.4-18.6)**
Diphenylhydantoin (18)	14.4 (13.9-15.1)*
Diphenylhydantoin (18) + CHA (2)	16.8@ (16.2-17.2)**
Diphenylhydantoin (18) + CPPene (2.5)	17.5@ (15.9-18.2)**
Phenobarbital (10)	17.6 (16.9-19.4)*
Phenobarbital (10) + CHA (2)	19.7@(17.6-20.9)**
Phenobarbital (10) + CPPene (2.5)	20.1@ (18.1-21.4)**
Carbamazepine (20)	18.1 (16.8-19.6)*
Carbamazepine (20) + CHA (2)	23.7@(19.6-24.9)**
Carbamazepine (20) + CPPene (2.5)	21.2@ (19.2-22.8)**

The electroconvulsive threshold was determined as CS50 (in mA with confidence limits for 95% probability) in groups of 10 animals per dose. Control groups received i.p. vehicle injection (data shown for control is the mean of four control groups with 10 mice each). Significant difference between control and drug-treated groups is indicated (P at least <0.05;** P at least < 0.01). @ indicate P < 0.05 as compared with the drug alone. The values and their statistical significance were calculated according to the method of Litchfield and Wilcoxon (32)*

In addition, both agents significantly ($P < 0.05$) reduced the ED50 of antiepileptic drugs against the maximal electroshock used (Table 2).

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Table 2: The influence of CHA and CPPene upon the protective effect (ED50 mg/kg, 95% fiducial limits) of sodium valproate, diazepam, diphenylhydantoin, phenobarbital and carbamazepine against electroshock-induced seizures.

	CHA (mg/kg, i.p.,)		CPPene (mg/kg, i.p.)	
Antiepileptic drug	0	2	0	2.5
Sodium valproate	327	194@	323	188@
	(294-364)	(174-216)	(289-366)	(170-211)
Diazepam	20.50	12.5@	21.40	11.40@
	(16.80-25.20)	(9.50-12.80)	(16.20-24.60)	(9.25-12.15)
Diphenylhydantoin	18.20	11.50@	17.80	10.80@
	(15.5-21.4)	(8.7-12.7)	(14.7-20.5)	(8.2-12.4)
Phenobarbital	32.20	24.50@	33.70	22.50@
	(28.9-35.8)	(19.7-27.8)	(26.9-36.5)	(19.4-26.1)
Carbamazepine	19.80	11.90@	17.90	12.40@
	(16-24.5)	(11.90-16.2)	(15-22.5)	(10.90-15.2)

The effective doses (with 95% confidence limits) and their statistical significance were calculated ccording to the method of Litchfield and Wilcoxon (32); @: P at least < 0.05 vs. control; n=10/group.

Table 3: Effect of CHA, CPPene and their co-administration with antiepileptic drugs on the threshold for different seizure types induced by i.v. infusion of PTZ in mice.

Treatment (mg/kg, i.p.)	PTZ threshold (mg/kg ± S.E.)		
	Initial myoclonic twitch	Generalized convulsions with loss of righting reflexes	Forelimb tonus
Control	30.9±1.1	38.1±2.3	73.5±3.6
CHA(2)	33.1±1.9	42.84±3.6	77.4±7.2
CHA(5)	35.4±1.2*	49.83±1.4*	84.35±4.1*
CPPene(2.5)	34.4±3.2	41.89±4.4*	76.85±7.7
CPPene(5)	36.4±3.2*	49.83±3.4*	88.85±5.1*
Diazepam(4.5)	38.7±2.9*	51.92±3.20*	90.60±4.6*
Diazepam(4.5)+CHA(2)	43.55±3.03**	58.5±5.4**@	96.5±6.4@
Diazepam(4.5)+CPPene(2.5)	44.40±3.3**	58.5±4.2**@	97.45±2.4@
Sodium valproate(300)	39.70±2.8*	52.95±3.2*	91.60±3.6*
Sodium valproate(300)+CHA(2)	44.60±3.7**	56.9±4.1**@	97.45±2.4@
Sodium valproate(300)+CPPene(2.5)	46.3±2.4**	58.5±4.5**@	101.55±4.2@
Diphenylhydantoin(18)	40.20±3.40*	58.7±2.9*	93.10±2.6*
Diphenylhydantoin(18)+CHA(2)	45.40±2.6**@	64.85±1.7**@	104.8±3.9**@
Diphenylhydantoin(18)+CPPene(2.5)	44.9±3.6**@	65.25±3.2**@	107.4±6.4**@
Phenobarbital(10)	41.8±1.90*	57.95±1.3*	93.70±2.15*
Phenobarbital(10)+CHA(2)	46.8±1.5**@	63.45±1.9**@	105.10±2.05**
Phenobarbital(10)+CPPene(2.5)	47.6±3.5**@	65.15±3.2**@	108.30±4.25**
Carbamazepine(20)	42.10±2.7*	58.15±2.1*	92.90±3.40*
Carbamazepine(20)+CHA(2)	52.6±1.08**@	71.20±3.3**@	103.70±1.7**@
Carbamazepine(20)+CPPene(2.5)	48.10±1.8**@	66.2±1.3**@	98.8±1.4**@

The seizure threshold was calculated as the dose of PTZ (± S.E.) which induced the respective seizure types in all animals of a group (n=10/group). Controls (n=10) received i.p. vehicle injection; data shown for control is the mean of three control groups with 10 mice each. Significant differences between respective control and drug-treated group are indicated (P <0.05; ** P< 0.01). @ indicate P < 0.05 as compared with the drug alone. The effective doses (with 95% confidence limits and their statistical significance were calculated according to the method of Litchfield and Wilcoxon (32)*

PTZ-induced convulsions

Effect of CHA, CPPene and their co-administration with antiepileptic drugs on the threshold for different seizure types induced by i.v. infusion of PTZ in mice is illustrated in Table 3. The influence of pretreatment with CHA and CPPene upon the protective effect of sodium valproate, diazepam, diphenylhydantoin and

phenobarbital against PTZ-induced convulsions are summarized in Table 4. CHA (2 mg/kg) and CPPene (2.5 mg/kg) significantly ($P < 0.05$) decreased the ED50 of the tested drugs required to antagonize the hind limb extension produced by the threshold dose of PTZ in mice.

Table 4: The influence of pretreatment with CHA or CPPene upon the protective effect (ED50) of the tested antiepileptic drugs against tonic convulsions induced by the threshold dose of PTZ

Antiepileptic Drugs	CHA (mg/kg, i.p.)		CPPene (mg/kg, i.p.)	
	0	2	0	2.5
Sodium valproate	283.60 (255-315.60)	259.97@ (236.10-276.10)	282.77 (257-312.68)	255.85@ (232.10-271.50)
Diazepam	5.20 (3.20-7.70)	2.60@ (1.98-3.30)	5.40 (3.35-7.66)	2.48@ (1.95-3.40)
Diphenylhydantoin	8.30 (5.50-10.70)	4.80@ (3.60-6.10)	7.55 (5.50-10.70)	4.25@ (3.10-5.97)
Phenobarbital	9.10 (7.10-11.90)	5.30@ (2.60-7.30)	10.30 (7.60-13.88)	4.70@ (2.40-7.10)
Carbamazepine	19.70 (14.70-25.60)	11.40@ (8.30-15.8)	20.66 (17.50-26.80)	14.40@ (8.89-16.8)

The effective doses (with 95% confidence limits) and their statistical significance were calculated according to the method of Litchfield and Wilcoxon (32); @: P at least <0.05 vs. control; n=10/group

Table 5: The effect of antiepileptic drugs and their combinations with CHA or CPPene on the performance of mice in the Chimney test

Treatment (mg/kg)	% of mice showing motor impairment
Control	0
CHA (2)	6
CHA (5)	18.9*
CPPene (2.5)	0
CPPene (5)	7.5
Diazepam (20.5)	29.7*
Diazepam (20.5) + CHA (2)	33.4*
Diazepam (20.5) + CPPene (2.5)	30.6*
Sodium valproate (300)	30.9*
Sodium valproate (300) + CHA (2)	39.4*
Sodium valproate (300) + CPPene (2.5)	32.9*
Diphenylhydantoin (18)	7.7
Diphenylhydantoin (18) + CHA (2)	12.9
Diphenylhydantoin (18) + CPPene (2.5)	9.7
Phenobarbital (10)	0
Phenobarbital (10) + CHA (2)	14
Phenobarbital (10) + CPPene (2.5)	8.3
Carbamazepine (20)	7.5
Carbamazepine (20) + CHA (2)	13.6
Carbamazepine (20) + CPPene (2.5)	8.8

*The data are expressed of mice that failed to perform the test. * P <0.05 vs. vehicle (n=10), Fisher's exact probability test; n=10/group.*

Gross Behavior

CHA (2 mg/kg, i.p.) and CPPene (2.5 mg/kg, i.p.), alone or in combination with the tested antiepileptic drugs produced no significant changes in their effects on gross behavior or on the locomotor activity of experimental animals.

Chimney test

The protective anticonvulsant doses of diphenylhydantoin, phenobarbital and carbamazine did not influence the performance of animals in this test. Sodium valproate and diazepam, however, caused motor impairment in 30% of animals (Table 5). Combinations of the antiepileptic drugs with CHA (2 mg/kg, i.p.) or CPPene (2.5 mg/kg, i.p.) did not significantly affect the motor performance of animals. CHA (5 mg/kg, i.p.), alone or in combination with the tested antiepileptic drugs, produced significant motor impairment.

Passive Avoidance Acquisition and Retention Testing

Diphenylhydantoin, phenobarbital, and carbamazepine when given at the protective anticonvulsant doses caused moderate impairment of the memory task. Sodium valproate and diazepam, however caused strong impairment of animal performance in this test. Combinations of the antiepileptic drugs with CHA (2 mg/kg, i.p.) or CPPene (2.5 mg/kg, i.p.) did not significantly affect the performance of animals in the test. CHA (5 mg/kg, i.p.), alone or in combination with the tested antiepileptic drugs produced significant impairment of long-term memory of animals (Table 6).

Table 6: The effect of antiepileptic drugs and their combinations with CHA or CPPene on the long-term memory of mice.

Treatment (mg/kg)	Time (s)
Control	175
CHA (2)	152.5±31.8
CHA (5)	119.6±17.5*
CPPene (2.5)	159.4±23.3
CPPene (5)	154.7±25.8
Diazepam (20.5)	95.5±16.8**
Diazepam (20.5) + CHA (2)	81.7±13.4**
Diazepam (20.5) + CPPene (2.5)	88.6±16.6**
Sodium valproate (300)	62.9±14.8**
Sodium valproate (300) + CHA (2)	55.4±12.9**
Sodium valproate (300) + CPPene (2.5)	58.9±16.4**
Diphenylhydantoin (18)	127.7±12.8*
Diphenylhydantoin (18) + CHA (2)	120.9±15.9*
Diphenylhydantoin (18) + CPPene (2.5)	125.3±13.5*
Phenobarbital (10)	145.8±11.8*
Phenobarbital (10) + CHA (2)	141.7±14.5*
Phenobarbital (10) + CPPene (2.5)	145.5±10.3*
Carbamazepine (20)	130.7±17.5*
Carbamazepine (20) + CHA (2)	122.8±13.6*
Carbamazepine (20) + CPPene (2.5)	127.5±18.8*

*The retention was calculated as the time interval, the mice were avoiding the dark compartment. Mann-Whitney's test was used for statistical analysis of the data. (*P<0.05, **P<0.01 vs. vehicle).*

Effect on Body Temperature

CHA (2 mg/kg, i.p.) or CPPene (2.5 mg/kg, i.p.), alone or in combination with the tested antiepileptic drugs, produced no significant changes in the body temperature of experimental animals. CHA (5 mg/kg, i.p.), however, alone or in combination with the tested antiepileptic drugs, produced significant decrease in body temperature.

Effects on blood pressure and heart rate

Urethane anesthesia decreased the heart rate and blood pressure of experimental animals. CPPene (2.5 mg/kg, i.p.) or CHA (2 mg/kg, i.p.), alone or in combination with the tested antiepileptic drugs produced no significant changes in their effects on the heart rate, systolic or diastolic blood pressure of experimental animals.

Influence of CHA and CPPene on the blood levels of antiepileptic drugs

CHA (2 mg/kg, i.p.) or CPPene (2.5 mg/kg, i.p.) did not alter the plasma levels of unchanged diazepam, sodium valproate, phenytoin, phenobarbital or carbamazepine, given i.p. at the median effective doses (Table 7).

Table 7: The influence of CHA and CPPene on the plasma levels of the unchanged antiepileptic drugs

Treatment (mg/kg, i.p.)	Plasma level (unchanged drug)
Diazepam (20.5)	3.6 ± 0.21
Diazepam (20.5) + CHA (2)	3.2 ± 0.17
Diazepam (20.5) + CPPene (2.5)	3.4 ± 0.23
Sodium valproate (300)	246.4 ± 22.5
Sodium valproate (300) + CHA (2)	243.7 ± 19.8
Sodium valproate (300) + CPPene (2.5)	241.8 ± 21.6
Diphenylhydantoin (18)	2.3 ± 0.14
Diphenylhydantoin (18) + CHA (2)	2.1 ± 0.11
Diphenylhydantoin (18) + CPPene (2.5)	2.15 ± 0.17
Phenobarbital (10)	1.4 ± 0.13
Phenobarbital (10) + CHA (2)	1.25 ± 0.10
Phenobarbital (10) + CPPene (2.5)	1.32 ± 0.15
Carbamazepine (20)	2.25 ± 0.2
Carbamazepine (20) + CHA (2)	2.31 ± 0.22
Carbamazepine (20) + CPPene (2.5)	2.12 ± 0.18

The values are the means (in μg/ml of plasma) of 8 determinations ±S.D. Student's t-test for unpaired data was used for statistical analysis.

DISCUSSION

In this study, the anticonvulsant profile of pretreatment with the adenosine A_1 agonist, cyclohexyladenosine, and the NMDA receptor antagonist (CPPene), in conjunction with sodium valproate, diazepam, diphenylhydantoin, phenobarbital and carbamazepine was investigated using two of the test models regarded as predictive of antiepileptic activity (30, 31, 35). Using the mouse and rat as animal models, allowed easy induction of electro convulsions and easy i.p. administration of both convulsant and anticonvulsant agents.

The intraperitoneal route was employed for the administration of CHA, CPPene and anticonvulsant drugs to

test their interactions when given systemically. Although the systemic administration of adenosine analogs has been proven to induce behavioral changes (36, 37), and anticonvulsant effects (8, 9, 11, 16), it is not clear to what extent they cross the blood brain barrier and act directly on central neurons. Durcan and Morgan (37), and Rosen and Berman (38), reported that adenosine analogs could cross blood brain barrier to produce central effects, while Brodie (2) reported the reverse. Several reports have previously described both pro- and anticonvulsant actions of acutely administered adenosine analogs in a variety of experimental models of epilepsy (8, 11, 16)

In the present study, the co-administration of CHA or CPPene with each of sodium valproate, diazepam, diphenylhydantoin, carbamazepine and phenobarbital resulted in an enhancement of their anticonvulsant activity in mice against electro- and PTZ-induced convulsions. The potentiating effect of CHA and CPPene upon antiepileptics used was not associated with an increase in their plasma levels, excluding pharmacokinetic interactions.

These findings support the early investigations (3-7), which suggested that the administration of adenosine analogs is associated with reduction in the severity of seizures and preventation of their recurrences.

In previous studies (8, 9), the intranigral injection of adenosine A_1 agonists had shown to attenuate established tonic-clonic seizures when produced by electro convulsive shock. According to Herberg et al. (9), the intranigral CHA injections appeared to affect subsequent convulsions, but not the acquisition of kindling.

Our findings also support the suggestion of Dragunow and Goddard (39) that each seizure itself releases adenosine that serves to end it, that of Dragunow (7) who concluded that adenosine might be the brain's natural anticonvulsant neurotransmitter, and that of Thorat, Kulkarni (40) who reported that adenosinergic agents such as adenosine, 2-chloro-adenosine, N6-cyclohexyladenosine produced dose-dependent protective effect against DMCM- and Ro 5-4864-induced convulsions and mortality. Here, CHA may produce an adenosine-like action at its specific receptors. This action may be potentiated by the presence of the tested antiepileptic drugs. The present investigation, are consistent with the early study of Weir et al. (41) who reported that carbamazepine is a selective ligand for A1 adenosine receptors in brain.

The present data are also consistent with the work of Chapman (42), who concluded that seizures can be provoked in epileptic and non-epileptic animals and humans by a wide number of glutamatergic molecular mechanisms in which the excitatory glutamatergic system plays a key role, and with the finding of Urbanska, et al. (24) who reported that the NM DA-mediated events are involved in the protective action of diazepam and carbamazepine against maximal electroshock-induced seizures.

This study further supports the notion that adenosinergic mechanisms and NMDA receptor antagonism mediate neuroprotective effects in the central nervous system.

In conclusion, our findings suggest that the administration of an adenosine A_1 agonist, or an NMDA antagonist in conjunction with the common antiepileptic drugs may enhance the drug effects and may allow their use at smaller doses with the least level of side effects, provided that such drug combinations does not interfere with the normal behavior or the heart function. However, additional experimental and clinical studies should be performed in order to elucidate the mechanism of interaction and to support this suggestion.

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