Studying effect of xanthines, alone and after CPA and CPCA pretreatment, on locomotor activity and forced performance of rats

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Abstract

Background and aim of the study

Clinical use of theophylline, as a representative of methylxanthines, has declined and limited to refractory cases. Its neurological effects range from mild symptoms e.g. headache, insomnia, irritability and restlessness to recalcitrant seizures. The present study is aimed at investigating how activation of adenosine receptors by the adenosine A1 agonist, N6-Cyclopentyladenosine (CPA) and the A2 receptor agonist, 5-(N-cyclopropyl) carboxamidoadenosine (CPCA), may affects theophylline effects on motor activity of rats.

Methods

The present study investigated the effect of theophylline , alone and after pretreatment of rats with CPA and CPCA on locomotor activity and forced performance of rats. In addition, the study calculated the median convulsive dose (CD50) of theophylline, alone and after pretreatment with CPA in rats.

Results

Intraperitoneal injection (i.p.) of CPA in a dose of 10 mg/kg 60 minutes before theophylline in a dose of 100 mg/kg produced significant inhibition of spontaneous activity and forced motor performance of rats. On the contrary; i.p. injection of CPCA in a dose of 10 mg/kg 60 minutes before theophylline in a dose of 100 mg/kg, does not affect significantly the

spontaneous activity and forced motor performance induced by theophylline. CPA produced significant elevation in CD50 of theophylline from 210 mg/kg to 225 mg/kg.

Conclusion

The present study showed that activation of A1 receptors, but not A2 receptors, has a marked inhibitory influence on the stimulant effects of theophylline on motor activity of rats.

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Introduction

Clinical use of theophylline, as a representative of methylxanthines, has immunomodulatory and anti-inflammatory activities. However, its therapeutic use in treatment of bronchial asthma and obstructive pulmonary diseases has declined and limited to refractory cases (Vassallo and Lipsky, 1998). Theophylline's effects in the central nervous system (CNS) range from mild symptoms headache, insomnia, irritability and restlessness to recalcitrant seizures and neurologic failure. Theophylline is a known CNS stimulant that produces profound CNS excitatory activity and causes marked increase of spontaneous and forced motor activity of rats.

Adenosine stimulates two major receptor subtypes, A1 and A2, which are linked to a multitude of effectors namely, adenylate cyclase, inositol phosphate, potassium channels, calcium channels and neurotransmitter release (Williams, 1990). Both positive and negative modulations of these effector systems have been documented depending on the receptor subtype activated. Thus, while A1 receptors inhibit, A2 stimulate adenylate cyclase activity (Londos et al., 1980). Adenosine, through activation of A1 receptors, has been implicated in sedative, anticonvulsant, anxiolytic, and locomotor depressant effects (Malhotra and Gupta, 1997).

The present study is aimed at investigating how activation of adenosine receptors by the adenosine A1 agonist, N6-Cyclopentyladenosine (CPA) and the A2 receptor agonist, 5-(N-cyclopropyl) carboxamidoadenosine (CPCA), may affects theophylline effects on motor activity of rats.

Materials and methods

Chemicals

The following chemicals were used and obtained from the sources indicated:

- **Theophylline [ICN biomedicals, Inc]:** was dissolved in warm saline
- **Adenosine [ICN biomedicals, Inc]:** were freshly prepared as solutions in distilled water
- **N^6-cylcopentyl adenosine (CPA)- A1 agonist [ICN biomedicals, Inc]:** it was dissolved in 8% ethanol
- **5-N(cyclopropyl) Carboxamidoadenosine (CPCA)- A_2 agonist [ICN biomedicals, Inc]:** it was suspended in 8% tween 20.

Animals

Adult male rats weighing 150-200g were used. The animals were group housed in plastic cages and maintained under standard laboratory conditions with a natural light-dark cycle. Rats were left to acclimatize to the environment for at least a week before the experiments. Food and water were allowed ad libitum.

Effect of theophylline, alone and after pretreatment with CPA and CPCA, on motor activity of rats

9 groups of rats each was comprised of 5 animals

Treatment schedules

Group A: was given 0.5 ml of 8% tween 20.

Group B: was given 0.5 ml of 8% ethanol. was given theophylline in therapeutic doses (100 mg/kg).

Group C: was given ADO 100 mg/kg

Group D: was given CPA 10 mg/kg

Group E: was given CPCA 10 mg/kg

Group F: theophylline 100 mg/kg

Group G: was given ADO 100 mg/kg 5 minutes before theophylline 100 mg/kg.

Group G: was given CPA10 mg/kg 60 minutes before theophylline 100 mg/kg.

Group H: was given CPCA 10 mg/kg 60 minutes before theophylline 100 mg/kg.

The motor activity was determined by

1. Activity cages: (For screening of locomotor activity)

Rats were placed inside an acrylic transparent cage that rests on a sensor platform. It detects ambulatory movements as well as stereotypic activity like grooming, scratching, digging, etc. Vibrations caused by the animal activity produce proportional electrical signals. These are electrically processed to generate trigger pulses and drive a digital counter. Every count registered is accompanied by a flash. Activity recording was continued for 180 minutes. Activity records were taken for 1 minute each at 1, 5, 30, 60, 120 and 180 minutes after giving the drugs mentioned above (Paul and Kazi, 1992).

Source: http://animal-activity.tripod.com/

2. Rotarod test: (For screening of forced motor performance)

Rats were allowed to remain on a rotating rod until falling off. The length of time the rat remained on the rod was recorded. The falling latency was recorded for each group at 1, 5, 15, 30, 60, 120 and 180 minutes after giving the drug (Dunham and Miya, 1957).

Source: http://www.biobserve.com

Calculation of the Median Convulsive Dose (CD50) of theophylline, alone and after pretreatment with CPA in rats

Computation of the median convulsive dose and its 95% confidence limits for theophylline , alone and after pretreatment with CPA in rats, were proceeded according to the method of Litchfield and Wilcoxon (1949). Two sets of groups, each comprised of 10 rats, were injected i.p. with each of graded doses of theophylline alone and CPA injected i.p in a dose of 5 mg/kg 60 minutes prior to theophylline. Percentage incidence of seizures in each group of the two sets was determined during a period of 30 minutes after theophylline administration. Percentage incidence of seizures in each group was determined during a period of 30 minutes after theophylline administration.

Statistical analysis of the results

The degree of variability, in results of assessment of the effect of on spontaneous coordinate activity and forced motor performance of rats, were expressed as the mean + standard error (X + SE). The significance of the differences (before and after treatment) was determined using the student's t-test. The difference was regarded as significant when $P < 0.05$ and as a highly significant when $P < 0.01$ (Snedecor, 1967).

Results

Effect of theophylline on spontaneous motor activity of rats (see table 1)

Effect of theophylline on spontaneous coordinate activity in rats:

Intraperitoneal injection of theophylline in a dose of 100 mg/kg in rats showed increase in spontaneous activity. The effect was maximal 1 minute after injection. This increase of motor activity continued up to 1 hour post-injection.

Effect of theophylline after administration of adenosine on the spontaneous coordinate activity of rats

Intraperitoneal injection of adenosine in a dose of 100 mg/kg 5 minutes before theophylline in a dose of 100 mg/kg produced inhibition of spontaneous activity. The maximal inhibition was achieved 5 minutes after theophylline, then returned to normal 2 hours post-injection.

Effect of theophylline after administration of N6-cyclopentyladenosine (CPA) "A1 agonist" on the spontaneous coordinate activity of rats :

Intraperitoneal injection of N6-cyclopentyl adenosine in a dose of 10 mg/kg 60 minutes before theophylline in a dose of 100 mg/kg produced inhibition of spontaneous activity. The maximal inhibition was recorded 30 minutes after theophylline injection. Then, motor activity returned to normal 3 hours post-injection.

Effect of theophylline after administration of 5-(N-cyclopropyl) carboxamindoadenosine (CPCA) "A2 agonist" on the spontaneous coordinate activity of rats :

Intraperitoneal injection of CPCA in a dose of 10 mg/kg 60 minutes before theophylline in a dose of 100 mg/kg, does not affect the action of theophylline. That means that theophylline produced the same significant increase of the spontaneous activity, starting 1 minute after its injection and continuing for 1 hour, as when injected alone.

Effect of theophylline after adenosine receptor modulation on the forced motor performance of rats (see table 2)

Effect of theophylline on the forced motor performance in rats

Intraperitoneal injection of theophylline in a dose of 100 mg/kg showed increase in the forced motor performance of rats. The effect was maximal 1 minute after injection and continued for 2 hours after injection.

Effect of theophylline on the forced motor performance of rats after administration of adenosine

Intraperitoneal injection of adenosine 100 mg/kg 5 minutes before theophylline in a dose of 100 mg/kg produced inhibition of the forced motor performance. This inhibition was maximal 1 minute after injection of theophylline then the motor performance returned to normal 1 hour post-injection.

Effect of theophylline on the forced motor performance of rats after administration of N6-cyclopentyladenosine (CPA)

Intraperitoneal injection of N6-cyclopentyladenosine in a dose of 10 mg/kg 60 minutes before theophylline in a dose of 100 mg/kg produced inhibition of forced motor performance of rats. This inhibition was maximal 30 minutes after injection of theophylline, then motor performance returned into normal 3 hours post-injection.

Effect of theophylline on the forced motor performance of rats after administration of 5-(N-cyclopropyl) carboxamidoadenosine (CPCA)

Intraperitoneal injection of 5-(N-cyclopropyl)carboxamido-adenosine in a dose of 10 mg/kg 60 minutes before theophylline 100 mg/kg does not affect significantly the forced motor performance induced by theophylline. This means that theophylline showed the same increase in the motor performance, which reached its maximal 1 minute after theophylline injection. But, it was observed that the increase in motor performance lasted for 1 hour only, instead of 3 hours as when it was administered alone.

Calculation of CD50 of theophylline in rats

The median convulsive dose (CD50) of theophylline injected intraperitoneally into rats was equivalent to 210 (188.34 – 234.15) mg/kg.

Effect of CPA pretreatment on (CD50) of theophylline

The median convulsive dose (CD50) of theophylline injected i.p into rats 60 minutes after CPA was equivalent to 225 (178.6 – 283.5) mg/kg.

Table 1: effect of I.P. Injection of theophylline, alone and after pretreatment with CPA and CPCA, on spontaneous activity of rats

Treatment	Mean number of spontaneous activity/min							
	Time after treatment- %inhibition or increase of motor activity							
	Control	**1 min**	**5 min**	**15 min**	**30 min**	**1hour**	**2hours**	**3 hours**
8%tween 20 (5 ml)	23±0.7	25±0.45	23±0.45	23±0.3	23±0.45	23±0.45	23±0.3	23±0.3
		0%	0%	0%	0%	0%	0%	0%
8%ethanol (0.5ml)	25±0.3	25±0	26±0.3	26±0.3	25±0.3	26±0.3	25±0	25±0.45
		0%	+4%	+4%	0%	+4%	0%	0%
Adenosine 100 mg/kg	19±1.86	7.6±2.32**	16.2±1.74	19±1.86	20±1.57	18±1.99	17.6±1.11	19±1.86
		0%	-14.74%	0%	+5.26	-5.26	-7.36	0%
CPA 10ml/kg	23±0.83	20±0.83	19±1**	15±0.95**	18±0.54**	19±0.77	23±1.22	23±0.95
		0%	-17.4%	-34.78%	-21.74%	-17.4%	0%	0%
CPCA 10 ml/kg	26±1.04	26±1	26±0.9	24±1.22	26±0.54	28±1.22	26±1	26±0.63
		0%	0%	-7.69%	0%	+7.69%	0%	0%
Theophylline 100mg/kg	**27±1.22**	**34±0.7****	**33±1.1****	**30±0.6***	**27.8±1.02**	**27.2±0.58**	**26.2±0.97**	**26±1.04**
		+25.9%	**+22.2%**	**+11.1%**	**+2.96%**	**+0.74%**	**-2.96%**	**-3.7%**
Adenosine 100 mg/kg before Theophylline 100mg/kg	29±0.45	24±0.45**	23±0.3**	25±0.45**	26.8±0.58	28±0.45	29±0.45	29.4±0.4
		-17.24%	+20.68%	-13.79%	-7.58%	-3.45%	0%	+1.38%
CPA 10ml/kg before Theophylline 100mg/kg	32±0.95	31.4±0.97	28.6±0.9*	27.4±0.6	25±0**	27±1.22**	29±0.45	29.4±0.4
		-1.87%	-10.6%	-14.37%	-21.8%	-15.6%	-9.37%	-8.12%
CPCA 10 ml/kg before Theophylline 100mg/kg	25±1.84	31±1.3**	28.8±1.32	27.6±1.43	26.4±0.92	26.8±0.97	24.6±1.32	23.2±0.97
		+24%	+15.2%	+10.4%	+5.6%	+7.2%	-1.6%	-7.2%

N.B: Data represent mean ± SE of 5 observations
* p<0.05 ** p<0.01
+% = percentage increase of motor activity
- % = percentage inhibition of motor activity

Table 2: effect of I.P. Injection of theophylline, alone and after pretreatment with CPA and CPCA, on forced motor performance in rats

Treatment	Mean time –in seconds- of rotation of rats							
	Time after treatment- %inhibition or increase of motor activity							
	Control	1 min	5 min	15 min	30 min	1hour	2hours	3 hours
8%tween 20 (5 ml)	45±0.83	45±0.3	46±0.62	46±0.3	46±0.62	45±0.2	45.6±0.2	45.4±0.5
		0%	+2.22%	+2.22%	+2.22%	+0.44%	+1.33%	+0.88%
8%ethanol (0.5ml)	45±0.45	45±0	45±0.3	46±0.3	46±0.0	45±0.54	46±0.45	46±0.3
		0%	0%	+2.22%	+2.22%	0%	+2.22%	+2.22%
Adenosine 100 mg/kg	47±1.33	40±1.7**	45.2±1.65	45.6±1.9	47.6±1.12	47±1.22	48±1.22	47±2.54
		-14.89%	-3.83%	-2.98%	+1.27%	0%	+2.127	0%
CPA 10ml/kg	44±3.9	40±3.16*	40±3.16*	34±1.86*	36±1.86**	38±1.22	40±1.85	42±1.99
		-9.09%	-9.09%	-22.72%	-18.8%	-13.63%	-9.09%	-4.5%
CPCA 10 ml/kg	45±1.86	45±1	43±1.86	44±2.24	45±1	44±1.52	43±1.86	45±1.86
		0%	-4.4%	-2.2%	0%	-2.2%	-4.4%	0%
Theophylline 100mg/kg	**29±2.44**	**43±3.08****	**39±1.86***	**38±1.22****	**35±1.6***	**31±1.86**	**29±1.2**	**27±1.22**
		+48.27%	**+43.48%**	**+31.03%**	**+20.7%**	**+6.89%**	**0%**	**-6.9%**
Adenosine 100 mg/kg before Theophylline 100mg/kg	29±1.37	21.6±1.2*	22.2±1.85**	23.2±0.58*	25.4±1.47	28±1. 5	27±1.22	28±1.54
		-22.8%	-20.7%	-17.14%	-9.28%	-3.45%	-3.6%	-3.45%
CPA 10ml/kg before Theophylline 100mg/kg	29±1.86	27±1.99	23±2.54**	21±1.68**	21±1.86**	23±1.99*	27±1.22	29±1
		-6.89%	-20.69%	-27.6%	-27.6%	-20.69%	-6.9%	0%
CPCA 10 ml/kg before Theophylline 100mg/kg	26±1.87	38±2.54*	35±2.73**	32±1.99**	30±1.57*	29±1.86	25±1.57	24±1.37
		+46.15%	+34.6%	+23.07%	+15.38%	+11.54%	-3.85%	-7.7%

N.B: Data represent mean ± SE of 5 observations
* p<0.05 ** p<0.01
+% = percentage increase of motor activity
- % = percentage inhibition of motor activity

Table(3): CD50 of theophylline in rats

Dose (mg/kg)	Convulsed / Tested	Observed % convulsed	Expected % convulsed	Observed - expected	Contribution to $(Chi)^2$
100	0/10	Zero	0.02	0.02	0.0000
150	2/10	20	5	15	0.4600
200	4/10	40	40	0	0.0000
250	8/10	80	80	0	0.0000
300	10/10	98.4	95	3.4	0.0280
				Total	**0.488**

Total animals = 50
Number of doses = 5
Animals/doses = 50/5 = 10
$(Chi)^2$ = 0.488 X 10 = 4.88
Degrees of freedom = 3
Exponent = 2.77/$\sqrt{30}$ = 2.77/5.47 = 0.51
$FCD_{50} = 1.24^{0.51} = 1.115$
CD_{50} X FCD_{50} = 234.15
CD_{50}/FCD_{50} = 188.34

Table(4): CD50 of theophylline in rats after CPA preatreament

Dose (mg/kg)	Convulsed / tested	Observed % convulsed	Expected % convulsed	Observed - expected	Contribution to $(Chi)^2$
100	0/10	1.3	4	2.7	0.0022
150	2/10	20	20	0	0.0000
200	4/10	40	40	0	0.0000
250	6/10	60	60	0	0.0000
300	8/10	80	72	8	0.0320
350	10/10	94.5	82	12.5	0.1150
				Total	**0.169**

Total animals = 60
Number of doses = 6
Animals/doses = 60/6 = 10
(Chi)2 = 0.169 X 10 = 1.69
Degrees of freedom = 4
Exponent = 2.77/$\sqrt{40}$ = 2.77/6.3 = 0.44
FCD50 = 1.60.44 = 1.26
CD50 X FCD50 = 283.5
CD50/FCD50 = 178.6
∴CD50 and its 95% confidence limits = 225 (178.6 – 283.5) mg/kg

Discussion

Theophylline is a methylxanthine derivative which was widely used in the treatment of asthma and bronchopulmonary obstructive diseases. It has a low therapeutic safety margin and its toxicity is manifested by restlessness, convulsions and possibly fatal arrhythmias leading to its limited clinical use to refractory cases (Vassallo and Lipsky, 1998). Many previous studies have reported that adenosine, through activation of A1 receptors, has sedative, anticonvulsant, anxiolytic, and locomotor depressant effects (Nikodijevic et al., 1991; Stone, 1991; Jain et al., 1995; Malhotra and Gupta, 1997).

Our findings, shown in this study, have observed that adenosine elicited a rapid inhibitory effect on spontaneous coordinate motor activity and forced motor performance of rats. CPA which is an adenosine A1 receptor agonist elicited a long-lasting inhibitory effect on motor activity while CPCA which is an agonist at the adenosine A2 receptor agonist didn't cause any change in the motor activity of rats and this possibly excludes any role of A2 receptors in controlling motor activity. These results are in agreement with those observed with other investigators, Popoli et al., 1998 have demonstrated that adenosine A1 antagonists stimulate motor activity and Latini et al., 1996 concluded that adenosine is an endogenous neuromodulator that exerts its depressant effects on neurons by acting on the A1 receptor subtype. In addition Marston et al., 1998 showed that adenosine A1 agonist, CPA reduced spontaneous motor activity and that CPA-induced locomotor depression was attenuated by adenosine A1 receptor selective antagonists such as DPCPX, FK 453 and FK 352 but not by the A2 receptor antagonist KF 17837. On the contrary, theophylline, a CNS stimulant produced profound CNS excitatory activity and caused restlessness and marked increase of spontaneous and forced motor activity of rats. This stimulant effect of theophylline was opposed by pretreating rats with adenosine and CPA (A1 agonist) while it was not changed after pretreatment with CPCA (A2 agonist).

In the present work, it is observed that CPA was ineffective for protection against theophylline- induced seizures, when given in a doses of 10, 20 and 30 mg/kg 60 minutes prior to theophylline in a dose of 200 mg/kg (produced 25% decrease in incidence of seizures). However, it produced significant elevation in CD50 of theophylline from 210 mg/kg to 225 mg/kg. Suggested explanations of these confused findings were that antagonism of A1 receptors by theophylline may be a mechanism of convulsant action of theophylline and so prior administration of adenosine and CPA (A1 agonist) causes increased threshold of seizure susceptibility of theophylline thus CD50 of theophylline was elevated.

These results were supported by some observations of other investigators. Hornfeldt and Larson, 1994 tested the hypothesis: does agonists acting at the adenosine A1 receptor can inhibit seizures caused by toxic doses of theophylline in mice. Results showed that pretreatment with the direct acting adenosine A1 agonists carbamazepine and cylcohexyladenosine and the indirect acting agonist dipyridamole, each failed to inhibit the ability of theophylline to cause tonic seizures. Failure of these drugs to protect against theophylline-induced seizures suggests that these seizures are produced by mechanisms other than that involving adenosine A1 receptor. Gupta and Malhotra, 1998 studied the effect of pretreatment with different doses of adenosine, the adenosine A1 receptor agonist N6-cyclopentyl adenosine (CPA) against seizures induced by theophylline in rats and observed that both these drugs, at all dose levels tested, failed to protect against theophylline seizures. Thus adensoinergic system is unlikely to be involved in mediating the convulsant action of theophylline. On the other hand, they found that the conventional antiepileptic drugs, diazepam, sodium valproate and phenobarbitone but not carbamazepine which is an adenosine A1 receptor agonist, afforded some protection.

In conclusion, the present study confirmed the suggestion of a possible antagonistic effect of theophylline on adenosine A1 receptors.

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