Effect of CPA and CPCA on forebrain neurotramitters and motor activity of rats

Romany Helmy Thabet[1], Abdullah Saud Muslah Almutairi[2], Mohammed Aqela Mosalam Alazmi[2], Ahmed Saad Hassan Alasmari[2], Nasser Jubran Saeed Aljurbui[2], Saad saleh aqil alghunaymi[2], Alanazi bander Mohammed M[2], Alotaibi Saleh Gazai B[2], Adel Nezar Tawfiq Alakkam[2]

[1]Pharmacology department, faculty of medicine, Northern Border University, Arar, Saudi Arabia
[2]Medical Student, faculty of medicine, Northern Border University, Arar, Saudi Arabia

Abstract

Background and aim of the study

Adenosine is a neuromodulator. Adenosine A1 receptors are present on glutamatergic terminals of the rat cerebral cortex. The present study is aimed at investigating the effects of the adenosine A1 agonist, N6-Cyclopentyladenosine (CPA) and the A2 receptor agonist, 5-(N-cyclopropyl) carboxamidoadenosine (CPCA) on motor activity of rats. The present work investigates also the effect of CPA and CPCA on the forebrain levels of the neurotransmitters glutamate and GABA in rats after challenge with pentylenetetrazole (PTZ) a commonly employed chemoconvulsant, used for screening drugs for anticonvulsant activity.

Methods

The present work investigated the effect of CPA and CPCA on locomotor activity and forced performance of rats and measured the forebrain levels of the neurotransmitters glutamate and GABA in rats pretreated with adenosine agonists after PTZ challenge.

Results

Cellular brain glutamate concentrations decreased significantly contrary to the significant increase in GABA levels ($P < 0.05$) in rats given ADO 1000 mg/kg 5 minutes prior to 60 mg/kg PTZ and the adenosine A1 receptor agonist, CPA, 10 mg/kg 60 minutes prior to 60 mg/kg PTZ compared with control group and PTZ-treated group. On the other contrary, the levels of both glutamate and GABA are insignificantly altered in rats given CPCA 10 mg/kg 60 minutes prior to 60 mg/kg PTZ compared with PTZ-treated group. CPA, but not CPCA exerts a significant inhibitory effect on rats motor activity.

Conclusion

The present study confirmed the neuromodulatory and inhibitory effect of adenosinergic agonists, A1 agonists e.g. CPA, on motor activity of rats

{**Citation:** Romany Helmy Thabet, Abdullah Saud Muslah Almutairi, Mohammed Aqela Mosalam Alazmi, Ahmed Saad Hassan Alasmari, Nasser Jubran Saeed Aljurbui, Saad saleh aqil alghunaymi, Alanazi bander Mohammed M., Alotaibi Saleh Gazai B, Adel Nezar Tawfiq Alakkam. Effect of CPA and CPCA on forebrain neurotramitters and motor activity of rats. American Journal of Research Communication, 2016, 4(2): 12-21} www.usa-journals.com, ISSN: 2325-4076.

Introduction

Adenosine is a purine nucleoside used as an antiarrhythmic agent, to treat supraventricular tachycardia. It is a neuromodulator, believed to play a role in promoting sleep and suppressing arousal. Adenosine also plays a role in regulation of blood flow to various organs through vasodilation.

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). Similarly activation of A1 receptors inhibits the release of acetylcholine (Ribeiro and Sebastiao, 1986) and glutamate (Dolphin and Archer, 1983) while activation of A2 receptors has an opposite effect i.e. stimulates ischemia-evoked release of acetylcholine and glutamate (Burke and Nadler, 1988; Brown et al., 1990).

Glutamate is the major excitatory neurotransmitter in the human neocortex release as their primary neurotransmitter (DeFelipe, 1993). Enhanced glutamatergic activity is coupled tightly to increased cerebral energy metabolism (Shen et al., 1999). On the contrary, GABA is the major inhibitory neurotransmitter in the adult human cortex (DeFelipe, 1993).

The present study is aimed at investigating the effects of the adenosine A1 agonist, N6-Cyclopentyladenosine (CPA) and the A2 receptor agonist, 5-(N-cyclopropyl) carboxamidoadenosine (CPCA) on motor activity of rats. The present work investigates also

the effect of CPA and CPCA on the forebrain levels of the neurotransmitters glutamate and GABA in rats after challenge with pentylenetetrazole (PTZ) a commonly employed chemoconvulsant, used for screening drugs for anticonvulsant activity.

Materials and methods

Chemicals

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

- **Adenosine (ICN biomedicals, Inc):** it was freshly prepared as a solution 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.
- Pentylenetetrazole **(PTZ) (Sigma, USA):** Soluble in normal saline and water.

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.

Measurement of Glutamate and GABA levels in rat forebrain

Glutamate and GABA levels were measured in forebrains of the following groups of rats:

- Control normal rats weighing 150-200g (negative group) comprised of 5 animals
- Positive control group challenged with PTZ in a dose of 60 mg/kg
- CPA administered i.p to rats in a dose of 10 mg/kg 60 minutes prior to acute challenge with PTZ in a dose of 60 mg/kg.
- CPCA administered i.p to rats in doses of 10 mg/kg 60 minutes prior to administration of PTZ in a dose of 60 mg/kg

Rats, of the aforementioned groups were decapitated and brains were quickly removed (<90 seconds) rostral to the cerebellum and frozen in liquid nitrogen.

Brain extraction method

Following method described by Petroff and his collegues and also by Laura and Ognen, frozen brains of the rats groups previously mentioned were extracted in 3.5 ml cold 12% perchloric acid (PCA) stock solution containing 7.7 mM dichloracetic acid and centrifuged at 3200×g for 15 minutes at 4 °C (Petroff et al., 1995, Laura and Ognen, 2003). The supernatant pH became was neutral with a solution containing 4.8 M KOH and 0.3 M K2HPO4 and centrifuged at 3200×g for 10 minutes at 4 °C. The neutral solution then was treated with 0.5 g chelating resin, filtered, and lyophilised. After that, the dried powder was dissolved in neutral 50 mM deuterated phosphate in D2O containing 2 mM isopropanol as previously described30. Dichloracetic acid was added to the extraction procedure as a concentration standard and chemical shift reference.

Effect of CPA and CPCA on motor activity of rats

5 groups of rats each was comprised of 5 animals.

Treatment schedules

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

Group B: was given i.p 0.5 ml of 8% ethanol.

Group C: was given adenosine i.p in a dose of 100 mg/kg.

Group D: was given CPA (A_1 agonist) i.p in a dose of 10 mg/kg.

Group E: was given CPCA (A_2 agonist) i.p in a dose of 10 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).

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).

Statistical analysis of the results

The degree of variability, in results of assessment of the effect of adenosine, CPA and CPCA on spontaneous coordinate activity and forced motor performance of rats, were expressed as the mean + standard error (X + SE). The significance of the differences 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

Glutamate and GABA levels in rat forebrain

Cellular brain glutamate concentrations decreased significantly contrary to the significant increase in GABA levels ($P < 0.05$) in rats given CPA10 mg/kg 60 minutes prior to 60 mg/kg PTZ compared with control group and PTZ-treated group. On the other hand the levels of both glutamate and GABA are insignificantly altered in rats given CPCA 10 mg/kg 60 minutes prior to 60 mg/kg PTZ compared with PTZ-treated group (See table 1).

Table (1) : Glutamate and GABA levels in rat forebrain after PTZ challenge

Group	Glutamate	GABA
Negative control	12.5 ± 0.3	2.28 ± 0.05
PTZ 60 mg/kg	14.25 ± 0.2*	1.75 ± 0.03*
CPA10 mg/kg 60 minutes prior to 60 mg/kg PTZ	11.2 ± 0.1*#	2.42 ± 0.03*#
CPCA 10 mg/kg 60 minutes prior to 60 mg/kg PTZ	14.1 ± 0.1*	1.83 ± 0.05*

Values represent the mean concentrations (mM) with ± SEM. * $p < 0.05$ versus control. # $P < 0.05$ versus PTZ 60 mg/kg using the student *t*-test

Effect of i.p injection of 8% Tween 20 , 8% ethanol , adenosine, CPA and CPCA on the spontaneous coordinate activity in rats (see table 2).

Effect of i.p injection of 8% Tween 20 and 8% ethanol on the spontaneous coordinate activity in rats

Intraperitoneal injection of o.5 ml of each of 8% Tween 20 and 8% ethanol showed no significant changes in the spontaneous coordinate activity of rats.

Effect of adenosine on the spontaneous coordinate activity in rats

Intraperitoneal injection of adenosine in a dose of 100 mg/kg produced significant decrease in spontaneous coordinate activity of rats. Maximal effect was noticed 1 minute after administration. The decrease of spontaneous activity 5 minutes after administration was less. Spontaneous activity returned to normal 15 minutes after administration.

Effect of CPA on the spontaneous coordinate activity in rats

Intraperitoneal injection of CPA in a dose of 10 mg/kg produced also a decrease in the spontaneous motor activity of rats. The effect was maximal 15 minutes after administration, and then motor activity returned to normal 2 hours post-injection.

Effect of CPCA on the spontaneous coordinate activity in rats

Intraperitoneal injection of CPCA in a dose of 10 mg/kg showed no significant changes in the spontaneous activity of rats.

Table 2: effect of I.P. Injection of 8% Tween 20, adenosine, CPA and CPCA each alone 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%

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

Effect of i.p injection of 8% Tween 20 , 8% ethanol , adenosine, CPA and CPCA on the forced motor performance of rats (see table 3).

Effect of i.p injection of 8% Tween 20 and 8% ethanol on the forced motor performance of rats

Intraperitonal injection of each of 8% Tween 20 and 8% ethanol produced no changes in the forced motor performance of rats.

Effect of adenosine on the forced motor performance of rats

Intraperitoneal injection of adenosine, in a dose of 100 mg/kg, produced inhibition of the forced motor performance of rats. The maximal inhibition was 1 minute after injection.

Effect of CPA on the forced motor performance of rats

Intraperitoneal injection of CPA, in a dose of 10 mg/kg, produced inhibition of the forced motor performance. The inhibition started 1 minute after injection. Maximum inhibition was 15 minutes after injection.

Effect of CPCA on the forced motor performance of rats

Intraperitoneal injection of CPCA in a dose of 10 mg/kg produced no significant changes in the forced motor performance in rats.

Table 3: effect of I.P . injection of 8% Tween 20, adenosine, CPA and CPCA each alone 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 (0.5 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%

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

Discussion

The present study illustrates how modulation of purinergic receptors alters the cellular brain levels of both the major excitatory neurotransmitter glutamate and the major inhibitory neurotransmitter GABA. Results have shown that cellular brain glutamate concentrations decreased significantly contrary to the significant decrease in GABA levels ($P < 0.05$) in rats given ADO 1000 mg/kg 5 minutes prior to 60 mg/kg PTZ, the adenosine analogue,2-CAD, 5 mg/kg 30 minutes prior 60 mg/kg PTZ and the adenosine A1 receptor agonist, CPA, 10 mg/kg 60 minutes prior to 60 mg/kg PTZ compared with control group and PTZ-treated group. On the other contrary, the levels of both glutamate and GABA are insignificantly altered in rats given CPCA 10 mg/kg 60 minutes prior to 60 mg/kg PTZ compared with PTZ-treated group. This is in agreement with the conclusion of Mario et al. that adenosine and adenosine receptor agonists modulate glutamate release by activating inhibitory A1 receptors present on glutamatergic terminals of the rat cerebral cortex (Mario et al., 2002).

Our finding agree with previous studies which reported that adenosine acting through its abundant adenosine receptors modulates glutamatergic neurotransmission in the striatum. The striatum shows presynaptically localized adenosine A2A receptors in glutamatergic nerve terminals that join GABAergic striatonigral dynorphinergic medium spiny neurons, which heterodimerize with adenosine A1 receptors. At the presynaptic location, low concentrations of adenosine were hypothesized to bind preferentially to adenosine A1 receptors and decrease glutamate release. By contrast, high concentrations of synaptic adenosine, obtained by strong cortico–limbic–thalamic input with high co-release of glutamate and ATP, will consecutively induce occupation of adenosine A2A receptors, and thus counteract the effects of adenosine A1 receptor stimulation and increase the probability of glutamate release (Kiran and Kulkarni, 2012). This is in line with our finding that showed the significant increase in glutamate release in rats given the adenosine A2 agonist, CPCA, compared with levels in normal control animals.

Our foregoing results have shown 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.

In conclusion, the present study confirmed the neuromodulatory and inhibitory effect of adenosinergic agonists, A1 agonists e.g. CPA, on motor activity of rats

References

Brown SJ,James S, Reddington M and Richardson PJ (1990): Both A_1 and A_{2a} Purine receptors regulate striatal acetylcholine release. J. Neurochem. 55: 31 – 38.

Burke SP and Nadler JV (1988): Regulation of glutamate and aspartate release from slices of the hippocampal CA1 area: Effects of adenosine and baclofen. J. Neurochem. 51: 1541 – 1551.

Dolphin AC and Archer CR (1983): An adenosine agonist inhibits and a cyclic AMP analogue enhanced the release of glutamate but not GABA from slices of rat dentate gyrus. Neurosci Lett 43: 49 – 54.

Dunham NW and Miya TS (1957): A note on a simple apparatus for detecting neurological deficit in rats and mice. J Am pharmac Ass (Sci-Ed) 46: 208 – 209.

Kiran Kumar Akula, SK Kulkarni (2012): Adenosinergic System. An Assorted Approach to Therapeutics for Drug Addiction. Future Neurology 2012;7(3):307-327.

Latini S, Pazzagli M, Pepeu G and Pedata F (1996): A2 adenosine receptors: their presence and neuromodulatory role in the central nervous system. Gen. Pharmacol. 27(6): 925 – 933.

Laura D Errante, Ognen A.C Petroff(2003). Acute effects of gabapentin and pregabalin on rat forebrain cellular GABA, glutamate, and glutamine concentrations. Seizure. 12: (5), 300–306

Londos C, Cooper DMF and Wolff J (1980): Subclasses of external adenosine receptors. Proc. Natl. Acad. Sci. USA. 77: 2551 – 2554.

Mario Marchi, Luca Raiteri, Francesca Risso, Annalisa Vallarino, Andrea Bonfanti, Angela Monopoli, Ennio Ongini ,Maurizio Raiteri (2002). Effects of adenosine A1 and A 2A receptor activation on the evoked release of glutamate from rat cerebrocortical synaptosomes British Journal of Pharmacology (2002) 136, 434 -440

Paul V and Kazi M (1992): Measuring spontaneous motor activity of rats using a vibration sensing device. Biomedicine Journal Volume 12(2).

Petroff O.A.C., Pleban L.A, Spencer D. D. Symbiosis between in vivo and in vitro NMR spectroscopy: the creatin, N-acetylaspartate, glutamate, and GABA content of epileptic human brain. Magnetic Resonance Imaging, 13 (1995), pp. 1197–1211

Popoli P, Reggio R, Pezzola A, Fuxe K and Ferre S (1998): Adenosine A1 and A2a receptor antagonists stimulate motor activity. Neurosci. Lett. 251(3): 201 – 204.

Ribeiro JA and Sebastiao AM (1986): Adenosine receptors and calcium: basis for proposing a third (A_3) adenosine receptor. Prog. Neurobiol. 26: 179 – 209.

Shen, J., Petersen, K.F., Behar, K.L. et al. Determination of the rate of the glutamate–glutamine cycle in human brain by in vivo NMR. Proceedings of the National Academy of Sciences of the United States of America. 1999; 96: 8235–8240

Snedecor GW (1967): “Statistical methods” Iowa state college press.

William M (1990): Purine nucleosides and nucleotides as central nervous system modulators. Ann. New York Acad. Sci. 603: 93 – 107.