Medical Journal of the
Islamic Republic of Iran

Volume 12
Number 4
Winter 1377
February 1999

ANTINOCICEPTIVE INTERACTION BETWEEN ADENOSINE AND CARBAMAZEPINE IN MICE

H. HOSSEINZADEH, Ph.D., AND M.E. SHOOSHTARI, Pharm.D.

From the Department of Pharmacodynamy and Toxicology, Faculty of Pharmacy, Mashhad University of Medical Sciences, P.O. Box 91775-1365, Mashhad, I.R. Iran.

ABSTRACT

There are several reports that adenosine and carbamazepine have pharmacodynamic interaction. In this study the antinociceptive interaction of these two agents was evaluated in mice by the hot plate test. Agents were injected intraperitoneally.

0.2 and 0.4 mg/kg doses of R-phenylisopropyladenosine (R-PIA) substantially showed antinociceptive effects. Carbamazepine had antinociceptive actions except at a dose of 0.8 mg/kg. Theophylline (12.5 mg/kg), as a non-selective antagonist of adenosine receptors, showed a nociceptive effect. Dipyridamole, an uptake blocker of adenosine, had no antinociceptive effect even with a dosage of 90 mg/kg. Theophylline did not decrease the antinociceptive effect of carbamazepine, while dipyridamole increased the antinociceptive effect of carbamazepine 30 minutes after administration. Carbamazepine (8 mg/kg) inhibited the antinociceptive effect of R-PIA.

This study supports the possibility that the interaction between adenosine and carbamazepine may be related to their actions at adenosine receptor sites in the brain.

MJIRI, Vol. 12, No. 4, 381-386, 1999

Keywords: Adenosine, R-PIA, Carbamazepine, Theophylline, Dipyridamole, Antinociceptive.

INTRODUCTION

Adenosine has effects on almost all kinds of mammalian tissue including the central nervous system. As a neuromodulator adenosine has a profound depressant action in the central nervous system. Its action may be mediated pre- and post-synaptically through receptor-mediated mechanisms, including effects on second messenger systems, transmembrane ion fluxes and neurotransmitter release.[2,5,19]

Carbamazepine has different clinical uses due to its anticonvulsant, antimanic and antinociceptive activities.[9] The mechanism of the anticonvulsant activity of carbamazepine is not fully clear. This drug, like phenytoin, inhibits voltage-dependent Na^+ channels.[13] Interest in the possibility that the anticonvulsant action of carbamazepine may relate to an interaction with adenosine receptors developed as a result of different observations.

Since the clinical properties of carbamazepine are reminiscent of the anticonvulsive, sedative, and anxiolytic properties of adenosine, an adenosine-agonistic activity of carbamazepine has been proposed. However, indirect evidence suggested a rather antagonistic activity of carbamazepine at adenosine receptors.[1,20] Carbamazepine inhibits the binding of adenosine analogs [^{3}H]L-N6-phenylisopropyladenosine and [^{3}H]cyclohexyladenosine to synaptosomal membranes in the rat brain.[7,16]

Carbamazepine was found to antagonize the A_1-receptor-

Correspondence:
H. Hosseinzadeh, Ph.D., Department of Pharmacodynamy and Toxicology, Faculty of Pharmacy, Mashhad University of Medical Sciences, P.O. Box 91775-1365, Mashhad, I.R. Iran.

mediated inhibition of cyclic AMP accumulation in cultured astroblasts and in GH3-cells. Carbamazepine also inhibited the adenosine-induced increase in the level of cyclic AMP in cultured astroblasts, which is mediated by low-affinity A_{2b}-receptors.[21]

In the present study we have investigated the antinociceptive interaction of carbamazepine with the adenosine receptor in the central nervous system.

MATERIAL AND METHODS

Animals

Male and female albino mice weighing 25-30g were obtained from a random bred colony maintained on a special diet (Khorassan Javane Co., Mashhad, I.R. Iran) in the animal lab of Mashhad University of Medical Sciences. Animals were housed in a room with a 12/12 hr light/dark cycle at 21±2°C. The animals had free access to food and water.

Measurement of analgesic activity

Antinociceptive activity was assessed by the hot-plate test. The temperature of a metal surface was maintained at 55±0.2°C. The latency to a discomfort reaction (licking paws or jumping) was determined before and after drug administration. The cut-off time was 40 sec. All drugs were injected intraperitoneally (ip). When two agents were used, injections in two sites of the peritoneum were with a 30 minute interval.

Carbamazepine was dissolved in 50% propylene glycol and 50% saline warmed on a water bath. The solvent of R-PIA was water. Theophylline was dissolved in NaOH and the final preparation was restored to a pH of 7.4. Dipyridamole was suspended in carboxymethyl-cellulose (1%). Control groups received only the solvent. Carbamazepine, dipyridamole and theophylline were gifts from CIBA-GEIGY, Thomae and Darupakhsh (I.R. Iran), respectively. R-PIA was purchased from Sigma.

Statistical analysis

The data were expressed as mean values±S.E.M. and tested with analysis of variance followed by the multiple comparison test of Tukey.

RESULTS

Effect of R-PIA on hot-plate latency

R-PIA significantly induced an antinociceptive effect and the maximum responses were observed at 0.4 mg/kg, 30 minutes after agent injection (Fig. 1).

Effect of carbamazepine on hot-plate latency

Intraperitoneal administration of different doses of carbamazepine to mice induced a dose-dependent

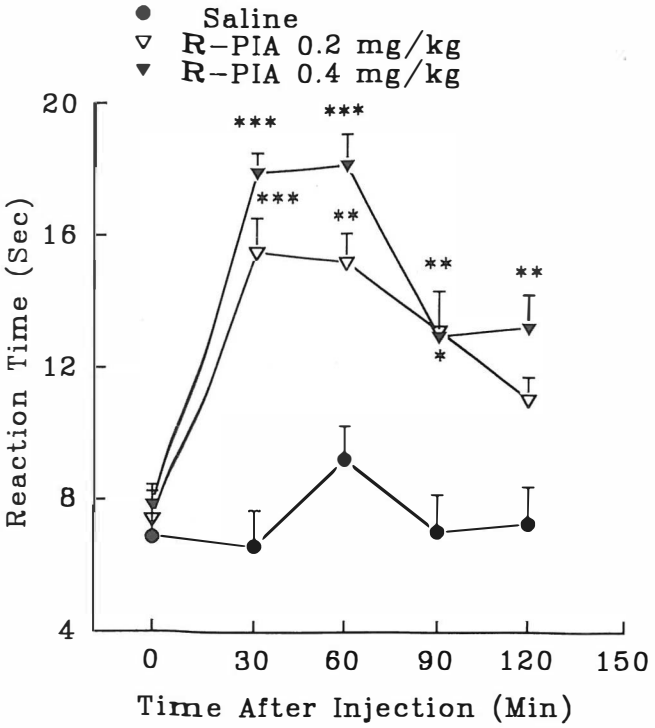

Fig. 1. Effect of intraperitoneal injection of R-phenylisopropyladenosine (R-PIA) on pain thresholds of mice in the hot-plate test. Each point represents the mean±S.E.M. of the reaction time of six mice. A Tukey-Kramer test was employed to determine the significance level relative to controls (*p<0.05, **p<0.01, ***p<0.001).

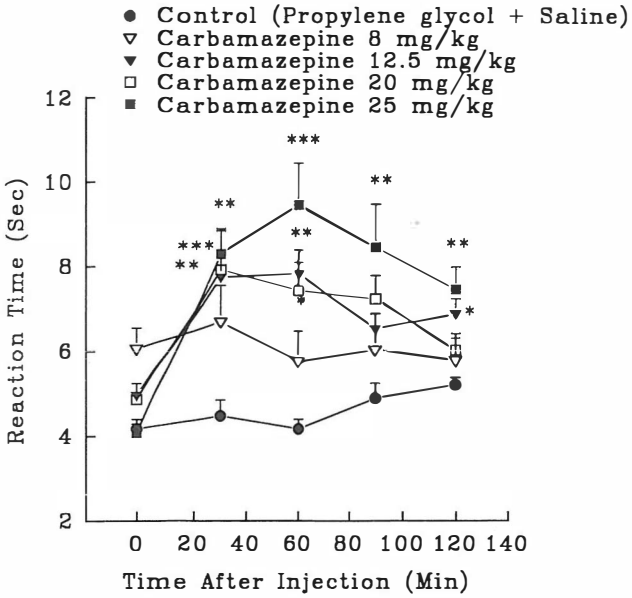

Fig. 2. Effect of intraperitoneal injection of carbamazepine (CBZ) on pain threshold of mice in the hot-plate test. Each point represents the mean±S.E.M. of the reaction time of six mice. A Tukey-Kramer test was employed to determine the significance level relative to controls (*p<0.05, **p<0.01, ***p<0.001).

Table I. Effect of theophylline on carbamazepine (CBZ) antinociception in the hot-plate test. All mice were injected intraperitoneally.

	Reaction Time (sec)				
Treatment (mg/kg)	**0 min**	**30 min**	**60 min**	**90 min**	**120 min**
Control (Theophylline)	6.28±0.39	7.12±0.36	6.13±0.45	6.75±0.30	7.05±0.55
Control (Carbamazepine)	4.17±0.23	4.47±0.38	4.17±0.23	4.90±0.36	5.22±0.17
Control (Theophylline+CBZ)	3.87±0.10	3.78±0.25	4.63±0.25	3.97±0.33	3.82±0.19
Theophylline (7)	5.19±0.38	4.84±0.35	4.27±0.23	3.93±0.58	4.57±0.56
Carbamazepine (20)	4.87±0.38	7.93±0.97	7.43±0.96	7.23±0.55	6.20±0.39
Carbamazepine (25)	4.08±0.21	8.30±0.55	9.46±0.93	8.45±1.02	7.45±0.53
Theophylline (7) + CBZ (20)	5.50±0.44	5.62±0.26	8.67±0.56	9.73±0.64	7.93±0.45
Theophylline (7) + CBZ (25)	5.64±0.22	5.76±0.17	11.63***± 0.43	10.49*± 0.33	8.59±0.48

*$p<0.05$, ***$p<0.001$; theophylline group compared with theophylline+CBZ 25 mg/kg group, Tukey-Kramer test, N= 6 mice; control group of carbamazepine and theophylline received propylene glycol+saline and sodium hydroxide, respectively.

antinociceptive effect (Fig. 2). The maximum response was observed with 25 mg/kg, 60 minutes after drug injection.

Effect of dipyridamole on hot-plate latency

Doses up to 90 mg/kg of dipyridamole had no effect on the pain threshold of mice (Fig. 3).

Effect of theophylline on hot-plate latency

Theophylline reduced hot plate latency, implying an enhancement of pain sensitivity. The maximum response was observed with 50 mg/kg, 30 minutes after drug injection (Fig. 4).

Effect of theophylline on carbamazepine-induced antinociception

Theophylline at 7 mg/kg increased the antinociceptive effect of carbamazepine (25 mg/kg) at 60 and 90 minutes after injection (Table I).

Effect of dipyridamole on carbamazepine-induced antinociception

Dipyridamole increased the antinociceptive effect of carbamazepine only at a dose of 90 mg/kg (Table II).

Effect of carbamazepine on R-PIA induced antinociception

Carbamazepine, at a concentration that had no antinociceptive activity (8 mg/kg), reduced the antinociceptive effect of R-PIA 30 and 60 minutes after its administration (Table III).

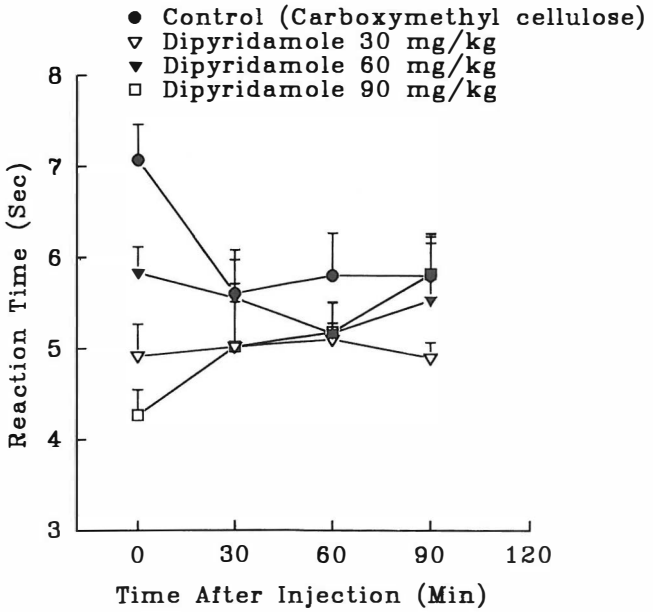

Fig. 3. Effect of intraperitoneal injection of dipyridamole on the pain threshold of mice in the hot-plate test. Each point represents the mean±S.E.M. of the reaction time of six mice. A Tukey-Kramer test was employed to determine the significance level relative to controls.

DISCUSSION

The results of this study indicate that carbamazepine inhibits the antinociceptive effect of R-PIA at a concentration that by itself has no effect.

Table II. Effect of dipyridamole on carbamazepine (CBZ) antinociception in the hot-plate test. All mice were injected intraperitoneally.

	Reaction Time (sec)				
Treatment (mg/kg)	**0 min**	**30 min**	**60 min**	**90 min**	**120 min**
Control (Dipyridamole)	7.08±0.39	5.60±0.48	5.83±0.45	5.80±0.46	4.58±0.24
Control (Carbamazepine)	4.17±0.23	4.42±0.43	4.17±0.28	4.90±0.39	5.22±0.24
Control (Dipyridamole+CBZ)	4.58±0.24	4.59±0.34	4.95±0.25	5.34±0.27	5.69±0.33
Carbamazepine (25)	4.08±0.21	8.30±0.55	9.46±0.99	8.45±1.02	7.45±0.53
Dipyridamole (60)	5.83±0.29	5.55±0.43	5.17±0.32	5.53±0.62	5.81±0.41
Dipyridamole (90)	4.27±0.28	5.02±0.69	5.18±0.33	5.82±0.42	5.32±0.65
Dipyridamole (60) + CBZ (25)	5.10±0.39	10.81±0.87	8.84±0.49	7.03±0.51	6.17±0.56
Dipyridamole (90) + CBZ (25)	5.98±0.32	11.58***± 0.64	10.18±0.43	8.90±0.42	8.90±0.42

***$p<0.001$; carbamazepine group compared with dipyridamole (90)+CBZ 25 mg/kg group, Tukey-Kramer test, N= 6 mice; control group of carbamazepine and dipyridamole received propylene glycol+saline and carboxymethyl cellulose, respectively.

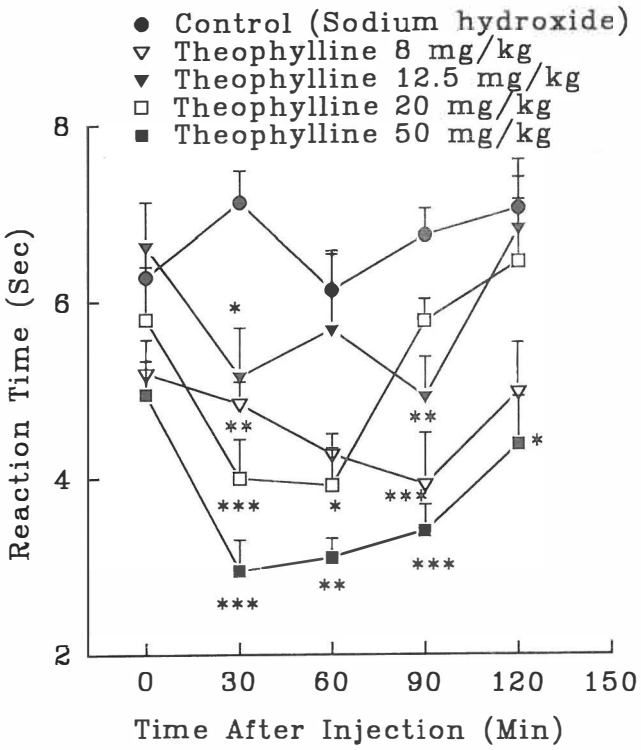

Fig. 4. Effect of intraperitoneal injection of theophylline on the pain threshold of mice in the hot-plate test. Each point represents the mean±S.E.M. of the reaction time of six mice. A Tukey-Kramer test was employed to determine the significance level relative to controls (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

The antinociceptive dose of carbamazepine in this study is similar to the dose which showed anticonvulsant activity against electroshock seizures in mice (18 mg/kg) or pentylenetetrazole-induced tonic extensor seizures in rats (10 mg/kg).[6,17] Thus carbamazepine—at anticonvulsant doses—may have antinociceptive effects.

Theophylline dose-dependently induced a reduction of hot-plate latency. This effect may relate to the blockade of A_1 adenosine receptors that have an analgesic effect. In this study theophylline increased the antinociceptive effect of carbamazepine. This interaction implies that the antinociceptive effect of carbamazepine was not mediated by adenosine receptors. The reason for this effect is not clear in this experiment. In the study of interaction of adenosine with the GABA system, theophylline increased the antinociceptive effect of baclofen. The authors of this article discussed that this effect may relate to changes in dopaminergic equilibrium.[14]

In this study intraperitoneal injections of dipyridamole, an adenosine uptake blocker, had no antinociceptive action in mice. This may indicate that there is not enough adenosine in the extracellular medium in the normal situation. This neuromodulator is liberated during situations such as hypoxia or seizures when dipyridamole can then be effective.[2,5] Dipyridamole increased the antinociceptive effect of carbamazepine only at 30 minutes after injection. The aim of this experiment was to study the role of adenosine in affecting the actions of carbamazepine. Since the effect of carbamazepine was increased only at 30 minutes, it is unlikely that adenosine has a role in the antinociceptive effect of carbamazepine. Carbamazepine blocks adenosine uptake and this may be potentiated by dipyridamole, but carbamazepine only has this effect with high doses.[15]

At a concentration that had no antinociceptive effect, carbamazepine inhibited the antinociceptive effect of the A_1 adenosine receptor agonist, R-PIA. This interaction is

Table III. Effect of carbamazepine (CBZ) on R-phenylisopropyladenosine (R-PIA) antinociception in the hot-plate test. All mice were injected intraperitoneally.

	Reaction Time (sec)				
Treatment (mg/kg)	**0 min**	**30 min**	**60 min**	**90 min**	**120 min**
Control (R-PIA)	7.87±0.56	7.57±0.61	8.23±0.55	8.04±0.58	8.33±0.51
Control (Carbamazepine)	8.33±0.67	7.25±0.43	8.10±0.61	8.33±0.48	7.10±0.42
Control (R-PIA+CBZ)	7.30±0.44	7.90±0.57	7.51±0.43	7.58±0.54	7.78±0.54
Carbamazepine (8)	6.97±0.50	7.77±0.55	9.16±0.42	9.49±0.47	8.31±0.34
R-PIA (0.4)	7.86±0.60	18.00±0.59	18.20±0.94	13.00±1.36	13.24±1.00
R-PIA (0.4) + Carbamazepine (8)	7.00±0.32	13.84***± 0.99	13.98**± 0.69	12.53±0.57	12.51±0.51

**p<0.01, ***p<0.001; R-PIA 0.4 mg/kg group compared with R-PIA+CBZ group, Tukey-Kramer test, N= 6 mice; control group of R-PIA and carbamazepine received saline and propylene glycol+saline, respectively.

similar to the results of Stone[18] who showed that carbamazepine blocked the inhibitory activity of 2-chloroadenosine on orthodromic potentials of hippocampal slices.

In view of this fact that both adenosine and carbamazepine have depressant actions on the central nervous system,[10,12] it is a paradox that carbamazepine blocks the antinociceptive effect of an adenosine agonist. However, other results such as the reduction of the anticonvulsant activity of adenosine on cortex neurons,[11] inhibition of the effect of adenosine on adenylate cyclase[16] and reduction of adenosine analogue effects on muscle contraction by carbamazepine[17] are consistent with our results. On the other hand, adenosine depresses the excitability of pyramidal neurons in the hippocampus. This effect was lost in calcium-free media[4] and carbamazepine restored sensitivity to adenosine in calcium-free medium.[3]

With chronic treatment, carbamazepine also increased adenosine A_1 receptor density in the rat brain.[7-8] Therefore, chronic carbamazepine treatment appears to upregulate adenosine receptors, suggesting that this drug may act as an adenosine antagonist. In contrast to other anticonvulsants such as phenobarbital, phenytoin, primidone, sodium valproate and ethosuximide, the anticonvulsant carbamazepine inhibited binding of cyclohexyladenosine, a selective adenosine A_1 receptor, to rat cerebral tissues.[22]

In summary, this study has demonstrated a reduction of adenosine antinociceptive effects by carbamazepine. Carbamazepine may antagonize adenosine A_1 receptors in the brain. Further basic and clinical studies are required to confirm this interaction.

ACKNOWLEDGEMENT

The authors are thankful to T.W. Stone, Professor of Pharmacology, Division of Neuroscience and Biomedical Systems, University of Glasgow, Scotland, for his guidance.

REFERENCES

1. Dragunow M: Purinergic mechanisms in epilepsy. Prog Neurobiol 31: 85-108, 1988.
2. Higgins MJ, Hosseinzadeh H, MacGregor DG, Ogilvy H, Stone TW: Release and actions of adenosine in the central nervous system. Pharm World Sci 16: 62-68, 1994.
3. Hosseinzadeh H, Stone TW: Mechanism of the hippocampal loss of adenosine sensitivity in calcium-free media. Brain Res 569: 221-225, 1994.
4. Hosseinzadeh H, Stone TW: The effect of calcium removal on the suppression by adenosine of epileptiform activity in the hippocampus: demonstration of desensitization. Br J Pharmacol 112: 316-322, 1994.
5. Hosseinzadeh H, Stone TW: Adenosine in the central nervous system. Med J Islam Rep Iran 9: 361-368, 1996.
6. Kulkarini C, Joseph T, David J: Inhibition of anticonvulsant action of carbamazepine by aminophylline and caffeine in rats. Indian J Exp Biol 27: 1048-1051, 1989.
7. Marangos PJ, Post RM, Patel J, Zander K, Parma A, Weiss S: Specific and potent interaction of carbamazepine with brain adenosine receptors. Eur J Pharmacol 93: 175-182, 1983.
8. Marangos PJ, Weiss SRB, Montgomery P, Patel J, Narang PK, Cappabinaca AM, Post RM: Chronic carbamazepine treatment increases brain adenosine receptors. Epilepsia 26: 493-498, 1985.
9. McNamara JO: Drugs effective in the therapy of the epilepsies. In: Hardman JG, Limbird LE, (eds), Goodman and Gilman's The Pharmacological Basis of Therapeutics. New York: McGraw-Hill, pp. 461-486, 1996.

10. Olpe HR, Baudry M, Jones RSG: Electrophysiological and neurochemical investigations on the action of carbamazepine on the rat hippocampus. Eur J Pharmacol 110: 71-80, 1985.
11. Phillis JW: Interaction of the anticonvulsant diphenylhydantoin and carbamazepine with adenosine on cerebral cortical neurones. Epilepsia 25: 765-772, 1984.
12. Phillis JW, Kostopoulos KG, Limacher JJ: A potent depressant action of adenine derivatives on cerebral cortical neurones. Eur J Pharmacol 30: 125-129, 1975.
13. Rogawaski MA, Porter RJ: Antiepileptic drugs: pharmacological mechanism and clinical efficacy with consideration of promising developmental stage compounds. Pharmacol Rev 42: 223-286, 1990.
14. Sabetkasai M, Zarrindast MR: Antinociceptive interaction between adenosine and GABA system. Arch Int Pharmacodyn 322: 14-22, 1993.
15. Skerritt JH, Davies LP, Johnstone GAR: A purinergic component in the anticonvulsant action of carbamazepine? Eur J Pharmacol 82: 195-197, 1982.
16. Skerritt JH, Davies LP, Johnstone GAR: Interactions of the anticonvulsant carbamazepine with adenosine receptors. 1. Neurochemical studies. Epilepsia 24: 634-642, 1983.
17. Skerritt JH, Johnstone GAR, Chen C: Interactions of the anticonvulsant carbamazepine with adenosine receptors. 2. Pharmacological studies. Epilepsia 24: 643-650, 1983.
18. Stone TW: Interaction of carbamazepine, chlomethiazole and pentobarbitone with adenosine on hippocampal slices. Gen Pharmacol 19: 67-72, 1988.
19. Stone TW, Simmonds HA: Purines: Basic and Clinical Aspects. London: Kluwer Academic Press, pp. 1-7, 69-89, 1991.
20. Van Calker D, Berger M: Possible role of adenosine receptors in psychiatric diseases. Drug Dev Res 28: 354-358, 1993.
21. Van Calker D, Steber R, Klotz KN, Greil W: Carbamazepine distinguishes between adenosine receptors that mediate different second messenger responses. Eur J Pharmaco Mol Pharmacol Sec 206: 285-290, 1991.
22. Weir RL, Padgett W, Daly JW, Anderson SM: Interaction of anticonvulsant drugs with adenosine receptors in the central nervous system. Epilepsia 25: 492-498, 1984.