ELSEVIER

European Journal of Pharmacology 310 (1996) 257–261

ejp

Adenosine A_2 receptor activation facilitates $^{45}Ca^{2+}$ uptake by rat brain synaptosomes

M. Lurdes Gonçalves *, J. Alexandre Ribeiro

Laboratory of Pharmacology, Gulbenkian Institute of Science, 2781 Oeiras, Portugal

Received 15 February 1996; revised 29 April 1996; accepted 14 May 1996

Abstract

Adenosine has been shown to increase the release of neurotransmitters by stimulation of adenosine A_2 receptors. This effect probably depends on Ca^{2+} entry into presynaptic nerve terminals. In the present work the ability of the mixed adenosine A_1/A_2 agonist, 2-chloroadenosine, to stimulate Ca^{2+} uptake into rat brain synaptosomes was investigated. $^{45}Ca^{2+}$ uptake was induced by 20 μM veratridine. In the absence of other drugs, 2-chloroadenosine (1 μM) decreased $^{45}Ca^{2+}$ uptake into synaptosomes. Blocking the adenosine A_1 receptor with 100 nM of 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), 2-chloroadenosine (1 μM) increased rather than decreased the uptake of $^{45}Ca^{2+}$ into synaptosomes. The excitatory effect of 2-chloroadenosine observed in the presence of DPCPX was reversed by 200 nM of ω-agatoxin-IVA, a specific P-type Ca^{2+} channel antagonist, but not by L-type (nifedipine, 100 nM to 1 μM ; methoxyverapamil 1–10 μM) or N-type (ω-conotoxin GVIA, 500 nM) Ca^{2+} channel antagonists. The adenosine A_{2A} selective agonist, 2-*p*-(2-carboxyethyl)-phenethylamino-5′-*N*-ethyl-carboxamido-adenosine (CGS 21680), did not significantly modify Ca^{2+} uptake induced by veratridine. In contrast, the selective adenosine A_2 receptor agonist, N^6-(2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethyl)-adenosine (DPMA), in concentrations ranging from 10 nM to 1 μM increased Ca^{2+} uptake induced by veratridine. The selective adenosine A_2 receptor antagonist 3,7-dimethyl-1-propargylxanthine (DMPX) at a concentration of 10 μM antagonized the stimulatory effect of DPMA (0.1 μM) on $^{45}Ca^{2+}$ uptake. In conclusion, activation of adenosine A_2 receptors increases Ca^{2+} uptake by synaptosomes depolarized by veratridine, which could explain the increase of neurotransmitter release observed when A_2 receptors are activated.

Keywords: Adenosine; Ca^{2+} uptake; Ca^{2+} channel; Brain synaptosome

1. Introduction

It is now well documented that adenosine can exert excitatory modulatory effects on the central nervous system via activation of adenosine A_2 receptors. For example, Sebastião and Ribeiro (1992) showed that the selective adenosine A_{2A} receptor agonist, 2-[4-(2-carboxyethyl)phenethylamino]-5′-*N*-ethylcarboxamidoadenosine (CGS 21680), increases the amplitude of evoked population spikes recorded from the CA1 pyramidal cells of the rat hippocampal slices. Excitatory effects of adenosine enhancing the release of neurotransmitters were found in rat cerebral cortical slices (Spignoli et al., 1984) as well as in the rat striatum (Brown et al., 1990), where the adenosine agonist, 5′-*N*-ethylcarboxamideadenosine (NECA), increased the release of acetylcholine. Other studies showed that the adenosine A_{2A} receptor agonist, CGS 21680, at low nanomolar concentrations, enhances acetylcholine release from striatal synaptosomes (Kirk and Richardson, 1994) and from the CA3 and dentate gyrus areas of the hippocampus (Cunha et al., 1994). Recent studies (Cunha et al., 1995) provide evidence showing that hippocampal cholinergic nerve terminals possess adenosine A_{2A} receptors, which, stimulated by veratridine, facilitate the release of acetylcholine. Stimulation of A_{2A} receptors also enhances the release of excitatory amino acids, namely glutamate and aspartate, from the ischemic rat cerebral cortex (O'Regan et al., 1992). Furthermore, in guinea-pig hippocampal slices, Okada et al. (1992) observed that adenosine at low nanomolar concentrations increases glutamate release and hippocampal excitability. Also by using the non-selective A_1/A_2 receptor agonist, the stable adenosine analogue 2-chloroadenosine, Mogul et al. (1993) identified, in the CA3 area of guinea-pig hippocampus, excita-

* Corresponding author. Tel.: 351-1-443 14 85; fax: 351-1-443 16 31.

PII S0014-2999(96)00383-4

tory adenosine A_2 receptors, probably of the A_{2B} subtype, which enhance P-type Ca^{2+} currents through a cyclic AMP-dependent mechanism.

As it is well established that the evoked release of neurotransmitters depends on the entry of Ca^{2+} ions into presynaptic nerve terminals, and because isolated nerve terminals (synaptosomes) are bioenergetically intact, retain the ability to release neurotransmitters in a Ca^{2+}-dependent manner, and are a good model to explore events occurring at nerve terminals (Nicholls, 1989), we tested, in the present work, whether stimulation of adenosine A_2 receptors could increase Ca^{2+} entry into rat brain synaptosomes.

A preliminary account of this work has been presented (Gonçalves and Ribeiro, 1995).

2. Materials and methods

2.1. Preparation of the synaptosomal fraction

Experiments were performed on rat brain synaptosomes prepared according to the method described by Hajõs (1975). In brief, brains from five male Wistar rats (about 2 months) were homogenized in 9 volumes of 0.32 M sucrose and 20 mM Tris-base (pH 7.4) and centrifuged at $1500 \times g$ for 10 min. The supernatant was saved, and the pellet was resuspended and centrifuged as before. The resulting supernatants were combined and centrifuged at $9000 \times g$ for 20 min. The pellet was washed and dispersed in 0.32 M sucrose and 20 mM Tris-base (pH 7.4). Five-milliliter samples of this suspension were layered carefully on 20 ml of 0.8 M sucrose and 20 mM Tris-base (pH 7.4) and centrifuged at $9000 \times g$ for 30 min. The particle fraction dispersed in the 0.8 M sucrose solution (synaptosomal fraction) was collected and adjusted to 0.4 M sucrose by addition of 20 mM Tris-base (pH 7.4) in a dropwise manner while stirring. This suspension was centrifuged at $20\,000 \times g$ for 30 min. The resulting pellet was suspended in an incubation medium containing 132 mM NaCl, 5 mM KCl, 1.3 mM $MgCl_2$, 1.2 mM NaH_2PO_4, 1.2 mM $CaCl_2$, 10 mM glucose and 20 mM Tris-base, pH 7.4. The synaptosomal protein concentration in the suspension was measured according to the method of Lowry as modified by Peterson (Peterson, 1977).

2.2. $^{45}Ca^{2+}$ uptake induced by veratridine

Fifty microliters of the synaptosomal suspension (about 0.30 mg of protein) were added to 890 μl of the incubation medium and pre-incubated in the presence and absence of the test drugs for a period of 20 min at 32°C. Ca^{2+} uptake into synaptosomes was initiated by addition of 60 μl of the incubation medium containing $^{45}CaCl_2$ (specific activity, 0.32 μCi/μmol of Ca^{2+}) and veratridine. The concentration of veratridine in the test tube was 20 μM. After 10 min the uptake was terminated by addition of 4 ml EGTA solution (120 mM NaCl, 5 mM KCl, 5 mM EGTA, and 20 mM Tris-base, pH 7.4) followed by filtration through GF/C glass fiber filters. The filters were washed three times with 5 ml of wash solution (132 mM NaCl, 5 mM KCl, 1.3 mM $MgCl_2$, 1.2 mM $CaCl_2$, and 20 mM Tris-base, pH 7.4). The radioactivity associated with the synaptosomes was determined in a scintillation spectrometer. The net stimulated $^{45}Ca^{2+}$ uptake is the difference between $^{45}Ca^{2+}$ uptake under stimulation conditions (20 μM veratridine) and without stimulation (basal conditions). In each experiment $^{45}Ca^{2+}$ uptake determinations were performed in triplicate. All the experiments were performed in the presence of adenosine deaminase (2 U/ml) in order to remove endogenous adenosine.

2.3. Drugs used

Adenosine deaminase (type VI EC 3.5.4.4), 2-chloroadenosine, veratridine, nifedipine and methoxyverapamil were from Sigma. N^6-[2-(3,5-Dimethoxyphenyl)-2-(2-methylphenyl)-ethyl]adenosine (DPMA), 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), 3,7-dimethyl-1-propargylxanthine (DMPX), ω-conotoxin GVIA and 2-*p*-(2-carboxyethyl)phenethylamino-5′-*N*-ethylcarboxamidoadenosine (CGS 21680) were from RBI. $^{45}CaCl_2$ (specific activity, 0.32 μCi/μmol of Ca^{2+}) was from Amersham. ω-Agatoxin-IVA was from the Peptide Institute. DPCPX was made up in a 5 mM stock solution in 99% dimethylsulfoxide (DMSO)/1% NaOH 1 M (v/v).

2.4. Statistics

The data are expressed as means ± S.E.M. from n (number of experiments). The significance of the differences was evaluated by Student's t test. $P < 0.05$ were considered to represent a significant difference.

3. Results

3.1. Excitatory and inhibitory effects of 2-chloroadenosine on $^{45}Ca^{2+}$ uptake

In these experiments $^{45}Ca^{2+}$ uptake by synaptosomes was induced by a concentration of 20 μM of veratridine. The mean value obtained for the net amount of Ca^{2+} taken up by synaptosomes in control conditions was 2.4 ± 0.5 nmol Ca^{2+}/mg protein ($n = 15$).

In order to know whether stimulation of adenosine A_2 receptors could increase Ca^{2+} uptake by rat brain synaptosomes, experiments were done where the effect of the adenosine agonist, 2-chloroadenosine, on $^{45}Ca^{2+}$ uptake induced by 20 μM veratridine was tested in the absence and in the presence of the selective adenosine A_1 receptor antagonist, 1,3-dipropyl-8-cyclopentylxanthine (DPCPX). Under these conditions, in the absence of DPCPX it was

observed that 2-chloroadenosine, tested at a concentration of 1 μM, decreased $^{45}Ca^{2+}$ uptake by 14.0 ± 5.0% ($n = 5$). When 2-chloroadenosine (1 μM) was tested in the presence of DPCPX (100 nM) it was observed that this adenosine agonist increased rather than decreased the uptake of Ca^{2+} into synaptosomes by 15.5 ± 3.0% ($n = 5$) (see Table 1).

3.2. Effect of ω-agatoxin-IVA

To know whether this excitatory effect of 2-chloroadenosine on $^{45}Ca^{2+}$ uptake observed in the presence of DPCPX could be due to activation of a P-type Ca^{2+} channel, experiments were designed where 2-chloroadenosine (1 μM) was applied together with DPCPX (100 nM) and ω-agatoxin-IVA, a specific P-type Ca^{2+} channel antagonist, in a concentration of 200 nM. Under these conditions, 2-chloroadenosine did not exert an excitatory effect on Ca^{2+} uptake but there was a slight reduction of 2.0 ± 5.7% ($n = 5$), which was not significantly different ($P > 0.05$) from the control (see Table 1).

3.3. L-type and N-type Ca^{2+} channel blockers

To validate the identification of the P-type Ca^{2+} channel involved in the excitatory effect of 2-chloroadenosine on $^{45}Ca^{2+}$ uptake, without participation of L- or N-type channels, experiments were done using the L-type Ca^{2+} channel blockers nifedipine (100 nM and 1 μM) and methoxyverapamil (1 μM and 10 μM) and as a N-type Ca^{2+} channel blocker, ω-conotoxin GVIA (500 nM). Neither L-type nor N-type Ca^{2+} channel blockers inhibited the excitatory effect of 2-chloroadenosine observed in the presence of DPCPX.

3.4. Effect of adenosine A_2 receptor agonists on $^{45}Ca^{2+}$ uptake

To test further whether adenosine A_2 receptor activation increases $^{45}Ca^{2+}$ uptake, experiments were done where synaptosomes were incubated in the presence of the adenosine A_{2A} selective receptor agonist, 2-*p*-(2-carboxyethyl)-phenethylamino-5′-*N*-ethylcarboxamidoadenosine (CGS 21680), as well as in the presence of the selective adenosine A_2 receptor agonist, N^6-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)-ethyl]adenosine (DPMA). The adenosine agonist CGS 21680 tested at concentrations ranging from 1 nM to 100 μM did not significantly modify the uptake of Ca^{2+} induced by veratridine into rat brain synaptosomes. In contrast, the agonist DPMA tested in concentrations ranging from 10 nM to 1 μM enhanced the Ca^{2+} uptake induced by veratridine. The maximal excitatory effect (21.5 ± 8.8% $n = 4$) was obtained with DPMA 100 nM; DPMA 1 μM did not cause any further increase in Ca^{2+} uptake into synaptosomes and it was even less effective, causing only a 17.2 ± 7.9% increase ($n = 4$). When applied in a higher concentration (10 μM), DPMA reduced the Ca^{2+} uptake induced by veratridine by 23.9 ± 9.0% ($n = 4$) (Table 1). This inhibitory effect is probably related to adenosine A_1 receptor activation, which is evident only when one uses DPMA in high concentrations (Bridges et al., 1988).

3.5. Effect of DMPX

The ability of 3,7-dimethyl-1-propargylxanthine (DMPX), a selective adenosine A_2 receptor antagonist, to antagonize the excitatory effect of DPMA on $^{45}Ca^{2+}$ uptake induced by veratridine was investigated in five experiments, in which synaptosomes were incubated with

Table 1
Effects of 2-chloroadenosine on $^{45}Ca^{2+}$ uptake in the presence of 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) and in the presence of DPCPX + ω-agatoxin IVA; and effects of N^6-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)-ethyl]adenosine (DPMA) in the absence and presence of 3,7-dimethyl-1-propargylxanthine (DMPX)

Condition	*n*	Change $^{45}Ca^{2+}$ uptake (%)
Control	5	0 ± 0
2-Chloroadenosine (1 μM)	5	−14.0 ± 5.0 *
2-Chloroadenosine (1 μM) + DPCPX (100 nM)	5	15.5 ± 3.0 *
2-Chloroadenosine (1 μM) + DPCPX (100 nM) + ω-agatoxin IVA (200 nM)	5	−2.0 ± 5.7
DPMA		
10 nM	4	6.0 ± 1.9 *
100 nM	4	21.5 ± 8.8 *
1 μM	4	17.2 ± 7.9 *
10 μM	4	−23.9 ± 9.0 *
DPMA (100 nM) + DMPX (10 μM)	5	−4.9 ± 3.8

Values are means ± S.E.M. and represent the percentage change of $^{45}Ca^{2+}$ uptake in relation to the control value (0%) obtained with 20 μM veratridine.
* Significantly different from the control ($P < 0.05$).

DPMA (100 nM) in the presence of DMPX (10 μM). As shown in Table 1, when the adenosine A_2 receptor antagonist DMPX was present in the incubation medium, the excitatory effect of DPMA was antagonized and Ca^{2+} uptake was not increased but reduced by $4.9 \pm 3.8\%$, a value which was not significantly different from the control ($P > 0.05$).

4. Discussion

The present work showed that the non-selective adenosine A_1/A_2 receptor agonist, 2-chloroadenosine, decreased Ca^{2+} uptake by synaptosomes but increased the uptake when the synaptosomes were incubated in the presence of the selective adenosine A_1 receptor antagonist, DPCPX. This indicates that this excitatory effect of 2-chloroadenosine on Ca^{2+} uptake can be observed only when the A_1 inhibitory receptors are blocked. This is consistent with the currently accepted view that 2-chloroadenosine has a greater affinity for A_1 than for A_2 receptors (see Fredholm et al., 1994). The observed inhibitory effect of 2-chloroadenosine on Ca^{2+} uptake by synaptosomes induced by veratridine is in agreement with previous studies showing that adenosine and its analogues decrease the uptake of Ca^{2+} by rat brain synaptosomes stimulated by potassium (e.g. Ribeiro et al., 1979; Wu et al., 1982) or by electrical stimulation (Gonçalves et al., 1991). In this work 2-chloroadenosine was used since it behaves like adenosine, but it is not degraded by adenosine deaminase and is a poor substrate for adenosine uptake (Jarvis et al., 1985).

The increase in Ca^{2+} uptake by presynaptic nerve terminals produced by adenosine A_2 receptor activation may play a significant role in the facilitation of neurotransmitter release in the central nervous system. The excitatory adenosine A_2 receptor subtype has a widespread distribution in the brain and has been divided into A_{2A} and A_{2B} receptors according to its high and low affinity for adenosine, respectively (Daly et al., 1983). The adenosine agonist, CGS 21680, a NECA (5′-*N*-ethylcarboxamidoadenosine) derivative, is a highly potent and selective agonist for the A_{2A} receptor (see Fredholm et al., 1994) and inactive as an agonist for the A_{2B} receptor (Yakel et al., 1993). The lack of excitatory effect of CGS 21680 on Ca^{2+} uptake by synaptosomes detected in the present work contrasts with data showing that this agonist increases evoked neurotransmitter release in different areas of the nervous system (Kirk and Richardson, 1994; Cunha et al., 1994, 1995). One possible explanation for the absence of effect of the selective adenosine A_{2A} agonist CGS 21680 on Ca^{2+} uptake detected in the present work is that the excitatory effect of 2-chloroadenosine on Ca^{2+} uptake could be due to stimulation of adenosine A_{2B} receptors, as suggested by Mogul et al. (1993), instead of A_{2A} receptors. Although in our experiments we verified that the selective adenosine A_2 receptor agonist DPMA stimulated Ca^{2+} entry into synaptosomes, this does not indicate whether the receptor involved is an A_{2A} or A_{2B} receptor because neither DPMA nor the adenosine A_2 receptor antagonist, DMPX, discriminate between these two types of adenosine receptor, and no adenosine A_{2B}-selective antagonists are available. It is unlikly that the absence of effect of CGS 21680 in nanomolar concentrations could be due to desensitization of the excitatory A_{2A} receptors as was suggested to occur in different areas of the central nervous system (Kirk and Richardson, 1994) as well as in canine thyroid-derived A_{2A} receptor in transfected Chinese hamster ovary cells, where a 30-min agonist exposure to higher concentrations of CGS 21680 is sufficient to cause impairment of the receptor-G protein interaction (Palmer et al., 1994).

In the present work we verified that ω-agatoxin-IVA, a specific P-type Ca^{2+} channel antagonist (Mintz et al., 1992; Turner et al., 1992), inhibited the excitatory effect of 2-chloroadenosine on Ca^{2+} uptake when the adenosine A_1 receptors were blocked by DPCPX. This result suggests that stimulation of excitatory adenosine A_2 receptors can activate the P-type Ca^{2+} channel and induce Ca^{2+} entry into synaptosomes, a finding which apparently supports previous data obtained with electrophysiological techniques by Mogul et al. (1993). These authors showed that in isolated pyramidal neurons from the CA3 area of the guinea-pig hippocampus, the activation of adenosine A_1 receptors inhibits the N-type Ca^{2+} current while activation of A_2 receptors results in a significant potentiation of a P-type Ca^{2+} current. Under the present experimental conditions, neither L-type nor N-type Ca^{2+} channels were involved in the excitatory effect of 2-chloroadenosine. This is consistent with the observation that in mammalian systems presynaptic Ca^{2+} channels are largely resistent to both ω-conotoxin GVIA and dihydropyridines (Hofmann and Habermann, 1990; Mintz et al., 1992). Additionally, L-type channels may not be very common in nerve terminals (Miller, 1987).

In conclusion, we have shown that adenosine A_2 excitatory receptor activation increases Ca^{2+} uptake by synaptosomes depolarized by veratridine, a finding that could explain why neurotransmitter release is also increased when adenosine A_2 receptors are stimulated. However, correlative experiments with the same temporal resolution have to be performed to provide more direct proof that this is the mechanism involved when adenosine facilitates Ca^{2+}-dependent transmitter release.

Acknowledgements

We gratefully acknowledge the skillful technical assistance of Mrs Dolores Constantino. The work was supported by Gulbenkian Foundation and JNICT (PBIC/C/CEN 1076/92). The authors participate in ADEURO (BIOMED I/EU network).

References

Bridges, A.J., R.F. Bruns, D.F. Ortwine, S.R. Priebe, D.L. Szotek and B.K. Trivedi, 1988, N^6-[2-(3,5-Dimethoxyphenyl)-2-(2-methylphenyl)-ethyl]adenosine and its uronamide derivatives. Novel adenosine agonists with both high affinity and high selectivity for the adenosine A_2 receptor, J. Med. Chem. 31, 1282.

Brown, S.J., S. James, M. Reddington and P.J. Richardson, 1990, Both A_1 and A_{2a} purine receptors regulate striatal acetylcholine release, J. Neurochem. 55, 31.

Cunha, R.A., E. Milusheva, E.S. Vizi, J.A. Ribeiro and A.M. Sebastião, 1994, Excitatory and inhibitory effects of A_1 and A_{2a} adenosine receptor activation on the electrically evoked [^{3}H]acetylcholine release from different areas of the rat hippocampus, J. Neurochem. 63, 207.

Cunha, R.A., B. Johansson, B.B. Fredholm, J.A. Ribeiro and A.M. Sebastião, 1995, Adenosine A_{2A} receptors stimulate acetylcholine release from nerve terminals of the rat hippocampus, Neurosci. Lett. 196, 41.

Daly, J.W., P. Butts-Lamb and W. Padgett, 1983, Subclasses of adenosine receptors in the central nervous system. Interaction with caffeine and related methylxanthines, Cell. Mol. Neurobiol. 1, 69.

Fredholm, B.B., M.P. Abbracchio, G. Burnstock, J.W. Daly, T.K. Harden, K.A. Jacobson, P. Leff and M. Williams, 1994, Nomenclature and classification of purinoceptors, Pharmacol. Rev. 46, 143.

Gonçalves, M.L. and J.A. Ribeiro, 1995, The excitatory effect of 2-chloroadenosine on Ca^{2+} uptake is antagonized by ω-agatoxin-IVA, Pharmacol. Res. 31 (Suppl.), 197.

Gonçalves, M.L., F. Pinto and J.A. Ribeiro, 1991, Effect of adenosine on $^{45}Ca^{2+}$ uptake by electrically stimulated rat brain synaptosomes, J. Neurochem. 56, 1769.

Hajõs, F., 1975, An improved method for the preparation of synaptosomal fractions of high purity, Brain Res. 93, 485.

Hofmann, F. and E. Habermann, 1990, Role of ω-conotoxin-sensitive calcium channels in inositolphosphate production and noradrenaline release due to potassium depolarization or stimulation with carbacol, Naunyn-Schmiedeberg's Arch. Pharmacol. 341, 200.

Jarvis, S.M., B.W. Martin and A.S. Ng, 1985,2-Chloro-adenosine, a permeant for the nucleoside transporter, Biochem. Pharmacol. 34, 3237.

Kirk, I.P. and P.J. Richardson, 1994, Adenosine A_{2a} receptor-mediated modulation of striatal [^{3}H]GABA and [^{3}H]acetylcholine release, J. Neurochem. 62, 960.

Miller, R.J., 1987, Multiple calcium channels and neuronal function, Science 235, 46.

Mintz, I.M., V.J. Venema, K.M. Swiderek, T.D. Lee, B.P. Bean and M.E. Adams, 1992, P-type Ca^{2+} channels blocked by the spider toxin ω-Aga-IVA, Nature 355, 827.

Mogul, D.J., M.E. Adams and A.P. Fox, 1993, Differential activation of adenosine receptors decreases N-type but potenciates P-type Ca^{2+} current in hippocampal CA3 neurons, Neuron 10, 327.

Nicholls, D.G., 1989, Release of glutamate, aspartate, and γ-aminobutyric acid from isolated nerve terminals, J. Neurochem. 52, 331.

Okada, Y., T. Sakurai and M. Mori, 1992, Excitatory effect of adenosine on neurotransmission is due to increase in neurotransmitter release in hippocampal slices, Neurosci. Lett. 142, 233.

O'Regan, M.H., R.E. Simpson, L.M. Perkins and J.W. Phillis, 1992, The selective A_2 agonist CGS 21680 enhances excitatory transmitter amino acid release from the ischemic rat cerebral cortex, Neurosci. Lett. 138, 169.

Palmer, T.M., T.W. Gettys, K.A. Jacobson and G.L. Stiles, 1994, Desensitization of the canine A_{2a} adenosine receptor: delineation of multiple processes, Mol. Pharmacol. 45, 1082.

Peterson, G.L., 1977, A simplification of the protein assay method of Lowry et al., which is more generally applicable, Anal. Biochem. 83, 346.

Ribeiro, J.A., A.M. Sá-Almeida and J.M. Namorado, 1979, Adenosine and adenosine triphosphate decrease ^{45}Ca uptake by synaptosomes stimulated by potassium, Biochem. Pharmacol. 28, 1297.

Sebastião, A.M. and J.A. Ribeiro, 1992, Evidence for the presence of excitatory A_2 adenosine receptors in the rat hippocampus, Neurosci. Lett. 138, 41.

Spignoli, G., F. Pedata and G. Pepeu, 1984, A_1 and A_2 adenosine receptors modulate acetylcholine release from brain slices, Eur. J. Pharmacol. 97, 341.

Turner, T.J., M.E. Adams and K. Dunlap, 1992, Ca^{2+} channels coupled to glutamate release identified by ω-Aga-IVA, Science 258, 310.

Wu, P.H., J.W. Phillis and D.L. Thierry, 1982, Adenosine receptor agonists inhibit K^+-evoked Ca^{2+} uptake by rat brain cortical synaptosomes, J. Neurochem. 39, 700.

Yakel, J.L., R.A. Warren, S.M. Reppert and R.A. North, 1993, Functional expression of adenosine A_{2b} receptor in *Xenopus* oocytes, Mol. Pharmacol. 43, 277.