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Short communication

Comparison of the potency of adenosine as an agonist at human adenosine receptors expressed in Chinese hamster ovary cells

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Abstract

The potency of adenosine and inosine as agonists at human adenosine receptors was examined in a functional assay using changes in cyclic AMP (cAMP) formation in intact Chinese hamster ovary (CHO) cells stably transfected with the human A_1, A_{2A}, A_{2B}, and A_3 receptors. Adenosine increased cAMP formation in cells expressing the A_{2A} (EC_{50}: 0.7 μM) and A_{2B} (EC_{50}: 24 μM) receptors and inhibited forskolin (0.3–3 μM)-stimulated cAMP formation in cells expressing the A_1 (EC_{50}: 0.31 μM) and A_3 receptors (EC_{50}: 0.29 μM). The potency of adenosine at the A_{2A} and A_{2B} receptors was not altered by the presence of the uptake inhibitor nitrobenzylthioinosine (NBMPR), whereas it was increased about 6-fold by NBMPR at the A_1 and A_3 receptors. In the presence of NBMPR, inosine was a potent agonist (EC_{50}: 7 and 0.08 μM at the A_1 and A_3 receptors, respectively), but with low efficacy especially at the A_3 receptors. No effect of inosine was seen at the A_2 receptors. Caffeine, theophylline, and paraxanthine shifted the dose–response curve for adenosine at the A_1, A_{2A}, and A_{2B} receptors. These results indicate that adenosine is the endogenous agonist at all human adenosine receptors and that physiological levels of this nucleoside can activate A_1, A_{2A}, and A_3 receptors on cells where they are abundantly expressed, whereas pathophysiological conditions are required to stimulate A_{2B} receptors to produce cyclic AMP.

Keywords: Cyclic AMP; Forskolin; Adenosine uptake; Inosine; Caffeine; Xanthines

1. Introduction

Most of the effects of the most widely used of all psychoactive drugs, caffeine, are believed to be secondary to the blockade of adenosine receptors [1]. Since caffeine is an antagonist, this implies that at least some adenosine receptors are occupied *in vivo* by the endogenous ligand. Four adenosine receptors, A_1, A_{2A}, A_{2B}, and A_3, have been cloned and pharmacologically characterized from several mammalian species [2]. It is generally assumed that adenosine is the natural ligand at all these receptors, but it was recently suggested that inosine may be a more important agonist than adenosine at the A_3 receptors [3]. It is also generally believed that adenosine is a much more potent agonist at A_1 and A_{2A} receptors than at A_{2B} and A_3 receptors [see e.g. Ref. 4]. However, this contention is largely based on studies on cells with widely different levels of receptor expression, and no direct comparison has been made. In addition, most studies on the potency of agonists at adenosine receptors are performed using binding assays. It is notoriously difficult to study the potency of adenosine itself in such a preparation [5–7], because adenosine is present in most membrane preparations and binds with very high affinity to the A_1 receptor in its high-affinity conformation in the absence of guanine nucleotides [8–10].

In the present series of experiments, we therefore examined the potency of adenosine and inosine to alter cAMP levels (increases in the case of A_{2A} and A_{2B} receptors; decreases in the case of A_1 or A_3 receptors) using intact CHO cells that express the human forms of the receptor. In addition, the potency of caffeine, theophylline, paraxanthine, and theobromine as antagonists was determined.

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Abbreviations: cAMP, cyclic adenosine 3′,5′-monophosphate; CHO, Chinese hamster ovary; NBMPR, nitrobenzylthioinosine; and NECA, 5′-*N*-ethyl carboxamido adenosine.

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2. Materials and methods

2.1. Materials

Forskolin, NBMPR, adenosine deaminase, caffeine, theophylline, and paraxanthine were from Sigma. Adenosine was from Aldrich-Europe and inosine from P-L Biochemicals. NECA and N^6-cyclopentyl adenosine (CPA) were from RBI. Rolipram (ZK 62711) was a gift from Schering A. Cell culture medium and additions were from GIBCO.

2.2. Cell culture

CHO cells transfected with the human forms of the adenosine A_1, A_{2B} and A_3 [11] and A_{2A} [12] receptors were cultured as described in these papers. Briefly, the cells were grown adherent in DMEM (Dulbecco's modified Eagle's medium)/Ham's F-12 medium containing 10% fetal bovine serum and 2 mM glutamine, 100 U/mL of penicillin, 100 μg/mL of streptomycin, and 200 μg/mL of geneticin.

2.3. cAMP assay

Cells were split 24 hr prior to assay into 24-well plates (150,000 cells per well). Prior to assay, the cells were washed twice with HEPES-buffered (20 mM) DMEM and cultured for 30 min in 0.4 mL of the same medium at 37°. Drugs to be tested were added in 0.1 mL of the same medium, which also contained the phosphodiesterase inhibitor rolipram at a final concentration of 30 μM. The potency of xanthines as antagonists was determined by adding 10–300 μM xanthine together with increasing concentrations of adenosine. The shift in the dose–response curve for adenosine was used to determine the potency. After 30-min incubation, the reaction was stopped by addition of perchloric acid (final concentration 0.4 M). The supernatant after centrifugation was neutralized with potassium hydroxide in Tris, and the cAMP concentration in the supernatant was determined using a competitive binding assay [13] modified for a 96-well format.

2.4. HPLC

To examine if inosine solutions were contaminated with adenosine, HPLC analysis as described [14] was performed.

2.5. Data analysis

Dose–response curves were calculated using procedures in GraphPad Prism. All results are presented as means and 95% confidence intervals to facilitate comparisons between groups or treatments.

3. Results

To examine the potency of adenosine as a receptor agonist at human adenosine receptors, we used changes in cAMP formation as a functional assay. In the case of A_{2A} and A_{2B} receptors this assay is straightforward, since agonists increase cAMP formation. However, both A_1 and A_3 receptors cause a decrease in the rate of cAMP formation. Therefore, a means to increase the rate of cAMP formation was needed. We chose forskolin as the agent to achieve this increase because in lower doses forskolin is known to facilitate the interaction between $G_{s\alpha}$ and the cyclase enzyme [15]. Forskolin (0.1–3 μM) caused a monophasic increase in cAMP accumulation in CHO cells transfected with the human A_1 receptor. Furthermore, the adenosine A_1 agonist cyclopentyladenosine (CPA; 1 μM) caused an inhibition that ranged between 65 and 82% over the entire range of forskolin concentrations. This means that adenosine receptor-mediated inhibition is equivalent over this range of concentration of forskolin. We found no difference in the EC_{50} value for adenosine to inhibit cAMP accumulation stimulated by 1 and 3 μM forskolin in A_1 receptor-transfected CHO cells: 0.26 (0.14–0.36) and 0.31 (0.18–0.53) μM, respectively (means and 95% confidence intervals). Similarly, in A_3 receptor-transfected CHO cells there was no difference in adenosine potency between cells stimulated with 0.3 or 1 μM forskolin: 0.34 (0.20–0.58) and 0.56 (0.17–2.1) μM, respectively. In experiments to study the effect of adenosine on A_1 or A_3 receptors, forskolin concentrations ranging from 0.3 to 3 μM were therefore used.

As shown in Fig. 1 (upper left panel), adenosine inhibited forskolin-stimulated cAMP accumulation by approximately 65% in cells that express the human A_1 receptor. There was a highly significant leftward shift in the presence of the adenosine transport inhibitor, NBMPR. It can also be seen that inosine caused a small inhibition of cAMP accumulation, which was also enhanced by NBMPR. In the absence of the uptake inhibitor, the potency of inosine was difficult to determine, but in its presence inosine was approximately 100-fold less potent than adenosine. HPLC analysis showed that after injection of 100 nmol inosine, 9 pmol adenosine was detected. Thus, inosine solutions are contaminated by less than one part in ten thousand by adenosine.

A similar potency of adenosine was observed when CHO cells transfected with A_3 receptors were studied (Fig. 1, upper right panel). Furthermore, the leftward shift in the dose–response curve in the presence of the uptake blocker NBMPR was similar. However, inosine was much more potent (in the presence of NBMPR) at A_3 than at A_1 receptors, even though the maximal response to inosine was much lower (20 vs 73 percent of the maximal inhibition afforded by adenosine). Hypoxanthine and xanthine were, however, ineffective, as was guanosine (not shown). 2′-Deoxyadenosine was weakly active (about 1000-fold less active than adenosine), but this could be explained by con-

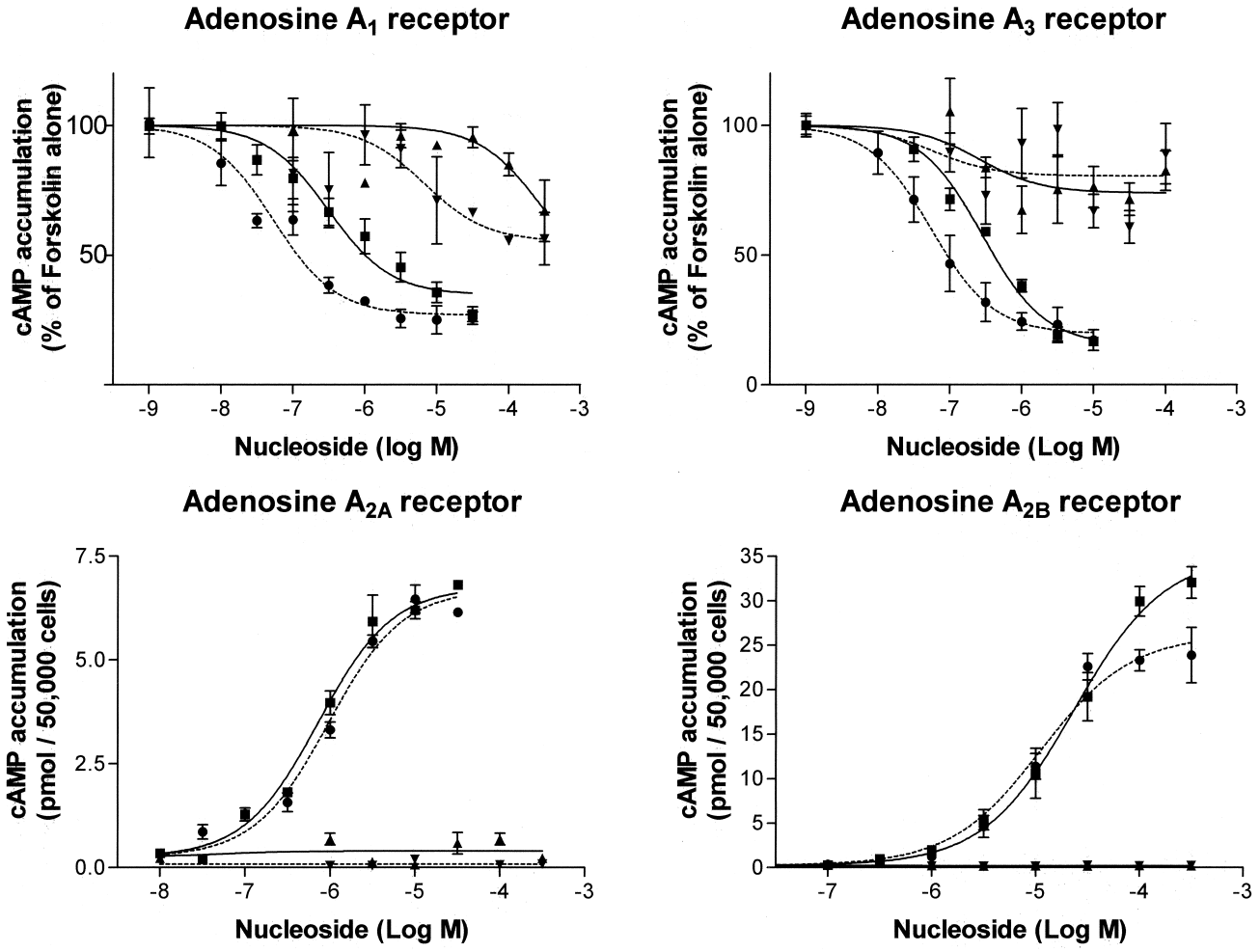

Fig. 1. Effect of adenosine (■) or inosine (▲) alone or in the presence of NBMPR (1 μM) (●: adenosine + NBMPR; ▼: inosine + NBMPR) in CHO cells expressing A_1 (upper left), A_3 (upper right), A_{2A} (lower left), or A_{2B} (lower right) receptors. Effects at A_1 and A_3 receptors were studied as inhibition of forskolin-stimulated cAMP accumulation, effects at A_{2A} and A_{2B} as increases in cAMP. Intact lines, experiments without NBMPR; broken lines, experiments with NBMPR.

tamination with adenosine (not shown). The fact that inosine had a weak inhibitory effect could indicate that it acted as a partial agonist. In order to test this, we examined if 10 μM inosine could shift the dose–response curve to adenosine. However, in two separate experiments, this was not the case. In the absence of inosine, adenosine had an EC_{50} value of 0.14 (0.099–0.21) μM. In the presence of 10 μM inosine, the corresponding values were 0.11 (0.068–0.16) μM. Similarly, in two separate experiments, inosine (0.03–30 μM) did not reduce the effect of 0.1 μM adenosine (results not shown).

As seen in Fig. 1, lower left panel, the potency of adenosine as an agonist at the A_{2A} receptor was not significantly influenced by NBMPR. It is also evident that inosine was ineffective as an agonist, both in the presence and absence of NBMPR. At the A_{2B} receptor, a 2-fold insignificant shift in potency was seen in the presence of NBMPR (Fig. 1, lower right). Inosine was ineffective.

For all receptors, adenosine was as efficacious as NECA (not shown), suggesting that both compounds act as full agonists. In agreement with previous results, adenosine and NECA did not have any significant effect on cAMP accumulation in CHO cells not transfected with adenosine receptors [12]. Adenosine deaminase (1 U/mL) markedly reduced the potency of exogenous adenosine, but did not significantly alter the basal level of cAMP.

Table 1 summarizes the data on the potency of adenosine and inosine in CHO cells transfected with all four forms of human adenosine receptor. This table also shows the effect of caffeine and its two active metabolites theophylline and paraxanthine. The potency of the xanthines as antagonists was determined by adding them at 10- or 100-μM concentration simultaneously with adenosine or NECA and recording the EC_{50} value for adenosine.

4. Discussion

Adenosine was, as expected, a full agonist at all four human adenosine receptors. The highest potency was ob-

Table 1
Comparison of the potency of adenosine, inosine, caffeine, theophylline, and paraxanthine at human adenosine receptors transfected into CHO cells

	Receptor			
	A_1	A_{2A}	A_{2B}	A_3
AGONISTS		EC_{50} (μM)		
Adenosine	0.31 (0.18–0.53) [13]	0.73 (0.56–0.95) [8]	23.5 (16.2–64.1) [8]	0.29 (0.18–0.53) [6]
Adenosine + NBMPR	0.054 (0.034–0.085) [5]	0.96 (0.67–1.37) [4]	11.3 (7.4–17.4) [4]	0.056 (0.032–0.098) [6]
Inosine	290 [3]	Inactive [4]	Inactive [3]	0.25 (0.025–2.5) [4]
Inosine + NBMPR	6.7 (1–45) [3]	Inactive [3]	Inactive [3]	0.081 (0.001–6.5) [5]
ANTAGONISTS		K_b (μM)		
Caffeine	33.8 (28–39)	12.3 (9.8–14.8)	15.5 (9.3–21.8)	>100
Theophylline	8.9 (6.5–14)	7.9 (6.3–10.0)	4.8 (3.9–5.8)	>100
Paraxanthine	15.8 (4.5–34)	5.3 (3.8–6.9)	5.5 (5.1–5.8)	>100

Results for agonists (upper part) are given as EC_{50} values in μM (means and 95% confidence intervals). Details regarding the determination of agonist potency are given in the legends to Fig. 1. To study antagonists, dose–response curves for adenosine or NECA were generated in the presence or absence of xanthines. Based on the shift in the dose–response curves and assuming a slope of the Schild plot not different from 1, K_b values were calculated (lower part). Data on the potency of caffeine, theophylline, and paraxanthine on A_{2A} receptors are from [12]. Data on the potency of the xanthines in cells expressing A_1 receptors are from 4 experiments at 10 μM and 7 experiments at 100 μM. Two experiments in duplicate were run for A_{2B} and A_3 receptors at each concentration of antagonist. Results are given as μM (means and 95% confidence interval). The number of experiments is given within brackets.

served at the A_1 and A_3 receptors, followed by the A_{2A} receptor. Adenosine was much less potent at the A_{2B} receptor. This study appears to be the first where the potency of adenosine as an agonist at all the human receptors has been directly compared. Using a luciferase construct under the control of multiple cyclic AMP response elements, an earlier study reported the effects of adenosine on CHO cells transfected with three of the adenosine receptors (A_1, A_{2A}, and A_{2B}) [16]. However, these authors did not report EC_{50} values for the agonist. As pointed out in the Introduction, this information can only be obtained in a functional assay such as the one used here, and not in binding assays. It is well known that adenosine deaminase must be included in binding assays for adenosine receptors in order to remove the endogenous ligand, which is formed by the membrane preparation [6]. However, in tests of the potency of adenosine the enzyme cannot be added, since it would only eliminate the agonist.

Basal adenosine levels in interstitial fluid are between 30 and 300 nM, and increase some 10-fold during hypoxia and 100- to 1000-fold in ischemia [17–20]. The present data therefore indicate that adenosine present under physiological conditions can activate A_1 and A_3 receptors to produce 10 to 50% of the maximal response. A_{2A} receptors can be expected to be marginally less activated by physiological levels of adenosine, whereas A_{2B} receptors are stimulated to produce cAMP only when adenosine levels are elevated, e.g. by hypoxia or ischemia. However, this is true only if the transfected CHO cells are representative of natural cells in terms of receptor number.

It is known, at least in the case of A_1 and A_{2A} receptors, that the position of the concentration–response curve is strongly affected by the number of receptors [21,22]. As discussed previously [23], transfected A_{2A} cells probably do not have a larger number of receptors than do striatopallidal cells or neutrophil leucocytes. By contrast, there are probably fewer A_1 receptors on most natural locations than in the transfected CHO cells. This is also the case for A_{2B} and A_3 receptors, both of which are sparser in most tissues. Thus, physiological levels of adenosine are likely to activate A_{2A} receptors at several locations and A_1 receptors at some locations, but it is unclear if A_{2B} and A_3 receptors can be activated except when adenosine levels are increased.

It is well known that adenosine is rapidly eliminated by cells and that uptake followed by phosphorylation intracellularly is the major mechanism occurring at physiological levels of the agonist [24,25]. To reduce the importance of the local elimination of adenosine from its site of action, experiments were also carried out in the presence of NBMPR, a known inhibitor of the ubiquitous *es*-transporter [24,25]. We found a sixfold leftward shift of the concentration–response curve for adenosine at A_1 and A_3 receptors when this inhibitor of adenosine uptake was added. No significant effect was, however, found in the case of A_{2A} and A_{2B} receptors. One possible explanation is that NBMPR had increased the level of adenosine, and another that the uptake of adenosine has a greater impact the higher the affinity of adenosine. However, there was little difference in potency of adenosine at A_1 and A_3 receptors compared to A_{2A} receptors in the absence of the uptake inhibitor. Yet, the potency of adenosine at A_{2A} receptors was not affected by NBMPR. Since all these observations were made in a common cellular background, cell-specific factors are also an unlikely explanation. Conceivably, the NBMPR-sensitive transporter is localized closer to A_1 or A_3 receptors than to A_{2A} or A_{2B} receptors. This and other possible explanations, including major kinetic differences between adenosine activation of G_i (A_1 and A_3 receptors)- and G_s (A_{2A} and

A_{2B})-coupled receptors, require closer scrutiny. In the absence of clear information about the mechanism of the selective enhancement, we cannot say with certainty if the values for the potency of adenosine obtained in the presence and the absence of an uptake inhibitor are the most relevant for an *in vivo* situation.

Interstitial levels of adenosine and inosine are roughly similar under both basal conditions and after hypoxia or ischemia [17–20]. Inosine was ineffective at the two A_2 receptors, but a weak agonist at the A_1 and A_3 receptors. At the latter receptor, it had a high potency but low efficacy. It follows that inosine cannot be considered a natural agonist at A_1, A_{2A}, and A_{2B} receptors. As pointed out previously [3], the situation is somewhat different in the case of the A_3 receptor. It was found in that study that inosine had an apparent affinity for the A_3 receptor close to 25 μM [3]. We have found here that it is even more potent when a functional assay is used. In fact, an EC_{50} of approximately 100 nM was observed when cellular uptake was prevented. This is close to the levels of interstitial inosine reported repeatedly under basal physiological conditions. However, we have found that adenosine was approximately twice as potent and close to four times as efficacious. Hence, even at the A_3 receptor, adenosine is likely to be the predominant natural agonist. Nevertheless, inosine may activate A_1 and A_3 receptors under some circumstances.

The fact that inosine is such a weak agonist is intriguing. Inosine was contaminated only to a minor degree by adenosine; furthermore, if contamination by adenosine had been the cause, then an equal efficacy but a lower affinity would have been expected. Another possibility is that inosine prevents adenosine from being degraded. This cannot be completely ruled out, but since inosine was active in the presence of the uptake inhibitor NBMPR, competition with adenosine for uptake can probably be ruled out. Since inosine appeared to have an effect in the assays where inhibition of adenylyl cyclase was the readout, but not in those assays where adenylyl cyclase stimulation was measured, it is possible that inosine directly inhibits the formation of cAMP. However, we would then expect it to be equally potent in the tests using CHO cells expressing A_1 and A_3 receptors. This was not the case, which renders direct interference with the assay system less likely. We also tested a fourth possibility, namely that inosine is a partial agonist. Partial agonists would be expected to antagonize the effects of a full agonist. We tested this in two ways: by varying the amount of the full agonist adenosine in the presence of a fixed concentration of the presumed partial agonist inosine, and by varying inosine concentrations in the presence of a fixed concentration of adenosine. As neither test revealed any inhibition, we therefore conclude that inosine is not a classical partial agonist. Consequently, we are left without an adequate explanation for the weak effect of inosine.

Caffeine antagonized, as expected, adenosine effects mediated via A_1, A_{2A}, and A_{2B} receptors, whereas responses mediated via A_3 receptors were not antagonized by concentrations of caffeine expected to be encountered in plasma. The same was true for theophylline and paraxanthine. The potency of these antagonists was similar to that reported earlier in studies using binding assays. Thus, the biological effects of caffeine and its metabolites in man are possibly mediated by A_1, A_{2A}, and A_{2B} receptors. Since caffeine acts by inhibiting endogenous adenosine, only the first two receptors are likely candidates, as suggested previously [see e.g. Ref. 4]. In humans, caffeine is rapidly metabolized to mainly paraxanthine [see Refs. 1 and 26], a reaction catalyzed by the 1A2 subform of cytochrome p450 (Cyp1A2) and which can be used to determine the activity of this enzyme *in vivo* [27,28]. It is therefore important to note that at the human A_1, A_{2A}, and A_{2B} receptors, paraxanthine is at least as active as caffeine. Hence, clearance of caffeine from plasma is not necessarily associated with a decreased effect, at least not in those instances when the effects are due to blockade of adenosine receptors.

In summary, we have provided an estimate of the relative potency of adenosine at human adenosine receptors. One surprising finding was that adenosine was as potent at A_3 as at A_1 receptors. At the A_3 receptor, inosine was also a potent albeit poorly efficacious agonist, whereas at the A_1 receptor inosine tended to be less potent but somewhat more efficacious. At the two A_2 receptors, inosine was essentially inactive. This suggests that adenosine is the natural ligand at all four receptors in the family. The data also provide additional evidence that caffeine exerts its low dose effects in man by antagonizing the actions of endogenous adenosine on cells that express abundant A_1 or A_{2A} receptors.

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References

[1] Fredholm BB, Bättig K, Holmén J, Nehlig A, Zvartau E. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol Rev 1999;51:83–153.
[2] Fredholm BB, Abbracchio MP, Burnstock G, Daly JW, Harden TK, Jacobson KA, Leff P, Williams M. Nomenclature and classification of purinoceptors. Pharmacol Rev 1994;46:143–56.
[3] Jin X, Shepherd RK, Duling BR, Linden J. Inosine binds to A_3 adenosine receptors and stimulates mast cell degranulation. J Clin Invest 1997;100:2849–57.
[4] Fredholm BB. Adenosine, adenosine receptors and the actions of caffeine. Pharmacol Toxicol 1995;76:93–101.
[5] Lohse MJ, Lenschow V, Schwabe U. Two affinity states of Ri adenosine receptors in brain membranes. Analysis of guanine nucleotide and temperature effects on radioligand binding. Mol Pharmacol 1984;26:1–9.
[6] Linden J. Adenosine deaminase for removing adenosine: how much is enough? Trends Pharmacol Sci 1989;10:260–2.

[7] Cohen FR, Lazareno S, Birdsall NJ. The affinity of adenosine for the high- and low-affinity states of the human adenosine A_1 receptor. Eur J Pharmacol 1996;309:111–4.
[8] Gerwins P, Nordstedt C, Fredholm BB. Characterization of adenosine A_1 receptors in intact DDT_1 MF-2 smooth muscle cells. Mol Pharmacol 1990;38:660–6.
[9] Parkinson FE, Fredholm BB. Magnesium-dependent enhancement of endogenous agonist binding to adenosine A_1 receptors: a complicating factor in quantitative autoradiography. J Neurochem 1992;58: 941–50.
[10] Prater MR, Taylor H, Munshi R, Linden J. Indirect effect of guanine nucleotides on antagonist binding to A_1 adenosine receptors: occupation of cryptic binding sites by endogenous vesicular adenosine. Mol Pharmacol 1992;42:765–72.
[11] Klotz KN, Hessling J, Hegler J, Owman C, Kull B, Fredholm BB, Lohse MJ. Comparative pharmacology of human adenosine receptor subtypes—characterization of stably transfected receptors in CHO cells. Naunyn Schmiedebergs Arch Pharmacol 1998;357:1–9.
[12] Kull B, Arslan G, Nilsson C, Owman C, Lorenzen A, Schwabe U, Fredholm BB. Differences in the order of potency for agonists, but not antagonists, at human and rat adenosine A_{2A} receptors. Biochem Pharmacol 1999;57:65–75.
[13] Nordstedt C, Fredholm BB. A modification of a protein-binding method for rapid quantification of cAMP in cell-culture supernatants and body fluid. Anal Biochem 1990;189:231–4.
[14] Fredholm BB, Sollevi A. The release of adenosine and inosine from canine subcutaneous adipose tissue by nerve stimulation and noradrenaline. J Physiol 1981;313:351–67.
[15] Seamon KB, Daly JW. Forskolin: its biological and chemical properties. In: Greengard P, Robison GA, editors. Advanced Cyclic Nucleotide and Protein Phosphorylation Research, Vol. 20. New York: Raven Press, 1986. p. 1–147.
[16] Borgland SL, Castanon M, Spevak W, Parkinson FE. Effects of propentofylline on adenosine receptor activity in Chinese hamster ovary cell lines transfected with human A_1, A_{2A}, or A_{2B} receptors and a luciferase reporter gene. Can J Physiol Pharmacol 1998;76:1132–8.
[17] Zetterström T, Vernet L, Ungerstedt U, Tossman U, Jonzon B, Fredholm BB. Purine levels in the intact rat brain. Studies with an implanted perfused hollow fibre. Neurosci Lett 1982;29:111–5.
[18] Hagberg H, Andersson P, Lacarewicz J, Jacobson I, Butcher S, Sandberg M. Extracellular adenosine, inosine, hypoxanthine, and xanthine in relation to tissue nucleotides and purines in rat striatum during transient ischemia. J Neurochem 1987;49:227–31.
[19] Dux E, Fastbom J, Ungerstedt U, Rudolphi K, Fredholm BB. Protective effect of adenosine and a novel xanthine derivative propentofylline on the cell damage after bilateral carotid occlusion in the gerbil hippocampus. Brain Res 1990;516:248–56.
[20] Ballarín M, Fredholm BB, Ambrosio S, Mahy N. Extracellular levels of adenosine and its metabolites in the striatum of awake rats: inhibition of uptake and metabolism. Acta Physiol Scand 1991;142:97–103.
[21] Gerwins P, Fredholm BB. Glucocorticoid receptor activation leads to up-regulation of adenosine A_1 receptors and down-regulation of adenosine A_2 responses in DDT_1 MF-2 smooth muscle cells. Mol Pharmacol 1991;40:149–55.
[22] Arslan G, Kull B, Fredholm BB. Signaling via A_{2A} adenosine receptor in four PC12 cell clones. Naunyn Schmiedebergs Arch Pharmacol 1999;359:28–32.
[23] Kull B, Ferré S, Arslan G, Svenningsson P, Fuxe K, Owman C, Fredholm BB. Reciprocal interactions between adenosine A_{2A} and dopamine D_2 receptors in Chinese hamster ovary cells co-transfected with the two receptors. Biochem Pharmacol 1999;58:1035–45.
[24] Geiger JD, Fyda DM. Adenosine transport in nervous system tissues. In: Stone TW, editor. Adenosine in the nervous system. London: Academic Press, 1991. p. 1–23.
[25] Thorn JA, Jarvis SM. Adenosine transporters. Gen Pharmacol 1996; 27:613–20.
[26] Bonati M, Latini R, Galletti F, Young JF, Tognoni G, Garattini S. Caffeine disposition after oral doses. Clin Pharmacol Ther 1982;32: 98–106.
[27] Fuhr U, Rost KL, Engelhardt R, Sachs M, Liermann D, Belloc C, Beaune P, Janezic S, Grant D, Meyer UA, Staib AH. Evaluation of caffeine as a test drug for CYP1A2, NAT2 and CYP2E1 phenotyping in man by *in vivo* versus *in vitro* correlations. Pharmacogenetics 1996;6:159–76.
[28] Miners JO, Birkett DJ. The use of caffeine as a metabolic probe for human drug metabolizing enzymes. Gen Pharmacol 1996;27:245–9.