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MOLECULAR PHARMACOLOGY, 43:909-914

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Characterization of Adenosine Receptors in Human Erythroleukemia Cells and Platelets: Further Evidence for Heterogeneity of Adenosine A_2 Receptor Subtypes

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Received September 28, 1992; Accepted March 23, 1993

SUMMARY

Adenosine receptors are present in platelets, and their activation results in accumulation of cAMP and inhibition of aggregation. The study of platelet adenosine receptors, however, is limited by the impossibility of maintaining these cells *in vitro*. Human erythroleukemia (HEL) cells express megakaryocytic/platelet markers and have been used as a model to study platelet receptors. Therefore, we sought to determine whether adenosine receptors were present in HEL cells. Adenosine agonists produced an accumulation of cAMP in HEL cells, implying the presence of A_2 receptors. Xanthine and nonxanthine adenosine receptor antagonists blocked this effect in a simple competitive manner (Schild analysis). Therefore, both platelets and HEL cells possess A_2 adenosine receptors. There were, however, significant differences between them. Adenosine agonists were, in general, less potent in HEL cells, compared with platelets. In particular, the adenosine analog CGS 21680, one of the most potent agonists in platelets, was virtually inactive in HEL cells. The orders of potencies for agonists (and their EC_{50} values for cAMP production) were 5′-*N*-ethylcarboxamidoadenosine (0.19 μM) = CGS 21680 (0.18 μM) > (*R*)-(−)-N^6-(2-phenylisopropyl)adenosine (0.5 μM) in platelets and 5′-*N*-ethylcarboxamidoadenosine (2.4 μM) > (*R*)-(−)-N^6-(2-phenylisopropyl)adenosine (160 μM) ≫ CGS 21680 (1600 μM) in HEL cells. In contrast to the decreased potency of agonists in HEL cells, the antagonist 1,3-dipropyl-8-*p*-sulfophenylxanthine was more potent in HEL cells, compared with platelets. Based on the striking differences in the rank orders of potencies of agonists and antagonists, we propose that HEL cells and platelets have different subtypes of adenosine A_2 receptors. We found CGS 21680 particularly helpful in distinguishing between these receptor subtypes.

There is growing evidence that adenosine acts as a modulator in many physiological functions. It has been implicated in the regulation of neural, cardiovascular, and metabolic processes, among others. These actions are mediated by activation of membrane-bound receptors. These receptors were first recognized and classified for their ability to inhibit (A_1) or activate (A_2) adenylate cyclase (1). It is now clear that A_1 adenosine receptors may interact with other intracellular effector systems in addition to adenylate cyclase (2). Characterization of adenosine receptors has been improved by the development of agonists and antagonists that bind preferentially to A_1 or A_2 receptors. Effective radioligands have been developed for A_1 receptors and have been used to investigate the coupling of this receptor to guanine nucleotide-binding proteins (3, 4) and its modulation by chronic exposure to agonists (5) or antagonists (6). Biochemical identification of these receptors has been aided by photoaffinity cross-linking (7, 8). Recently, a previously cloned protein that contains seven transmembrane helices, characteristic of guanine nucleotide-binding protein-coupled receptors, has been identified as an A_1 receptor (9). Nearly identical A_1 receptors have been cloned using polymerase chain reaction techniques (10, 11).

Our understanding of A_2 adenosine receptors, by comparison, lags considerably behind that of A_1 receptors. A_2 receptors are found primarily in the central nervous system, vascular smooth muscle, neutrophils, and platelets and have been implicated in the functions of these cells. The difficulty in studying these receptors lies, in part, in the relatively low affinity of these receptors for currently known agonists and antagonists. This has precluded the development of effective radioligands. The exception to this rule is the A_{2a} receptor found in the rat striatum, which has a greater affinity for adenosine than do

This work was supported by Grants RR00095 (Clinical Research Center) and HL14192 (Specialized Centers of Research in Hypertension) from the National Institutes of Health and by a grant from the International Life Science Institute.

ABBREVIATIONS: HEL, human erythroleukemia; NECA, 5′-*N*-ethylcarboxamidoadenosine; (*R*)-PIA, (*R*)-(−)-N^6-(2-phenylisopropyl)adenosine; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; DPSPX, 1,3-dipropyl-8-*p*-sulfophenylxanthine; CGS 21680, 2-[*p*-(carboxyethyl)phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine hydrochloride; (*S*)-PIA, (*S*)-(+)-N^6-(2-phenylisopropyl)adenosine; N-0861, (±)-N^6-endonorbornan-2-yl-9-methyladenine; 880316, 1,3-dipropyl-8-(*trans*-4-acetamidomethylcyclohexyl)xanthine; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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other A_2 receptors. This receptor subtype has been characterized by radioligand binding techniques (12, 13) and was recently cloned from a dog thyroid library (14).

In this report we describe an A_2 adenosine receptor found in HEL cells. These cells were originally derived from a patient with Hodgkin's disease and erythroleukemia (15). HEL cells express megakaryocyte/platelet membrane markers (16) and, for this reason, have been used as a model to study platelet receptors (17, 18). We examined the characteristics of adenosine receptors in HEL cells and compared the receptors with those found in platelets. We found that adenosine analogs produced accumulation of cAMP in both cell types, as expected for A_2 receptors, but that adenosine receptors in HEL cells are remarkably different from platelet receptors in their relative affinity for agonists and antagonists.

Materials and Methods

Cells. HEL cells (TIB 180) were obtained from the American Type Culture Collection (Rockville, MD) and maintained in suspension culture at a density of 3–9 × 10^5 cells/ml by dilution with RPMI 1640 medium supplemented with 10% (v/v) fetal calf serum, 10% (v/v) newborn calf serum, and 2 mM glutamine. Cells were kept under a humidified atmosphere of air/CO_2 (19:1) at 37°. Before each experiment, cells were harvested, washed by centrifugation (100 × *g* for 10 min), and resuspended to a concentration of 10^7 cells/ml in RPMI 1640 medium without phenol red pH indicator.

HEL cell membranes were prepared after lysis of the cells in 5 ml of hypotonic phosphate-buffered saline containing 0.1 mM phenylmethylsulfonyl fluoride and were frozen at −70°. Thawed lysates were homogenized with a Polytron homogenizer (20,000 rpm) three times for 10 sec, on ice. After centrifugation at 1000 × *g* for 10 min the supernatant was collected, the pellet was resuspended in phosphate-buffered saline containing 0.1 mM phenylmethylsulfonyl fluoride, and the homogenization and centrifugation steps were repeated. The combined supernatants were centrifuged at 100,000 × *g* for 1 hr. The resultant membrane pellet was resuspended in 50 mM Tris·HCl, pH 7.4, containing 5 mM $MgCl_2$ and 1 mM EDTA, and was stored at −70° until use.

For the preparation of platelets, fresh blood was obtained from healthy, medication-free volunteers who abstained from methylxanthine-containing beverages for at least 12 hr before the study. After the first milliliter was discarded, blood was drawn into an acid citrate dextrose solution (9:1, v/v) (25 g/liter dextrose, 22 g/liter sodium citrate, 8 g/liter citric acid, in water, pH 5) at room temperature and in the presence of indomethacin (5 μg/ml). In ancillary studies, we demonstrated that indomethacin had no influence on the action of adenosine on platelets. Platelet-rich plasma was obtained by centrifugation at 200 × *g* for 20 min at room temperature. The supernatant was placed over 50% albumin and centrifuged at 1000 × *g* for 10 min. The platelet layer was resuspended in a buffer containing 150 mM NaCl, 2.7 mM KCl, 0.37 mM NaH_2P0_4, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 5 g/liter D-glucose, 10 mM HEPES-NaOH, 50 units/ml heparin, 0.35% bovine serum albumin, 4 mM phosphocreatinine, and 8 units/ml creatine phosphokinase, pH 6.55. After incubation for 30 min at room temperature, washed platelets were obtained by centrifugation over 50% albumin at 1000 × *g* for 10 min. The platelet layer was resuspended in the same buffer except that the pH was 7.4 and the buffer contained 2 mM $CaCl_2$ and no heparin (buffer B). Platelet concentration was determined with a cell counter (Coulter Electronics, Inc., Hialeah, FL) and adjusted to 100,000 platelets/μl.

Measurement of cAMP and adenylate cyclase activity. HEL cells (2 × 10^6/tube) or platelets (2 × 10^7/tube) were preincubated for 2 min at 37° in a total volume of 200 μl of RPMI 1640 medium or buffer B, respectively, containing the cAMP phosphodiesterase inhibitor papaverine (0.1 mM). In ancillary studies, we determined that the increase in cAMP produced by NECA was not different whether HEL cells were incubated in RPMI 1640 medium or in buffer B. cAMP accumulation in response to adenosine agonist was measured after the addition of the agonist (2 μl) to the cell suspension. Cells were then mixed with a vortex mixer and incubated for 2 min at 37°. The reaction was stopped by addition of 50 μl of 25% trichloracetic acid. To determine the effect of adenosine receptor antagonists on cAMP accumulation, antagonists (2 μl) were added 2 min before activation of adenylate cyclase with NECA. Trichloroacetic acid-treated extracts were washed five times with 10 volumes of water-saturated ether, and cAMP concentrations were determined by competition binding of tritium-labeled cAMP to a protein derived from bovine muscle that has high specificity for cAMP (19) (cAMP assay kit TRK.432; Amersham, Arlington Heights, IL).

In experiments utilizing HEL cell membranes, adenylate cyclase activity was determined by the method of Solomon (20). Incubations were initiated by the addition of membrane suspensions (10 μg of membrane protein). Each reaction mixture (50 μl) contained 0.5 mM [α-^{32}P]ATP (5 × 10^6 cpm), 10 μM GTP, 1 mM dithiothreitol, 50 μM cAMP, 5 mM $MgCl_2$, 0.1 mg/ml bovine serum albumin, 25 mM Tris·HCl, pH 7.6, 100 μM papaverine hydrochloride, 5 mM creatine phosphate, and 50 units/ml creatine kinase. Reactions were carried out for 15 min at 30° and stopped by the addition of 100 μl of 21% sodium lauryl sulfate, 45 mM ATP, and 1.3 mM cAMP and then boiling for 3 min. After addition of [8-^{3}H]cAMP (1 × 10^5 cpm) to each tube, the cAMP was purified by sequential chromatography over Dowex-50 resin and neutral alumina, and the ^{32}P and ^{3}H content was determined by liquid scintillation counting. The [^{32}P]cAMP content was corrected for recovery based on [^{3}H]cAMP content. Protein was determined by the Bio-Rad protein assay, with bovine serum albumin as a standard.

Drugs. NECA, (*R*)-PIA, DPCPX, DPSPX, and CGS 21680 were purchased from Research Biochemicals, Inc. (Natick, MA). (*S*)-PIA was purchased from Boehringer Mannheim Gmbh (Germany). N^6-Cyclohexyladenosine, papaverine, caffeine, and theophylline were obtained from Sigma Chemical Co. (St. Louis, MO). The nonxanthine receptor antagonist N-0861 was a gift from Whitby Research, Inc. (Richmond, VA). Forskolin was purchased from Calbiochem Corp. (La Jolla, CA). 880316 was a gift from Dr. J. Wells (Department of Pharmacology, Vanderbilt University, Nashville, TN).

Data analysis. Calculation of EC_{50} and IC_{50} values from dose-response curves and data analysis by nonlinear regression were done using ALLFIT 2.6 software (Laboratory of Theoretical and Physical Biology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD) and InPlot 4.0 software (GraphPad Software, San Diego, CA) on a microcomputer. Results are presented as mean ± standard error.

Results

Adenosine analogs produced a dose-dependent accumulation of cAMP in both HEL cells and human platelets (Fig. 1). All agonists tested were, in general, more potent in platelets than in HEL cells. Furthermore, there were significant differences in the rank orders of potencies of agonists between these cells. In particular, CGS 21680 was as potent as NECA in platelets, while being a very poor agonist in HEL cells (Fig. 1). EC_{50} values for these and other agonists are shown in Table 1. It must be noted that maximal responses to (*R*)-PIA and CGS 21680 were not obtained in HEL cells. Higher concentrations were not tested because of lack of solubility of the compounds. For this reason, the maximal response obtained with NECA was used as the A_1 parameter of ALLFIT in the calculation of EC_{50} values for all agonists. Analysis using InPlot gave similar EC_{50} values.

In platelets, maximal responses to agonists were observed at a concentration of 100 μM, but a reduction in effectiveness was

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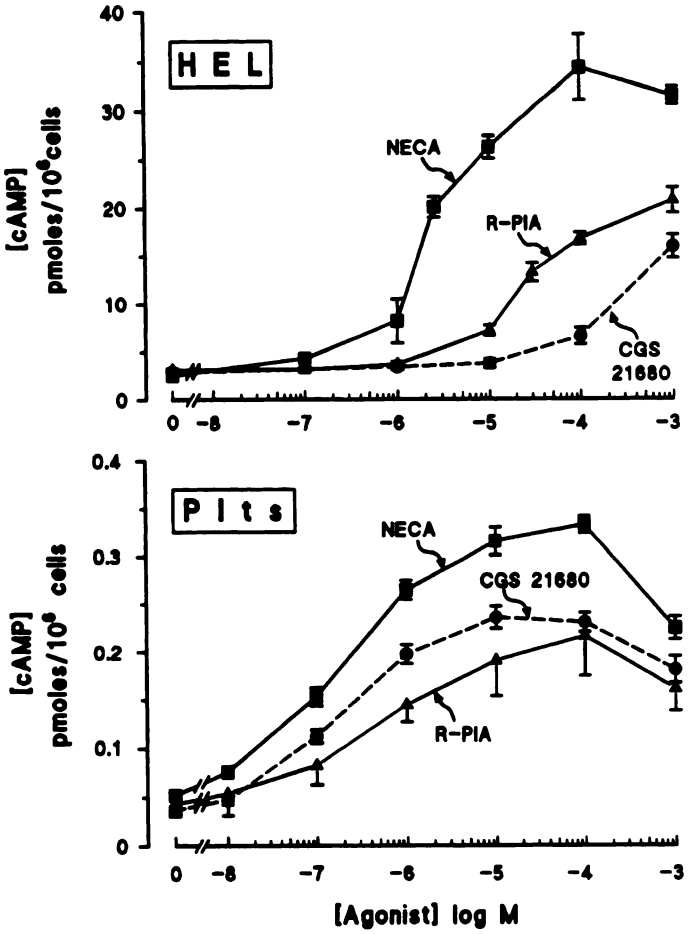

Fig. 1. Effect of increasing concentrations of adenosine receptor agonists on cAMP accumulation in HEL cells (*upper*) (six experiments) and platelets (*Plts*) (*lower*) (12 experiments). See Materials and Methods for details. EC_{50} values for these and other agonists are shown in Table 1. Agonists were, in general, more potent in platelets than in HEL cells. In particular, CGS 21680 was 1200-fold more potent in platelets.

TABLE 1

Potency (EC_{50} or IC_{50}) of agonists and antagonists at adenosine A_2 receptors (accumulations of cAMP)

	EC_{50} or IC_{50}			
	HEL cells[a]	Fibroblasts[b]	Platelets[a]	Striatum[c]
		μM		
Agonists				
NECA	2.4	2.6	0.2	0.156
(*R*)-PIA	160	150	0.53	
N^6-Cyclohexyladenosine	280	160		
(*S*)-PIA	950	750		
CGS 21680	1600		0.18	0.306
Antagonists				
DPSPX	0.25		4.0	2
DPCPX	0.55		1.4	
880316	0.54			
Theophylline	45	4.8		18
N-0861	126			
Caffeine	300	13	50	43

[a] Data obtained from the present study, using 10 μM NECA in HEL cells and 1 μM NECA in platelets to determine IC_{50} values.
[b] Data from Ref. 12, using NECA in fibroblasts to determine K_i of antagonists.
[c] Data from Ref. 26, using CGS 21680 in striatal membranes to determine K_i of antagonists.

observed at higher concentrations. Analysis of EC_{50} values were done using InPlot, without making assumptions on maximal responses. Data were best fitted by using a two-component model with a stimulatory component and an inhibitory component. The EC_{50} values for the stimulatory component are shown in Table 1. The reasons for this apparent biphasic effect of NECA on adenylate cyclase are unclear. Its importance is uncertain, considering that this phenomenon is observed only at very high concentrations of agonists and that NECA-induced inhibition of aggregation, which is mediated by activation of cAMP production (21), is not biphasic. Previous studies have not examined the high concentrations of adenosine analogs used in this study (22). High doses of authentic adenosine reportedly inhibit cAMP production in platelets through activation of the P site located in the catalytic subunit of adenylate cyclase (23). Activation of the P site requires integrity of the purine ring (24); however, adenosine analogs used in this study are not active at the P site.

Differences were also found between HEL cells and platelets in the rank order of potencies of antagonists (Fig. 2). For these studies, accumulation of cAMP was induced with NECA and inhibited with increasing concentrations of antagonists. The concentration of NECA was selected to produce 75% of its

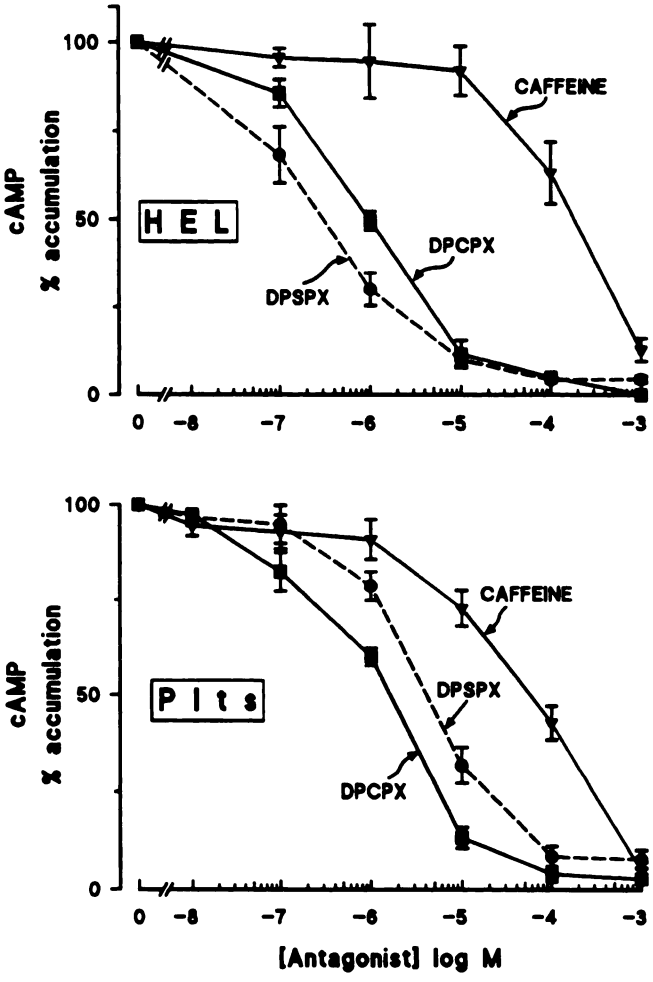

Fig. 2. Effect of increasing concentrations of adenosine receptor antagonists on cAMP accumulation produced by 10 μM NECA in HEL cells (*upper*) (six experiments) and by 1 μM NECA in platelets (*Plts*) (*lower*) (12 experiments). See Materials and Methods for details. IC_{50} values for these and other antagonists are shown in Table 1.

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maximal effect in either HEL cells (10 μM) or platelets (1 μM). Although caffeine is a relatively weak antagonist in both cell types, it is a particularly poor antagonist at HEL adenosine receptors. Conversely, DPSPX was more potent in HEL cells, compared with platelets. It is noteworthy that adenosine receptors in HEL cells were uniformly less sensitive to adenosine agonists, whereas they were more sensitive to the antagonist DPSPX. The calculated IC_{50} values for these and other adenosine antagonists are shown in Table 1.

To further characterize the adenosine receptor found in HEL cells, dose-response curves for NECA were repeated in the absence and in the presence of increasing concentrations of the antagonist DPSPX. Fig. 3 shows that increasing concentrations of the antagonist produced parallel rightward shifts of the dose-response curve for the agonist. Schild regression analysis revealed a slope approximating unity (0.997), consistent with simple competitive antagonism (25). The intercept, an approximation of the K_i, was 141 nM. These results confirm that DPSPX acted as a competitive antagonist of NECA, as would be expected for adenosine receptors. A similar Schild analysis for DPSPX in platelets revealed an apparent K_i of 1.2 μM, supporting the conclusion derived from IC_{50} values that this antagonist is more potent in HEL cells than in platelets. However, a true determination of the K_i for antagonists in platelets was not possible because the dose-response curve for NECA was not a simple sigmoidal relationship, which is required for the Schild analysis to be valid (26).

In undifferentiated HEL cells, 100 μM NECA produced a 14-fold increase in cAMP, from 2.5 ± 0.3 to 34.4 ± 3.2 pmol/10^6 cells, and 100 μM forskolin produced an 18-fold increase in cAMP (Fig. 4). Differentiation of HEL cells with phorbol ester has been shown to alter the expression of membrane-bound receptors (18). To determine whether differentiation had any effect on adenosine receptors, HEL cells were exposed to 1.6 μM 12-*O*-tetradecanoylphorbol-13-acetate for 3 days. Under these conditions, HEL cells underwent the well characterized change in morphology and increased adherence. Basal cAMP decreased slightly after differentiation with phorbol ester, from 2.0 ± 0.1 to 1.1 ± 0.2 pmol/10^6 cells. In differentiated cells

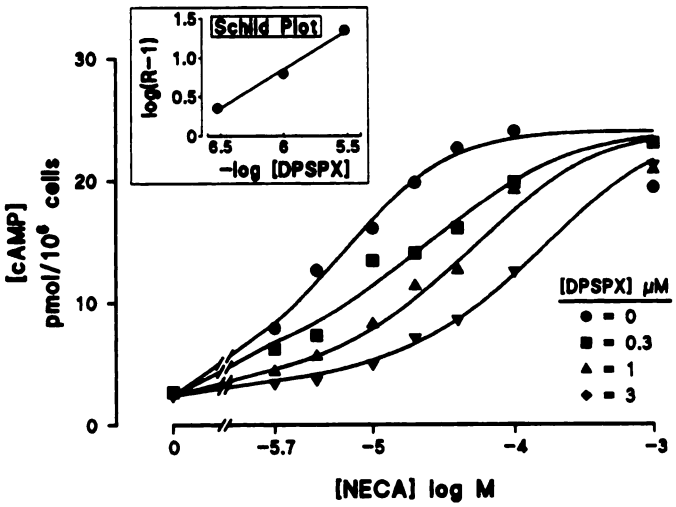

Fig. 3. Dose-response curves for accumulation of cAMP produced by NECA in HEL cells. Dose-response curves were repeated in the absence and in the presence of increasing concentrations of the adenosine receptor antagonist DPSPX, which produced a progressive shift to the right. Schild analysis of these data revealed a linear relationship (*inset*), implying competitive antagonism at HEL cell adenosine receptors.

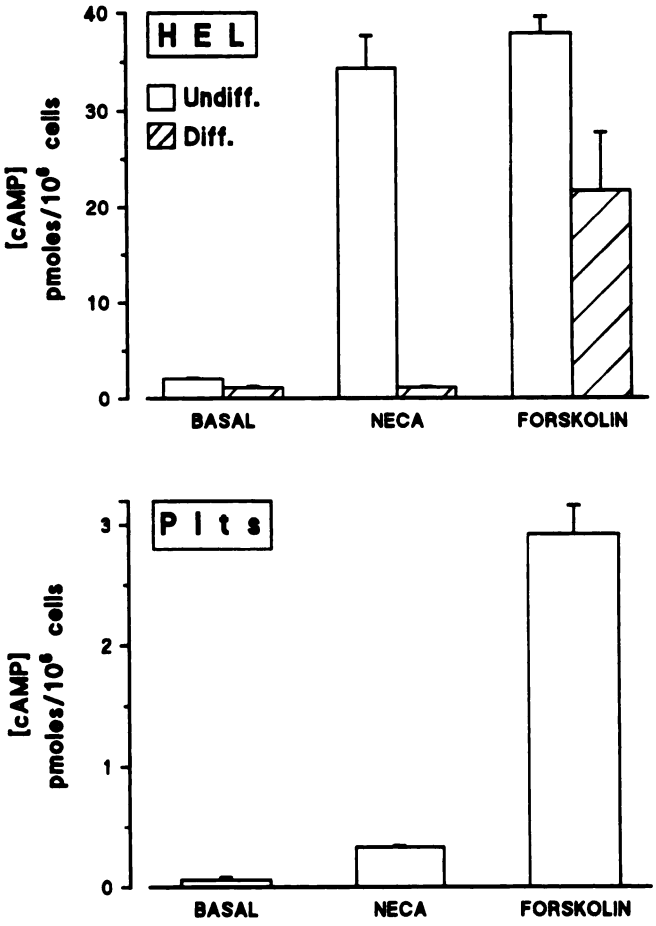

Fig. 4. Intracellular concentration of cAMP in HEL cells (*upper*) and in platelets (*Plts*) (*lower*) under resting conditions (*BASAL*) or after stimulation with 100 μM NECA or 100 μM forskolin. HEL cells were studied in the undifferentiated state (*Undiff.*) (six experiments) or after differentiation with phorbol ester (*Diff.*) (three experiments). Note that the accumulation of cAMP induced by NECA in undifferentiated HEL cells was lost upon differentiation. Results presented are the mean of three experiments.

NECA, up to a concentration of 1 mM, had no effect on cAMP levels (Fig. 4). The absolute level of cAMP accumulation produced by 100 μM forskolin was lower in differentiated cells, compared with undifferentiated cells, but the relative increase was similar in differentiated (20-fold) and undifferentiated cells (18-fold) because of the decrease in base-line cAMP levels in differentiated cells. In platelets, 100 μM NECA increased cAMP levels 6-fold, from 52 ± 5 to 333 ± 9 fmol/10^6 cells. The magnitude of this increase was similar to previously published data (22) but was lower than that produced by 100 μM forskolin (49-fold) in platelets.

A_{2b} receptors can be demonstrated in brain slices but not in brain membranes (27). For this reason we investigated whether receptor-effector coupling was preserved in membranes prepared from HEL cells. NECA produced a dose-dependent activation of adenylate cyclase (Fig. 5) with an EC_{50} of 7.22 μM, which correlates with that found in intact HEL cell preparations (2.4 μM). NECA (100 μM) produced a 1.3-fold increase in membrane adenylate cyclase activity. By comparison, 1 μM iloprost produced a 44-fold accumulation of cAMP in intact HEL cells and an 8-fold increase in adenylate cyclase activity in cell membranes; 100 μM forskolin produced an 18-fold increase in cAMP in inctact HEL cells and a 13-fold increase in adenylate cyclase activity in cell membranes.

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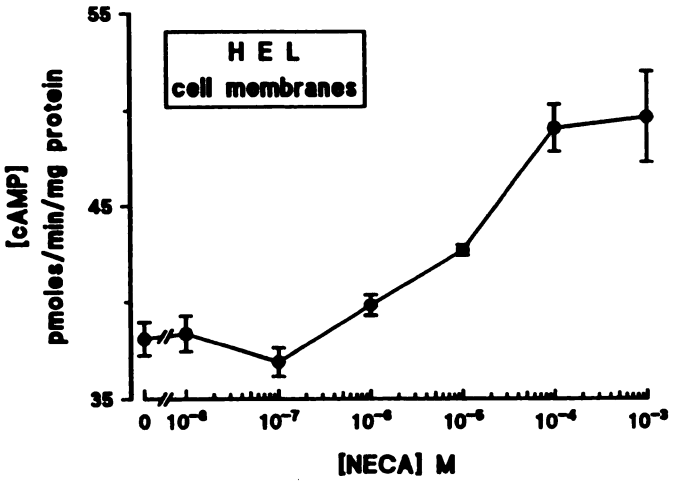

Fig. 5. Effect of increasing concentrations of the stable adenosine analog NECA on adenylate cyclase activity in HEL cell membranes (three experiments).

Discussion

Since the initial recognition of adenosine receptors, two main receptor types, A_1 and A_2, have been identified based on their differential coupling to adenylate cyclase (1) and on their relative affinities for adenosine analogs. The fact that these receptors are distinct molecular entities was recently confirmed by the cloning of A_1 receptors from dog thyroid (9) and rat brain (10, 11) libraries and of A_{2a} receptors from a dog thyroid library (14). The development of more potent and selective adenosine agonists and antagonists has allowed a more detailed characterization of adenosine receptors.

The presence of different subtypes of A_1 receptors in peripheral autonomic ganglia and in the central nervous system has been suggested on the basis of differences in the orders of potencies of agonists between certain tissues (28). The existence of subtypes of A_1 receptors, however, is not universally accepted, in part because differences in experimental protocol between studies may potentially account for the observed differences in agonist and antagonist potencies (2). On the other hand, the existence of subtypes of A_2 receptors is more widely accepted. This concept was proposed initially by Daly *et al.* (27) and emerged from the finding of high affinity A_2 receptors in rat striatum and of low affinity A_2 receptors throughout the brain. Activation of either receptor resulted in accumulation of cAMP. However, high affinity A_2 receptors increased cAMP only in membrane preparations of the striatum, whereas low affinity A_2 receptors increased cAMP only in brain slices and not in membrane preparations. The reasons for this phenomenon remain unclear. These high and low affinity receptor subtypes were later designated as A_{2a} and A_{2b}, respectively (12).

The brain striatal high affinity A_2 receptor remains the prototypical A_{2a} receptor. Radioligand binding to this receptor has been possible because of its relatively high affinity for agonists (12), in particular CGS 21680 (13). Although this analog is no more potent than NECA at A_2 receptors, it is highly selective because of its ineffectiveness at A_1 receptors. The K_d for CGS 21680 and NECA was found to be in the 2–18 nM range with striatal A_{2a} receptors (12, 13), which is a remarkably high affinity, compared with those of other known A_2 receptors. The recently cloned A_2 receptor derived from a dog thyroid library has been identified as an A_{2a} receptor, based on its localization in the striatum by *in situ* hybridization and its high affinity for CGS 21680 (14). A_{2a} receptors have also been described in rat pheochromocytoma PCR cells (26). The functional role of brain A_{2a} receptors remains unclear. CGS 21680 has been found to depress neuronal activity in the cerebral cortex (29) and to act centrally to depress locomotor activity *in vivo*. Other studies have not identified a physiological effect resulting from activation of A_{2a} receptors in the striatum (30).

Identification of the low affinity A_2 receptor has lagged behind that of the A_1 and high affinity A_{2a} receptors, in part because of the lack of effective radioligands and because these low affinity A_2 receptors were initially identified only in brain slices and not in cellular or membrane preparations. Bruns *et al.* (12, 31) used a human fibroblast cell line as a prototype of the so-called A_{2b} receptor. Whether the receptor type found in fibroblasts is the same as the low affinity brain receptor has not been clearly defined, in our view.

The initial purpose of this study was to determine whether HEL cells could be used as a model of platelet adenosine receptors. We found that both cell types possess A_2 adenosine receptors. However, it is clear from our results that HEL cells and platelets possess distinct subtypes of adenosine receptors. The differences between them can be summarized as follows. 1) Adenosine agonists are, in general, less potent at HEL cell receptors than at platelet receptors, with NECA being approximately 10-fold more potent in platelets; this was particularly true for CGS 21680, which was almost as potent as NECA in platelets but was virtually inactive in HEL cells. 2) In contrast to the decreased potency of agonists, some antagonists, and in particular DPSPX, are more potent in HEL cells than in platelets. 3) The rank orders of potencies of both agonists and antagonists are different in HEL cells and in platelets. It could be suggested that, because of the lower affinity for agonists and the higher affinity for antagonists, the HEL cell adenosine receptor is not a distinct protein but a low agonist affinity state of a high affinity A_2 receptor and that these two receptors may differ only in their coupling to intracellular effector systems. However, the striking difference in the rank orders of potencies of agonists between these receptors argues against this possibility (12, 31).

The order of potencies of agonists and antagonists found for the HEL cell adenosine receptor is very similar to that reported for the VA13 human fibroblast receptor used as the prototype of the A_{2b} receptor (12) (Table 1). Following those guidelines, therefore, the adenosine receptor present in HEL cells could be tentatively classified as an A_{2b} receptor subtype. We found that CGS 21680 was virtually inactive in HEL cells. Recent studies have also found CGS 21680 to be inactive as an agonist of A_2 receptors found in rat pineal gland and in chromaffin cells (32, 33). CGS 21680 is also inactive in some vascular smooth muscle preparations that are responsive to NECA (34), suggesting that those vascular beds also contain A_{2b} receptors. Another adenosine analog, CV1674, was also found to be inactive in fibroblast A_{2b} receptors (31). Both CGS 21680 and CV1674, therefore, have been shown to be useful in differentiating A_{2b} from other A_2 receptors. It has been proposed that the low affinity A_2 receptor present in the brain is an A_{2b} subtype, but direct studies with these newer analogs have not been made. It has been found, however, that CGS 21680 is ineffective in increasing cAMP in hippocampal slices, whereas NECA increased cAMP levels 4-fold in this preparation (30).

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Given our finding that CGS 21680 is inactive at A_{2b} receptors, this observation argues in favor of the existence of A_{2b} receptors in the hippocampus and the presumption that the low affinity adenosine brain receptor is indeed an A_{2b} receptor.

Low affinity A_2 receptors have been found in brain slices but not in membrane preparations (27), raising the possibility of a loss of the A_{2b} receptor or its coupling to the effector system during membrane preparation. Therefore, we determined whether adenosine receptors could be demonstrated in HEL membranes. NECA produced a 1.3-fold increase in cAMP levels in HEL cell membranes with an EC_{50} (7.2 μM) similar to that found in intact HEL cells (2.4 μM). The magnitude of this effect is comparable to that reported in platelet membranes (35) but significantly smaller than that found in intact HEL cells (14-fold). However, a similar decrease in effectiveness was observed with iloprost, which increased cAMP 44-fold in intact HEL cells but increased adenylate cyclase activity only 8-fold in membrane preparations. On the other hand, the effectiveness of forskolin remained relatively preserved in membrane preparations, suggesting that adenylate cyclase was maintained in membranes but its coupling to receptors was modified. We could not demonstrate, therefore, that this putative loss of receptor-effector coupling in cell membranes is unique to the adenosine A_{2b} receptor.

In summary, adenosine receptors are present in HEL cells and platelets. Adenosine receptor activation increases cAMP levels in both cell types, as expected for A_2 receptors, but the relative potencies of analogs and antagonists differ widely. In particular, HEL cells do not respond to the agonist CGS 21680 but have a greater affinity for the antagonist DPSPX. We found these agents particularly helpful in differentiating between A_2 receptor subtypes. Thus, adenosine receptors in HEL cells have biochemical characteristics different from those of receptors present in platelets and probably correspond to the A_{2b} subtype.

Acknowledgments

The authors would like to thank Dr. Jack Wells for useful discussions on the design and interpretation of the studies and Ms. Dorothea Boemer and Ms. Jane Estrada for editorial assistance.

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