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ADENOSINE RECEPTORS OF HUMAN LEUKOCYTES—II

CHARACTERIZATION OF AN INHIBITORY P-SITE

GIANNI MARONE, ROSARIA PETRACCA, SERGIO VIGORITA and VINCENZO CASOLARO
Department of Medicine, Division of Clinical Immunology, University of Naples Federico II, II School of Medicine, Via S. Pansini 5, 80131 Naples, Italy

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Abstract—We have investigated the effects of 2′,5′-dideoxyadenosine (DDA), 9′-β-D-arabinofuranosyladenine (ARA), and 9-β-D-xylofuranosyladenine (XFA), which have been classified as P-site adenosine agonists, on the cyclic adenosine 3′,5′-monophosphate (cAMP) metabolism of human lymphocytes and polymorphonuclear leukocytes (PMNs). DDA (10^{-5}–2×10^{-4} M), ARA and XFA caused a dose-dependent decrease in cAMP content of human lymphocytes. In addition to decreasing lymphocyte cAMP levels, DDA, ARA and XFA markedly inhibited the effects of many adenylate cyclase-stimulating agents including β-adrenergic stimuli, prostaglandin E_1 (PGE_1), histamine, adenosine, forskolin and cholera toxin. Theophylline and 3-isobutyl-1-methylxanthine, which are known antagonists of adenosine A_1/R_i and A_2/R_a receptors, did not modify the inhibiting effects of DDA. Mn^{2+} (1 mM) increased the sensitivity to inhibition of adenylate cyclase agonists by DDA. We also searched for the presence of adenosine P-sites in human PMNs. DDA caused a significant decrease of PMN cAMP levels only at the highest concentrations used (2×10^{-4} M). In contrast, even low concentrations of DDA (10^{-6}–10^{-4} M) concentration-dependently blocked the stimulatory effect of PGE_1 and forskolin on PMN cAMP accumulation. The results support the existence of a purine P-site that regulates cAMP metabolism of human lymphocytes and PMNs.

Increasing evidence suggests that adenosine may function as an important immunoregulatory autacoid [1]. Adenosine inhibits the mitotic response of human T lymphocytes [2], lymphocyte-mediated cytolysis [3], immunoglobulin synthesis [4], chemotaxis [5], histamine release from human basophils [6–8], platelet aggregation [9] and toxic oxygen radicals by human polymorphonuclear leukocytes (PMNs) [10–12]. Adenosine has been implicated in the pathophysiology of patients with severe combined immune deficiency associated with a deficit of adenosine deaminase [13], with systemic lupus erythematosus [14], with asthma [15, 16] and with alcoholism [17].

The mechanism(s) responsible for the modulation by adenosine of immune responses has been the subject of considerable controversy. Proposed mechanisms include an alteration of cAMP metabolism in human inflammatory cells induced by adenosine [18, 19] and by methylation [5, 20]. Adenosine receptors that modulate adenylate cyclase have been reported in many tissues [21–23]. There are at least two subclasses of adenosine receptors that modulate either inhibition or stimulation of adenylate cyclase [24]. For instance, the inhibitor receptor (called R_i or A_1), which requires very high concentrations of agonists (20–100 nM) and prefers (−)-R-PIA over NECA, and the stimulatory receptor (R_a or A_2), which is effective over a range of 0.1 to 30 μM and prefers NECA over (−)-R-PIA [25, 26]. Both subclasses are antagonized by methylxanthines and are located on the outer cell surface [22, 24]. We and others have described the presence of adenosine A_2/R_a receptors on different subpopulations of human leukocytes [11, 22, 27, 28].

In addition to these two subclasses of adenosine receptors, an inhibitory adenosine-activated P-site has also been identified [24]. 2′,5′-Dideoxyadenosine (DDA) and certain ribose modified analogues of adenosine interact with the P-site [24]. Interactions at the P-site inhibit adenylate cyclase activity and this inhibitory activity is enhanced in the presence of Mn^{2+} [24]. The P-site is probably located on the cytoplasmic surface of the plasma membrane. In contrast to A_1 and A_2 receptors, activation of the P-site is not reversed by methylxanthines [24]. The studies described in this paper were undertaken to characterize an inhibitory P-site effect of adenosine analogues on human lymphocytes and PMNs. The results support the hypothesis that an adenosine P-site is present on subpopulations of human lymphocytes that possess receptors for histamine, prostaglandin E_1 (PGE_1), β-adrenergic agonists and adenosine A_2/R_a receptors. The adenosine P-site also appears to be present on human PMNs, although it is more sensitive to agonists of the adenosine P-site than the P-site of lymphocytes.

MATERIALS AND METHODS

Materials. DL-Isoproterenol hydrochloride, adenosine, cholera toxin, forskolin, histamine dihydrochloride, ARA, 2′,5′-dideoxyadenosine, theophylline, cAMP, PGE_1, and PIPES, were obtained from

the Sigma Chemical Co. (St Louis, MO). XFA was obtained from the National Cancer Institute (Bethesda, MD). Fetal calf serum (FCS) was purchased from Flow Laboratories (McLean, VA), and RPMI 1640 medium from Gibco (Grand Island, NY). All drugs were prepared immediately before use. [^{3}H]cAMP (sp. act. 50 Ci/mmol) was purchased from New England Nuclear (Boston, MA). NECA was donated by Byk Gulden Italia (Milan, Italy).

PMN preparation. PMNs were isolated from venous blood of healthy adult volunteers as described previously [29].

Lymphocyte preparation. Mononuclear cells were isolated from venous blood of healthy adult volunteers as described previously [29]. The leukocyte fraction obtained in this way contained 80% lymphocytes and 20% monocytes. Platelet contamination was less than one platelet per nucleated cell. Aliquots of unfractionated mononuclear cells [$\simeq 5 \times 10^7$ cells in 7 mL of RPMI 1640 containing 10% fetal calf serum (FCS)] were incubated in 20 × 100 mm tissue culture (Falcon Plastics, Oxnard, CA) for 90 min at 37° in a humidified atmosphere of 95% air 5% CO_2. The non-adherent cells ($\simeq$75% of the cells applied to the dishes) were then collected and the plates were washed twice with RPMI 1640 containing 10% FCS. The pooled cells were used as the lymphocyte-enriched fraction and usually contained 80% T-lymphocytes and 15% B-lymphocytes. Monocyte contamination was less than 5%.

Incubation conditions. Lymphocytes or PMNs (2 to 5×10^6 viable cells in 0.5 mL final volume) were added to 12 × 75 mm plastic Falcon tubes and preincubated at 37° for 5 min. Over this range of cell density, variation in cell concentrations did not alter the basal or stimulated cAMP levels (data not shown). The incubation was initiated by adding the agents under investigation in a volume of 0.1 or 0.2 mL. All experimental conditions were performed in triplicate, and six drug-free control tubes were used in each experiment to ascertain baseline cAMP levels (three tubes each at the beginning and at the end of each set). When theophylline was used, the cells were preincubated at 37° with this agent for 5 min before stimulation. Incubations were stopped by adding 0.5 mL of 10% TCA and centrifuging (2 min, 1000 *g*, 22°). After centrifugation, the cAMP content of the supernatants was determined as indicated.

cAMP assay. The cAMP content was determined as previously described [30, 31].

Drug solutions. All drugs were immediately prepared before use. Isoproterenol was dissolved in PC containing 3 μg/mL of sodium metabisulfite. This concentration of sodium metabisulfite itself did not alter basal cAMP levels of PMNs or lymphocytes. PGE_1 was dissolved at 3×10^{-2} M in 95% ethanol and further dilutions were made in PC. Appropriate ethanol controls were performed in each experiment.

RESULTS

The effects of P-site agonists on the level of cAMP in human lymphocytes

DDA, XFA, and ARA are adenosine analogues that interact with the P-site [24]. DDA, XFA, and ARA at low concentrations caused a small increase in lymphocyte cAMP levels in four of twelve experiments (data not shown). Above 10^{-5} M, DDA caused a dose-dependent decrease in the cAMP content of human lymphocytes (Table 1). XFA and ARA, two P-site effectors, were less potent than DDA (data not shown), as previously observed for the P-site in other tissues [24, 32]. These experiments were carried out with a 10-min incubation period based on kinetic studies indicating that the inhibitory effect of P-site effectors is extremely rapid (data not shown).

Table 1. The effect of DDA on intracellular cyclic AMP levels in human lymphocytes*

	Expt. 1	Expt. 2	Expt. 3
	Cyclic AMP (pmol/10^7 cells)		
No drug	11.1 ± 0.9	40.5 ± 3.6	11.5 ± 0.8
DDA (M)			
10^{-6}	14.4 ± 0.3	ND	11.5 ± 1.3
10^{-5}	10.8 ± 0.3	39.1 ± 2.9	7.3 ± 2.5‡
10^{-4}	5.2 ± 0.2†	25.3 ± 2.6†	4.4 ± 1.2†
2×10^{-4}	3.7 ± 0.0†	16.3 ± 1.0†	ND

* The cells were incubated 8 min with the indicated concentrations of DDA. Each value represents the mean ± SE of triplicate determinations.

† $P < 0.01$ when compared with the corresponding value of no drug.

‡ $P < 0.05$ when compared with the corresponding value of no drug.

ND, not done.

The effects of P-site agonists on the cAMP increase caused by PGE_1, isoproterenol, histamine, and adenosine

PGE_1, isoproterenol, histamine and adenosine all increase lymphocyte cAMP levels, each presumably by interacting with a specific membrane receptor [29, 30]. Therefore, we examined the effects on lymphocyte cAMP levels of these adenylate cyclase agonists plus various concentrations of DDA. In a series of twelve experiments we found that DDA (10^{-6}–2×10^{-4} M) decreases the cAMP content of human lymphocytes and blocks the stimulatory effect of both PGE_1 (2×10^{-7} M) and isoproterenol (2×10^{-7} M). Figure 1 shows that DDA concentration-dependently blocked the stimulatory effect of both PGE_1 (2×10^{-7} M) and isoproterenol (2×10^{-7} M) on cAMP metabolism. The inhibitory effect of DDA was observed even at low concentrations (10^{-6}–10^{-5} M) that do not decrease basal level of cAMP in lymphocytes.

Histamine and adenosine increase lymphocyte cAMP levels, presumably by interacting with specific H_2 and A_2/R_a receptors, respectively [22, 30]. In a group of ten experiments we evaluated the possible interaction between DDA and the cAMP metabolism activated by histamine and adenosine. Figure 2 shows a typical experiment in which DDA almost completely blocked both histamine- and adenosine-induced increases of lymphocyte cAMP levels. The inhibitory effect of a low concentration (10^{-6} M) of

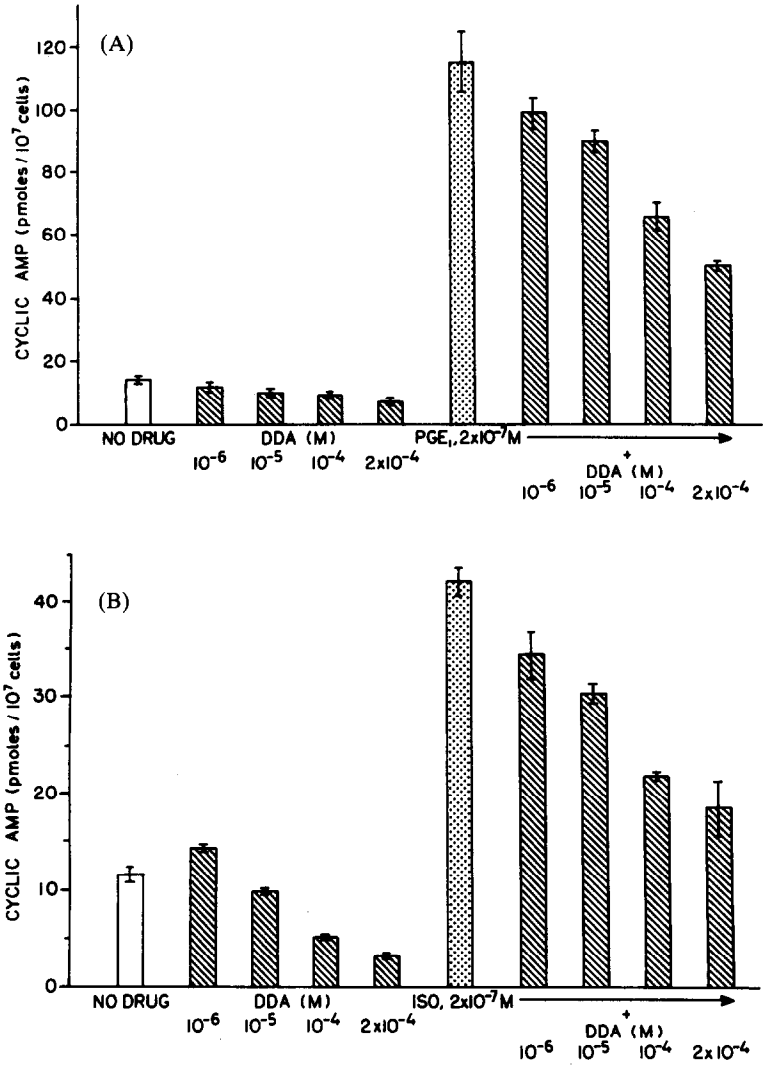

Fig. 1. The effect of DDA, alone, and in combination with PGE_1 (A) or isoproterenol (ISO) (B) on the level of cyclic AMP in human lymphocytes. The cells were preincubated 5 min with the indicated concentrations of DDA. PGE_1 or ISO was then added and the cells were incubated for an additional 8 min. Each bar represents the mean ± SE of triplicate determinations.

DDA was more marked in this case presumably because histamine and adenosine produced only a two- to three-fold increase in cAMP content of lymphocytes compared to the four- to six-fold increase caused by isoproterenol and PGE_1, respectively.

These experiments included a 5-min preincubation period with DDA, because kinetic studies (see Fig. 3) showed that the inhibitory effect of DDA is extremely rapid. The kinetics of the response induced by 10^{-7} M PGE_1 indicates that the cAMP levels increased significantly at the earliest time assayed (1 min), but decreased promptly (within ≈1 min) if DDA (2×10^{-4} M) was added to the cells (Fig. 3). Similar results were obtained in two other experiments. The kinetics of inhibition of activated adenylate cyclase by DDA in lymphocytes is similar to the characteristics of the P-site in different systems [32].

The ability of P-site effectors to inhibit the effect of adenylate cyclase agonists was not confined to DDA. XFA and ARA, which have been classified as P-site adenosine agonists [24, 32], markedly inhibited the effect of PGE_1 on lymphocyte cAMP levels (data not shown).

The effect of theophylline

Theophylline and other methylxanthines block the effect of adenosine and its analogues on both the stimulatory (A_2/R_a) and the inhibitory (A_1R_i) receptors [6, 9], and this inhibition is apparently due to competition for these receptors [1, 25, 26]. In contrast, the P-site, which is probably located on the cytoplasmic surface of the plasma membrane [24], is insensitive to methylxanthines [24]. In a series of five experiments, we examined the effect of low concentrations of theophylline, which do not inhibit cAMP phosphodiesterase (PDE) activity, on

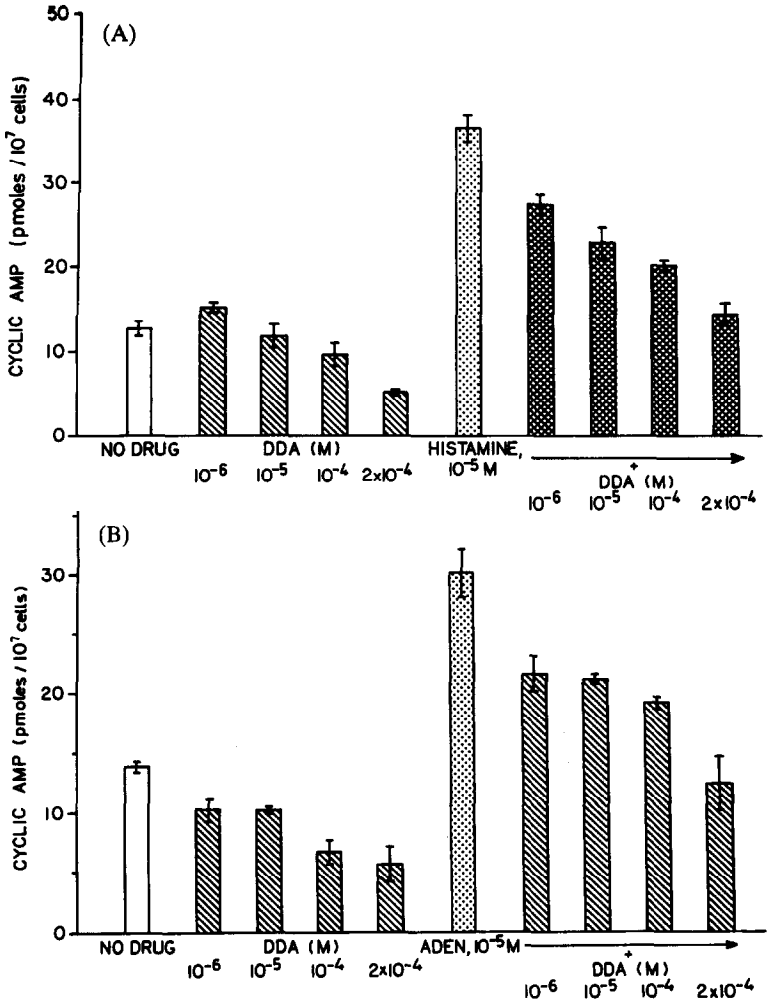

Fig. 2. The effect of DDA, alone, or in combination with histamine (A) or adenosine (B), on the level of cyclic AMP in human lymphocytes. The cells were preincubated 5 min with the indicated concentrations of DDA. Histamine or adenosine was then added and the cells were incubated for an additional 8 min. Each bar represents the mean ± SE of triplicate determinations.

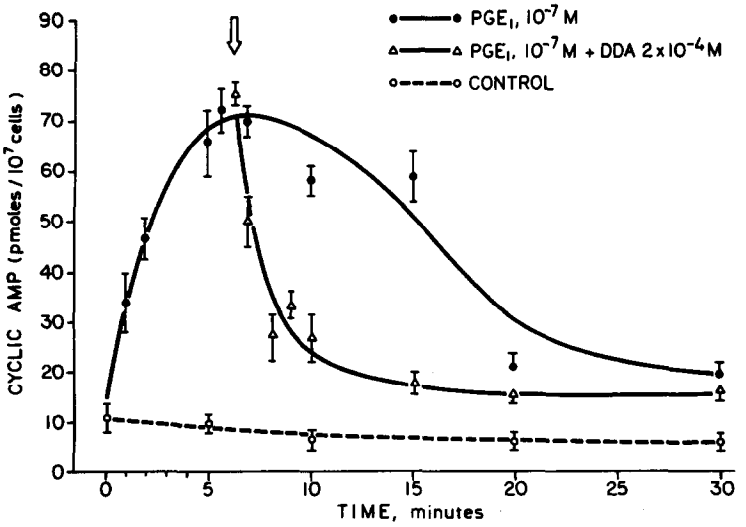

Fig. 3. Intracellular cyclic AMP levels in human lymphocytes incubated with (●——●) or without (○——○) PGE_1 (10^{-7} M) for varying time intervals. Cyclic AMP levels were also determined in lymphocytes incubated with PGE_1 for 7 min, after which DDA (2×10^{-4} M) was added (note arrow at the top of figure). Each symbol represents the mean ± SE of triplicate determinations.

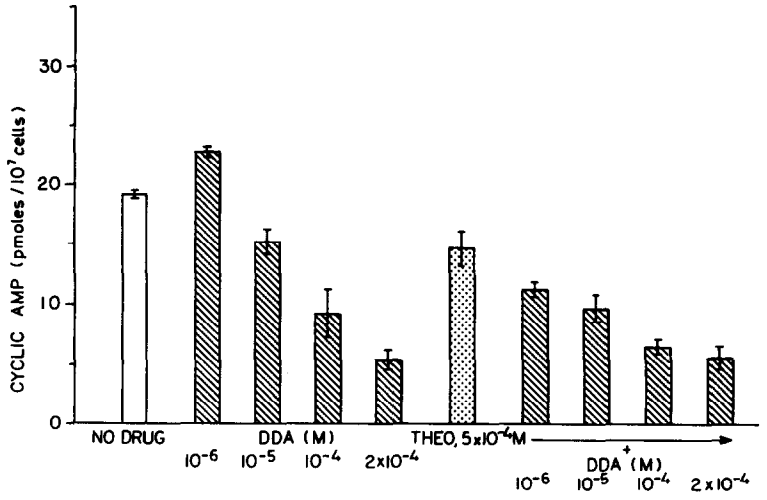

Fig. 4. The effect of DDA, alone, and in combination with theophylline (THEO), on intracellular cyclic AMP levels in human lymphocytes. The cells were preincubated 5 min with THEO. DDA was then added and cells were incubated for an additional 8 min. Each bar represents the mean ± SE of triplicate determinations.

cAMP changes induced by DDA in human lymphocytes. Figure 4 shows the effects of 5×10^{-4} M theophylline on both intracellular cAMP and on the inhibitory effect of DDA. This concentration of theophylline caused a small but consistent decrease in lymphocyte cAMP levels, probably by antagonizing endogenous adenosine that modulates the basal nucleotide level in these cells [33]. In contrast, theophylline did not block the inhibitory effect of DDA on cAMP production, which is probably mediated by interaction of the purine P-site. Similar results were obtained with 3-isobutyl-1-methylxanthine, another methylxanthine known to block the effect of adenosine [7] (data not shown).

In a parallel group of six experiments, we also examined the effect of theophylline on the inhibitory effect of DDA on lymphocyte cAMP changes induced by adenylate cyclase agonists. As shown in Fig. 5, preincubation with theophylline (10^{-4} M) did not modify the inhibitory effect of DDA (10^{-4} M) on cAMP changes induced by isoproterenol (10^{-7} M). Therefore, the inhibition of the effect of adenylate cyclase agonists by DDA is apparently unrelated to interaction with the A_1/R_i receptor, which is antagonized by methylxanthines [1, 6, 25, 26].

The effect of DDA on the activation of lymphocyte adenylate cyclase by forskolin and cholera toxin

Forskolin activates cAMP production in a variety of mammalian tissues and cell types without binding to a membrane receptor [34, 35]. It has been proposed that forskolin activates adenylate cyclase

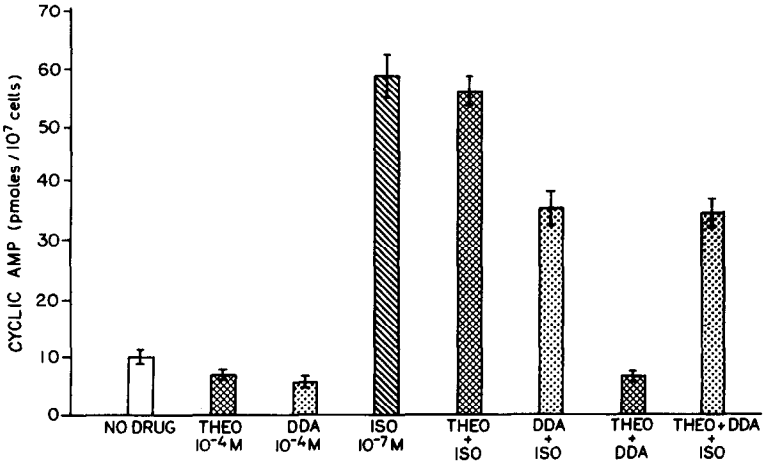

Fig. 5. The effect of DDA, alone and in combination with theophylline, on the elevation by isoproterenol (ISO) of the level of cyclic AMP in human lymphocytes. The cells were preincubated 5 min with THEO. DDA was then added and 5 min later ISO was incubated with the cells for an additional 8 min. Each bar represents the mean ± SE of triplicate determinations.

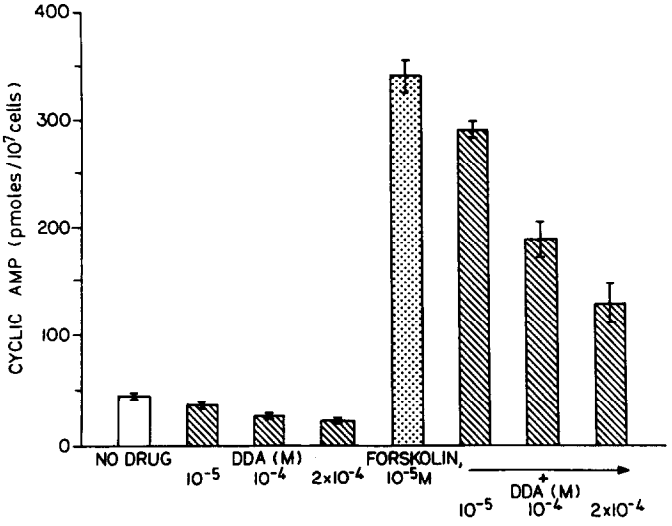

Fig. 6. The effect of DDA, alone and in combination with forskolin, on the level of cyclic AMP in human lymphocytes. The cells were preincubated 5 min with DDA. Forskolin was then added and the cells were incubated for an additional 8 min. Each bar represents the mean ± SE of triplicate determinations.

via direct interaction with the enzyme [35, 36]. In five experiments we investigated the interaction between forskolin and DDA in human lymphocytes. Figure 6 shows that forskolin (10^{-5} M) produced a seven-fold increase in the intracellular concentration of cAMP in lymphocytes; DDA (10^{-5}–2×10^{-4} M) concentration-dependently inhibited the cAMP accumulation produced by forskolin in human lymphocytes.

Cholera toxin increases intracellular cAMP by directly activating adenylate cyclase without binding to hormone receptor [37]. The accumulation of cAMP in human lymphocytes, as well as in other intact cells, shows a lag period of 10 to 45 min [30, 38]. In a group of three experiments we investigated the possible interaction between DDA and cholera toxin with respect to the cAMP metabolism of lymphocytes. Figure 7 shows the results obtained in a typical experiment performed to evaluate the kinetics of the effects of DDA alone (2×10^{-4} M), cholera toxin alone (1 ng/mL) and DDA + cholera toxin. DDA alone caused a rapid decrease in the cAMP content of lymphocytes that persisted up to 60 min of incubation. Cholera toxin

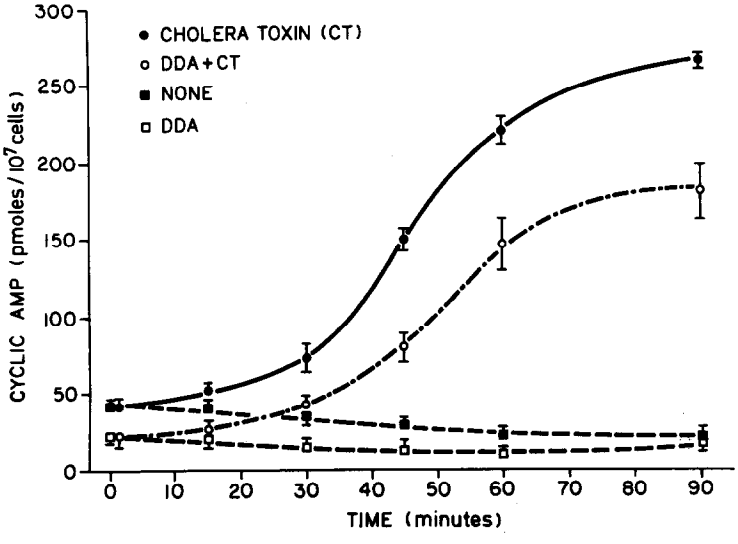

Fig. 7. Kinetics of the cyclic AMP levels in human lymphocytes incubated with buffer (■---■), DDA (2×10^{-4} M) (□---□), cholera toxin (1 ng/mL) (●——●) and DDA + cholera toxin (○-·-○). The cells were preincubated 5 min with DDA before the addition of cholera toxin. Each symbol represents the mean ± SE of triplicate determinations.

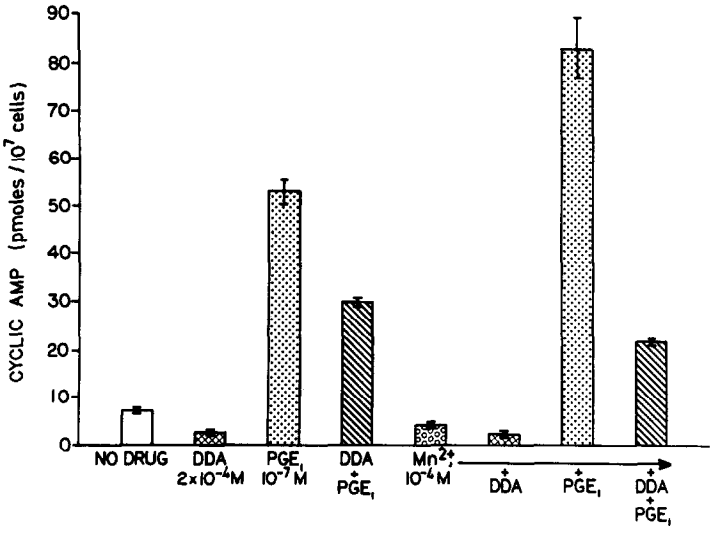

Fig. 8. The effect of DDA, alone and in combination with Mn^{2+}, on the elevation by PGE_1 of cyclic AMP levels in human lymphocytes. The cells were preincubated 5 min with Mn^{2+}. DDA was then added and 5 min later PGE_1 was incubated with the cells for an additional 8 min. Each bar represents the mean ± SE of triplicate determinations.

showed the typical lag period of 15 min, after which the cAMP content of lymphocytes started to increase and continued increasing up to 90 min of incubation. Incubation with DDA (2×10^{-4} M) inhibited also the cAMP accumulation produced by cholera toxin (1 ng/mL) in human lymphocytes. The results obtained with forskolin and cholera toxin suggest that the inhibitory effect of DDA is mediated by a negative influence on adenylate cyclase rather than at the level of membrane receptors.

The effect of Mn^{2+}

A characteristic feature of P-site-mediated inhibition is that the sensitivity to P-site effectors is increased by agents that increase adenylate cyclase activity, such as Mn^{2+} ions [39–41]. In a series of six experiments we evaluated the effect of Mn^{2+} on the inhibitory effect of DDA on cAMP metabolism. Figure 8 shows the results of a typical experiment in which we evaluated the effects of DDA, Mn^{2+} and PGE_1 alone or in combination on the cAMP levels on human lymphocytes. In fact, PGE_1 (10^{-7} M) produced a five-fold increase in the intracellular cAMP concentration of lymphocytes, whereas the presence of Mn^{2+} in the incubation buffer induced an over three-fold increase in cAMP lymphocyte concentration caused by PGE_1. Furthermore, as previously shown, DDA (2×10^{-4} M) inhibited to approximately 50% the stimulatory effect of PGE_1; in the presence of Mn^{2+} the inhibition was over 75%. Thus, Mn^{2+} potentiated the increase in cAMP induced by PGE_1. Mn^{2+} (10^{-4} M) alone or in combination with DDA (2×10^{-4} M) did not modify the basal level of cAMP in lymphocytes. Therefore, human lymphocytes appear to contain a P-site that exhibits the characteristic cation dependence seen in other systems [40, 41].

The effect of DDA on the activation of adenylate cyclase of human PMNs

Previous studies have demonstrated that the cAMP metabolism of human PMNs differs from that of human lymphocytes [29, 30]. In particular, many adenylate cyclase agonists such as β_2-agonists and histamine H_2-agonists, in the absence of PDE inhibitors, do not increase the cAMP content of human PMNs [29]. Only PGE_1 and forskolin are able to increase the cAMP content of human PMNs in the absence of PDE inhibitors [29, 42]. We have investigated the presence of an inhibitory adenosine P-site in human PMNs by evaluating the interaction between DDA and both PGE_1 and forskolin. Figure 9 shows a typical result obtained in a series of twelve experiments. Low concentrations (10^{-6}–10^{-4} M) of DDA did not significantly modify the basal level of cAMP in PMNs. Only the highest concentration (2×10^{-4} M) of DDA produced a significant decrease in the intracellular level of cAMP in PMNs. In contrast, lower concentrations of DDA (10^{-6}–10^{-5} M) concentration-dependently inhibited the effects of PGE_1 (10^{-6} M) (Fig. 9A) and of forskolin (Fig. 9B). A consistent finding of these experiments was the observation that the highest concentrations of DDA (10^{-4}–2×10^{-4} M) almost completely suppressed the stimulatory effect of both PGE_1 and forskolin on cAMP accumulation in human PMNs. The effects of DDA in human PMNs were not blocked by low concentrations of theophylline (data not shown). These results suggest that human PMNs possess an adenosine P-site.

DISCUSSION

We have previously shown that adenosine and its analogues, in micromolar concentrations, increase intracellular cAMP in human lymphocytes and PMNs by activating a membrane receptor endowed with properties similar to those of an adenosine A_2/R_a receptor [22, 28]. The present results indicate that adenosine analogues modified in the ribose moiety inhibit the effects of several agonists of adenylate cyclase in human lymphocytes and PMNs presumably

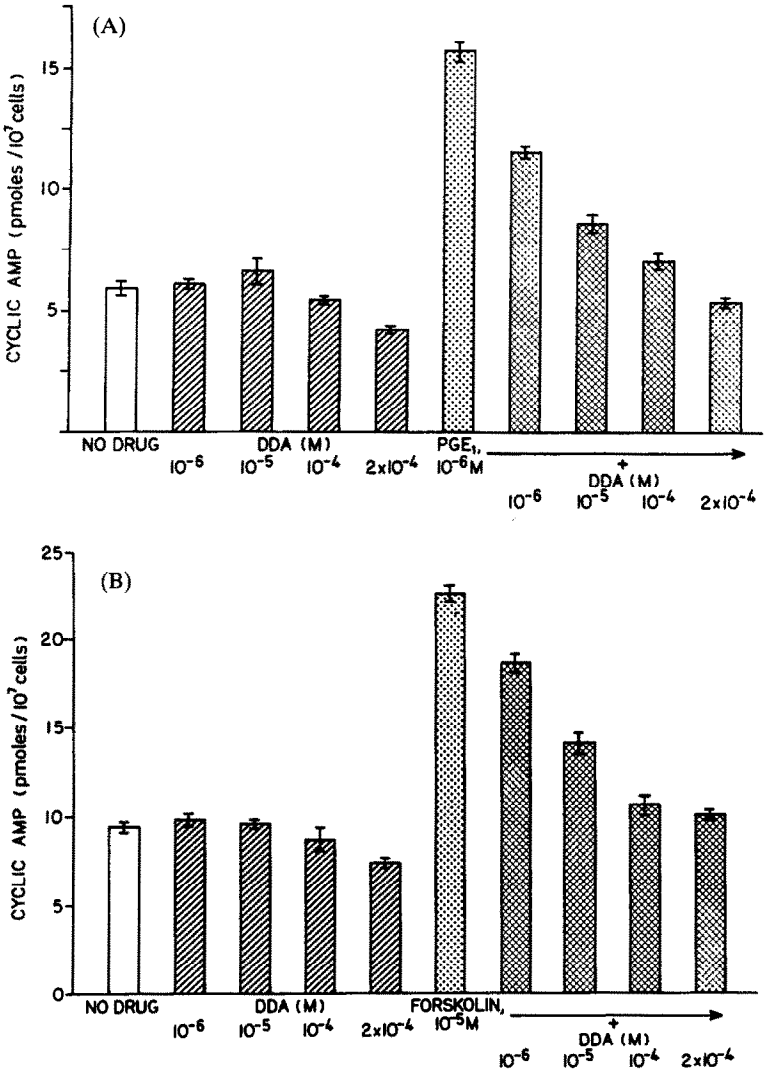

Fig. 9. The effect of DDA, alone and in combination with PGE_1 (A) or forskolin (B), on the level of cyclic AMP in human polymorphonuclear leukocytes. The cells were preincubated 5 min with DDA. PGE_1 or forskolin were then added and the cells were incubated for an additional 8 min. Each bar represents the mean ± SE of triplicate determinations.

by activating an inhibitory adenosine P-site. Several observations support the existence of an inhibitory P-site in human lymphocytes and PMNs. First, the inhibitory effect on cAMP metabolism of these cells is observed using three ribose-modified analogues of adenosine, DDA, ARA and XFA, which are known agonists of the P-site in a variety of tissues [24]. The rank order of potency DDA ≫ ARA = XFA is also consistent with their different affinity for the P-site in other tissues [24, 41]. Second, the inhibitory effect of DDA is particularly evident when adenylate cyclase activity was stimulated by a variety of agonists including isoproterenol, PGE_1, histamine, adenosine, cholera toxin and forskolin, as has been demonstrated in other systems [24, 41]. Third, the inhibitory effect of DDA on activated adenylate cyclase is extremely rapid, as previously shown by Lad *et al.* [32]. Fourth, the inhibitory effect of DDA on the activation of adenylate cyclase caused by several agonists is potentiated by the presence of Mn^{2+}—previously shown in other systems [39, 40].

Finally, the inhibition of cAMP metabolism caused by DDA in human lymphocytes and PMNs is not blocked by theophylline, a competitive agonist of the adenosine receptors in mast cells [1, 22].

The inhibitory effect of DDA, ARA and XFA on cAMP accumulation induced by adenylate cyclase agonists is clearly distinct from the activation of the inhibitory adenosine A_1/R_i receptor. Its effect is not blocked by theophylline or by other methylxanthines, which are, in low concentrations, specific antagonists of adenosine A_1/R_i receptors in human lymphocytes [1]. Furthermore, the shape of the dose–response curve of (−)-R-PIA on adenylate cyclase agonists is clearly different from that of DDA [41]. Finally, DDA is active at the P-site in submillimolar

concentrations, whereas the activation of adenosine A_1/R_i receptor requires nanomolar concentrations of the agonists [41].

The adenosine P-site is probably present on different subpopulations of human peripheral blood lymphocytes. In fact, we have shown that DDA inhibits the effects of many adenylate cyclase agonists including isoproterenol, histamine, adenosine, and PGE_1. Each of these agonists acts on different hormone receptors present on the membrane of human lymphocytes. The existence of subpopulations of human lymphocytes bearing different membrane receptors for these agonists has yet to be demonstrated [43]. However, DDA has a qualitatively similar effect on the accumulation of lymphocyte cAMP induced by the activation of different receptors. In quantitative terms, we have observed that the inhibitory effect of DDA is more marked against the activation of adenylate cyclase caused by histamine and adenosine than that caused by PGE_1 and isoproterenol. This phenomenon might be because the latter agonists induce a more marked accumulation of intracellular cAMP in lymphocytes. It is also interesting that DDA modulates the stimulatory effect of its parent compound, e.g. adenosine, which is exerted through the activation of adenosine A_2/R_a receptor on human lymphocytes [27, 28].

The inhibitory effect of DDA is probably not exerted at the level of the different membrane hormone receptors. In fact, DDA also inhibits the effect of forskolin and cholera toxin that stimulate cAMP production activating adenylate cyclase via direct interaction with the enzyme [35, 36]. Therefore, the effect of DDA on lymphocyte cAMP accumulation is probably exerted at the level of adenylate cyclase, as demonstrated in other systems [32]. Having used intact cells, we could not do biochemical studies to determine the site of interaction of DDA in the adenylate cyclase system in human leukocytes. However, studies on other systems are consistent with our results suggesting that DDA interacts with a site on the catalytic unit of adenylate cyclase that is Mn^{2+}-sensitive [39, 40].

The adenosine P-site appears to be present also on human PMNs. The effect of DDA in human PMNs is quantitatively different from that on lymphocytes. Only high concentrations of DDA (2×10^{-4} M) significantly decreased the cAMP content of human PMNs. In contrast, lower concentrations of DDA (10^{-6}–10^{-5} M), which did not modify the basal level of cAMP in PMNs, concentration-dependently suppressed the stimulating effect of PGE_1 and forskolin. Similar concentrations of DDA only partially inhibited the effects of adenylate cyclase on human lymphocytes. This is yet another difference of the modulation of the adenylate cyclase system between human lymphocytes and PMNs [29, 30, 37].

The properties of adenosine P-site have been characterized in a variety of tissues [24, 32, 39–41]. The present report describes the presence of an adenosine P-site on human inflammatory cells with properties similar to that previously described by Londos and co-workers in other systems [24, 41]. The biological significance of the P-site in human inflammatory cells is unknown. It has been demonstrated that DDA decreases the inhibition of PGE_1 of platelet aggregation induced by ADP as well as the associated increases in platelet cAMP [44, 45]. Local concentrations of adenosine can increase following hypoxia [46, 47] and bronchial challenge [48]. Furthermore, activation of mast cells and neutrophils can lead to intracellular accumulation of adenosine [49, 50]. Therefore, it is possible that, under appropriate circumstances, an increased extracellular or intracellular level of adenosine may play an immunoregulatory effect by interacting at the P-site of human leukocytes. This in turn suggests that its activation might play a role in the modulation of inflammatory reactions.

The present and preceding reports [19, 28] demonstrate that adenosine and its analogues may interact with at least one subclass of membrane receptors, A_2/R_a, and a P-site, of human leukocytes. The activation of these receptors modulates in a complex fashion the cAMP metabolism of human inflammatory cells. It has been suggested that intracellular cAMP levels are probably of physiological importance in modulating several aspects of immune responses [43, 51]. The results of this group of investigations, therefore, might suggest that adenosine, a natural nucleoside, plays a role in the control of immune response of man by modifying cAMP levels in inflammatory cells.

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