ADENOSINE RECEPTORS ON HUMAN LYMPHOCYTES

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INTRODUCTION

Evidence is accumulating that adenosine functions as an important immunoregulatory autacoid (1,2). Adenosine inhibits the mitotic response of human lymphocytes (3,4), lymphocyte-mediated cytolysis (5), superoxide anion generation by neutrophils (6), platelet aggregation (7) and mediator release from human basophils (8-10). The nucleoside has been shown to modulate a number of T lymphocyte responses (11,12). Adenosine has been implicated in the pathophysiology of patients with severe combined immune deficiency associated with a deficit of adenosine deaminase (13), in systemic lupus erythematosus (14), in bronchial asthma (15,16) and in a variety of cardiovascular diseases (17).

The mechanism underlying adenosine-induced modulation of immune responses has been the subject of considerable controversy. Among the various mechanisms postulated is an adenosine-induced alteration of cAMP metabolism in human inflammatory cells (1,4,12,18). Over the past few years adenosine receptors that modulate adenylate cyclase have been found in a variety of tissues (18,19). There are at least two subclasses of adenosine receptors that modulate either inhibition or stimulation of adenylate cyclase. For instance, the inhibitor receptor (called R1 or A1), which is more sensitive to the agonists (20-100 nM) and prefers \((-\text{N}^6\text{-}(R\text{-phenylisopropyl})\text{-adenosine \{(-)-R-PIA\} over 5\text{-N-ethylcarboxamideadenosine (NECA) (20-22), and the stimulatory receptor (R}_{A2}, which is effective over a range of 0.1-100 \mu M and prefers NECA over \((-\text{R-PIA (20-22). Both subclasses are antagonized by methylxanthines and are located on the outer cell surface (10,18,20-22). We have recently reported the presence of both adenosine A1/R1 and A2/Ra receptors on different subpopulations of human leukocytes (1,2).}

In addition to these two subclasses of adenosine receptors, an inhibitory adenosine-related P-site has also been identified (20). 2',5'-dideoxyadenosine (DDA), high concentrations of adenosine and certain modified ribose analogs of adenosine interact with the P-site (20-23). Interactions at the P-site inhibit adenylate cyclase activity and the inhibition is enhanced in the presence of Mn^{2+} (20-22). The P-site is probably located on the cytoplasmic surface of the plasma membrane. In contrast to A1/R1 and A2/Ra receptors, the P-site is not inhibited by methylxanthines (20-22).
We have recently investigated different subpopulations of human leukocytes for the presence of the P-site. The results support the hypothesis that an adenosine P-site is present on subpopulations of human lymphocytes that possess adenosine A2/Ra receptor and receptors for histamine and β-adrenergic agonists. The adenosine P-site also appears to be present on human polymorphonuclear leukocytes (PMNs).

EFFECT OF P-SITE AGONISTS ON THE LEVEL OF cAMP IN HUMAN LYMPHOCYTES

2',5'-dideoxyadenosine (DDA), 9-β-D-xylofuranosyladenosine (XFA), and 9'-β-D-arabinofuranosyladenosine (ARA) are adenosine analogs that interact with the P-site (20-22). Concentrations above 10^-5 M of DDA caused a dose-dependent decrease in the cAMP content of human lymphocytes. XFA and ARA, two P-site effectors, were less potent than DDA, as previously observed in other tissues (20-22).

EFFECT OF P-SITE AGONISTS ON THE cAMP INCREASES INDUCED BY PGE1, ISO-PROTERENOL, HISTAMINE, AND ADENOSINE

PGE1, isoproterenol, histamine and adenosine all increase lymphocyte cAMP levels presumably by interacting with a specific membrane receptor (18,23-25). Therefore, we examined the effects on lymphocyte cAMP levels of these adenylate cyclase agonists in the presence of various concentrations of DDA. DDA (10^-6 - 2 x 10^-4 M) decreased the cAMP content of human lymphocytes and blocked the stimulatory effect of PGE1. The inhibitory effect of DDA was not confined to interaction with PGE1: DDA also blocked the stimulatory effect of isoproterenol on cAMP metabolism, which requires the activation of the β-adrenergic receptor. Histamine and adenosine increased lymphocyte cAMP levels, presumably by interacting with specific H2 and A2/Ra receptors, respectively (2,18,25). DDA almost completely blocked both histamine- and adenosine-induced increases of lymphocyte cAMP levels. All experiments were preceded by a 5-min preincubation period with DDA, kinetic studies having indicated that the inhibitory effect of DDA is extremely rapid. The kinetics of inhibition of activated adenylate cyclase by DDA in lymphocytes is similar to the characteristics of the P-site in different systems (26).

The ability of P-site effectors to inhibit the effect of adenylate cyclase agonists was not confined to DDA. Interestingly enough we found that high concentrations of adenosine itself dose-dependently inhibited the effect of many adenylate cyclase agonists. Figure 1 shows that adenosine (10^-5 - 10^-3 M) also blocked the stimulatory effect of NECA on cAMP metabolism. NECA is a selective agonist of adenosine A2/Ra receptor on human lymphocytes (1,2). Therefore, the results of the experiment shown in Figure 1 indicate that high concentrations of adenosine might activate the P-site and modulate the stimulation of adenosine A2/Ra receptor. The biological significance of this remarkable observation is unknown and deserves further investigation. We also found that high concentrations of adenosine blocked the stimulatory effect of isoproterenol, histamine and PGE1 on cAMP metabolism (2).

EFFECT OF THEOPHYLLINE

Theophylline and other methylxanthines block the effect of adeno-