FUNCTIONAL AND BIOCHEMICAL EVIDENCE OF A SPECIFIC ADENOSINE A_1/R_1 RECEPTOR ON HUMAN PLATELETS

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There is now compelling evidence that activated platelets may play a role as mediators of tissue injury in such pathophysiological conditions as myocardial infarction 13, the complications of shock, such as 'shock lung' 48, 52, and allergic diseases 27, 50. Aggregation is one of the most important functions of platelets, but the mechanism of specific platelet-platelet recognition and its modulation is not clear 23. Adenosine, prostaglandin E_2, prostacyclin, prostaglandin D_2 and forskolin inhibit the aggregation of human platelets, presumably by increasing the intracellular concentration of cyclic adenosine monophosphate (cAMP) 9, 14, 19, 36.

An increase in intracellular cAMP results in a general, but not universal 80, 29, inhibitory effect on platelet functions. It thus seems that platelet responses to extracellular signals are mediated and modulated by changes in the intracellular concentration of one or more second messengers 29.

Adenosine, a natural nucleoside, has recently been implicated in the stimulus-response coupling of a variety of inflammatory cells. Adenosine receptors and/or hormone-like responses to adenosine have been reported in lymphocytes 2, 39, 40, polymorphonuclear leukocytes 7, 8, 58, basophils 34, 36, 37, 43, 44, mast cells 45, 46, 47 and macrophages 53.

A variety of adenosine analogs have been used to classify adenosine receptors into A_1/R_1 and A_2/R_2 subtypes 31. The former is a low affinity membrane receptor that activates adenylate cyclase 65, while the latter is a high affinity receptor that exerts an inhibitory effect on adenylate cyclase 30, 63. Both types of

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receptors are inhibited by methylxanthines \(^{31,36,39,40}\) and both show an absolute
dependence on the presence of guanosine triphosphate (GTP) \(^5,6\) as would be
expected for receptor-mediated modulations of adenylate cyclase \(^{54,65}\). High
affinity specific binding of adenosine analogs and antagonists has been ob-
erved in membranes from several cells \(^4,16,26\). These studies led to the charac-
terization of a number of adenosine analogs that bind with typical characteris-
tics to \(A_\gamma/R_\alpha\) or \(A_\alpha/R_\alpha\) receptors \(^{11}\). The inhibitory receptor, \(A_\gamma/R_\alpha\), is more sensi-
tive to the agonists (20-100 nM) and prefers \((-)-N^\alpha-(R-phenyl-isopropyl)-adenos-
ine \((-)-R-PIA\) to \(5^\prime-N\)-ethylcarboxamidoadenosine (NECA), whereas the stim-
ulatory receptor, \(A_\alpha/R_\alpha\), is effective over a range of 0.1-30 \(\mu\)M and prefers
NECA to \((-)-R-PIA\) \(^{11,30,65}\). Human platelet adenosine \(A_\gamma/R_\alpha\) receptors have re-
cently been characterized by radioligand binding techniques \(^{35}\).

In the present study we have shown that adenosine and its analogs inhibit
platelet aggregation and increase cAMP levels with a rank order compatible
with those of an \(A_\gamma/R_\alpha\) receptor. In addition, we found that methylxanthines
block the cAMP accumulation and the inhibition of platelet aggregation caus-
ed by adenosine.

We have drawn on the evidence presented in all the aforementioned
studies to demonstrate that adenosine and a series of its analogs inhibit plate-
let aggregation by activating a membrane receptor whose properties conform
to those of an \(A_\gamma/R_\alpha\) receptor.

MATERIALS AND METHODS

Materials - Adenosine, adenosine diphosphate (ADP), 2-chloroadenosine,
epinephrine, piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES) and theophyl-
line were purchased from Sigma Chem. Co. (St. Louis, MO). NECA was donat-
ed by Byk Gulden Italia (Milano, Italy). Erythro-9-(2-hydroxy-3-nonyl)-adenine
(EHNA) was obtained from Burroughs Wellcome and Co. (Triangle Park,
N.C.). Ro 20-1724 was provided by Dr. Herbert Sheppard, Hoffman-La-Roche,
Inc. (Nutley, N.J.). 6-nitrobenzylthioinosine (NTBI), \((-)-R-PIA\) and \((+)-N^\alpha-(S-
phenyl-isopropyl)-adenosine \((+)-S-PIA\) were purchased from Boehringer
Mannheim (Milano, Italy). 8-phenyltheophylline was obtained from Calbio-
chem-Behring Corp. (San Diego, CA). Dipyridamole was a gift from Boehrin-
ger Ingelheim Ltd. (Elmsford, N.Y.).

Buffers - The PIPES buffer used in these experiments was made up of 25
mM PIPES, 110 mM NaCl, 5 mM KCl, pH 7.35 \(^{41}\).

Blood collection - Peripheral venous blood samples were collected from nor-
mal human volunteers (age 23 to 42 years) without the application of tourni-
quet pressure \(^{34}\). The use of human volunteers was approved by the Joint Com-
mmittee on Clinical Investigation of the University of Naples, II School of Medi-
cine, and involved informed consent. None of the volunteers had taken plate-
let- or prostaglandin-active drugs in the 10 days prior to blood collection.

Platelet aggregation - Platelet-rich plasma was obtained by centrifugation
(250 \(\times\) g for 15 min at 22°C). Platelets were pelleted at 1,400 \(\times\) g for 15 min at
22°C and, after washing, resuspended in PC (PIPES buffer containing 1.0 mM
CaCl\(_2\) and 1.0 mM MgCl\(_2\)) at 3-5 \(\times\) 10^\(\text{\textdegree}\) cells/ml \(^{34}\). Platelet aggregation was
determined on a platelet aggregometer (Elvi 840). In brief, 0.25 ml of samples