Active transport of adenine and adenosine by blood platelets and cultured endothelial cells

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Blood platelets accumulate adenine and adenosine by active transport (Sixma, Holmsen & Trieschnigg, 1973; Sixma, Trieschnigg & Holmsen, 1973), and adenosine uptake is inhibited by dipyridamole (Rozenberg, Ledwidge, Wilcken & McKeon, 1971). We have tested a range of drugs as potential inhibitors of these transport processes, extended the studies to endothelial cells in culture, and developed a cytotoxicity assay based on adenine uptake.

Adenine and adenosine uptake by platelets was measured in 0.1 ml samples of human citrated platelet-rich plasma at 37°C, incubating for 3 min with 0.1 μM (8-^{3}H) adenine or for 12 min with 0.5 μM (U-^{14}C) adenosine.

Adenine transport was unaffected by a range of thio reagents with different actions: *N*-ethyl maleimide (NEM), dithiothreitol, iodoacetate, 5,5′-dithiobis (2-nitrobenzoic acid), and 2-amino (4-isothio-ureylmethylene)-thiazol dihydrochloride (Ag 307) did not significantly inhibit uptake at concentrations up to 3 mM. Adenosine transport was inhibited only by NEM (IC_{50} = 0.2 mM). Dipyridamole and its derivatives 2,6-bis(diethanolamino)4-piperidino-pyrimido-(5,4d)-pyrimidine (RA 233), 2-(2 amino-ethylamino)4-morpholinothieno-(3, 2d)-pyrimidine dihydrochloride (VK 744) and 2-piperazino-thieno-(3,2d)-pyrimidine dihydrochloride (VK 774) had little effect on adenine uptake at concentrations up to 0.3 mM, but dipyridamole and RA 233 (though not VK 744 or VK 774) inhibited adenosine transport by 80% at 50 μM. Prostaglandins D_2, E_1, E_2 and $F_{2\alpha}$ did not affect either transport process at concentrations up to 10 μM.

Porcine endothelial cells in culture (de Bono, 1974) were plated in 6 mm diameter wells (10^4 cells/well; 0.2 ml medium). Uptake of (8-^{3}H) adenine was linear for at least 30 min at 0.1 μM and 5 μM. The transport process was saturable, with values for K_m and V_{max} of approximately 0.8 μM and 2.5 nmol per 10^6 cells/h respectively. The uptake of adenine was unaffected by the presence of 20% v/v autologous heat-inactivated serum. Preliminary experiments have established that these cells also actively transport adenosine, this process being inhibited by NEM (0.1 mM), and abolished by dipyridamole (50 μM) or in the presence of 20% heat-inactivated serum.

Adenine uptake was reduced in endothelial cells damaged by antibody and complement, or by allergized lymphocytes, and this reduction correlated well both in time of onset and degree of effect with morphologically assessed cytotoxicity.

We have found that fibroblasts and smooth muscle cells in culture also transport adenine, and it seems likely that the active uptake of purine bases is an essential attribute of mammalian cells. The development of the terminal adenine uptake assay described above may therefore prove valuable as a means of assessing cytotoxicity in a wide range of cell types.

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