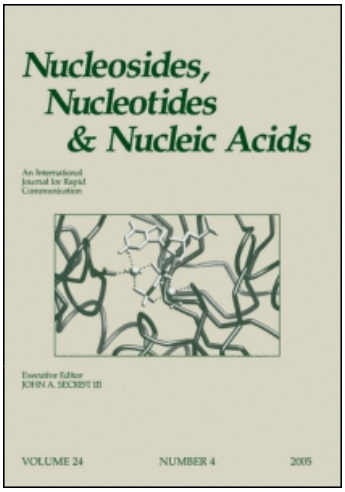

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Effect of ATP and ADP on U-937 Promonocyte Cell Adhesiveness and Intracellular Ca Levels

M. A. Ventura[a]; P. Thomopoulos[a]
[a] INSERM U-282, CRETEIL, (France)

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EFFECT OF ATP AND ADP ON U-937 PROMONOCYTE CELL ADHESIVENESS AND INTRACELLULAR CA^{++} LEVELS.

Ventura* M.A.and Thomopoulos P., INSERM U-282, Hôpital Henri Mondor, 94010 CRETEIL (France).

Abstract. In U-937 cells, Ca^{++} entry was activated by ADP or low doses of ATP, whereas intracellular Ca^{++} mobilization required high doses of ATP. The stimulation of cell adhesiveness correlated better with Ca^{++} entry than with Ca^{++} mobilization.

The adhesion of monocytes to endothelial cells is the first step in their migration through the blood vessel wall. Activated platelets and damaged cells release ATP and ADP. ATP activates phospholipase C (PLC) in polymorphonuclear cells (PMN)[1] and raises intracellular Ca^{++} concentrations ($[Ca^{++}]i$) of monocytes and PMN[2]. Other PLC-mediated effectors like PAF (platelet-activating factor), LTB_4 (leukotriene B_4) and fMLP (chemotactic peptide) stimulate monocyte adhesiveness[3]. We studied the effect of ADP and ATP on the adhesiveness and the intracellular Ca^{++} concentrations ($[Ca^{++}]i$) of the human promonocytic cell line U-937, differentiated with vitamin D (undifferentiated cells do not adhere).

To estimate adhesiveness, cells preincubated overnight with ^3H-thymidine were transfered to petri dishes and treated with ATP or analogs for 15 min. The supernatant was discarded and the radioactivity remaining in the dishes was measured. $[Ca^{++}]i$ was determined in cell populations using Fura-2 spectrofluorometry.

ADP and ATP stimulated cell adhesiveness to the same extent, but ATP was more potent,with an EC_{50} of 10^{-6} M versus 10^{-5} M for ADP (Fig 1). Adenosine deaminase potentiated the effects of ADP and ATP. Adenosine inhibits cell adhesion in this system[3] .This could explain in part the bell-shaped dose-response curve. The order of potency of the analogs tested was: ATP>ATPγS>ADP>>2-methyl-thio-ATP>ADPγS>>AMP-PCP. This rank corresponds neither to P_{2X} nor to P_{2Y} receptor types, but is similar to that found in neutrophils[4].

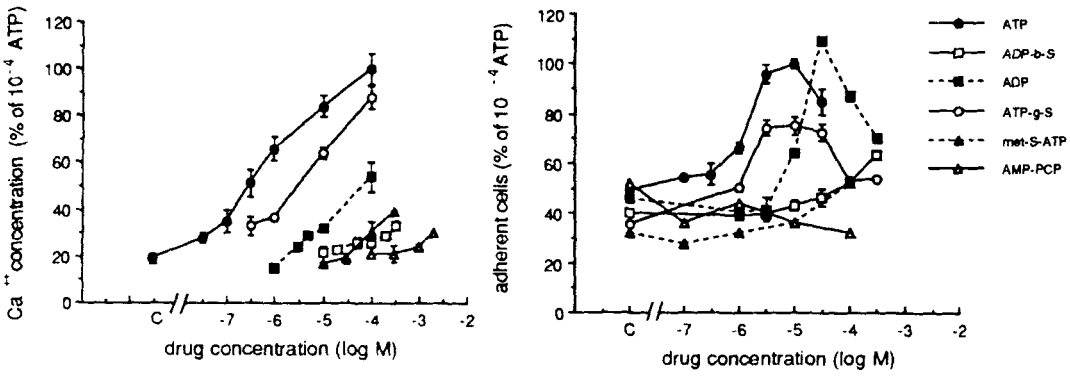

Fig-1.-Effect of P_2 purinergic agonists on cell adhesion (right) and intracellular Ca^{++} levels (left) of U-937 cells.

Both ADP and ATP raised $[Ca^{++}]i$, although the kinetics were drug- and dose-dependent. Maximal $[Ca^{++}]i$ were induced by 10^{-4} M ATP (478 nM vs 91 nM basal). None of the analogs tested induced these levels, thus precluding the calculation of EC_{50} (Fig 1). The order of potency of the analogs was similar to that obtained in adhesion experiments. ATP always induced a rapid peak, the height of which was dose-dependent (EC_{50}= $6x10^{-7}$). The peak was followed by a plateau at intermediate doses or, beyond 10^{-5} M, by a sharp fall to baseline, followed by a plateau,. The response to ADP was different: ADP raised $[Ca^{++}]i$ slowly to a plateau at a dose-dependent rate. At high doses (10^{-4} M), a peak followed by a plateau was observed. The response to ADP was inhibited in "Ca^{++}-free" medium and in the presence of 6.6 mM EGTA or 10^{-4} La^{+++}, a Ca^{++}-channel blocker. Thus, the rise in $[Ca^{++}]i$ induced by ADP showed a strict dependence on extracellular Ca^{++}. As for ATP, all these conditions shifted the dose-response curve of the peak to the right, and abolished the plateau component of the response. Thus, not only the plateau phase but, to some extent,the peak phase, both depend on extracellular Ca^{++}. Moreover, in our system, Ca^{++} entry seems to precede and to be independent of Ca^{++} mobilization from internal stores, at least for ADP and low doses of ATP. Another explanation would be that nucleotide binding requires Ca^{++}.

In conclusion: a) the P_2 purinergic receptor of U-937 promonocytic cells closely ressembles that of neutrophils, on the basis of the rank of ATP analogs, tested on cell adhesion and $[Ca^{++}]i$, b) Ca^{++} entry is activated by ADP or low doses of ATP, whereas intracellular Ca^{++}

mobilization requires high doses of ATP and c) the stimulation of cell adhesiveness correlates better with Ca^{++} entry than with Ca^{++} mobilization.

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