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嘉南藥理科技大學補助專題研究計畫成果報告

嘌呤類受體對細胞生長之影響

計畫類別：個別型計畫

計畫編號：90-PH-03

執行期間：90/1/1－90/12/31

計畫主持人：韓建華

執行單位：嘉南藥理科技大學藥學系

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1. Abstract

ADP is an adenine nucleotide that plays a central role in a number of physiological events of vascular biology. In addition to activating platelets, it is able to stimulate endothelial cells, induce phagocytosis, and cause vascular smooth muscle contraction. It is also postulated that ADP may modulate angiogenesis in diseases such as tumor growth and diabetic retinopathy. Most of these events are thought to involve cell surface receptors called P_2 receptors. Effect of ADP on mitogenesis in cardiovascular system is rarely documented and the underlying mechanisms remain to be determined. My studies demonstrated that ADP is able to induce mitogenesis in human erythroleukemia (HEL) cells.

Keywords: ADP, mitogenesis, P_2 receptor

2. Background and Significance

Adenosine nucleotides such as ADP and ATP are stored in secretory granules in platelets and upon activation can be released from these cells after activation by thrombin, collagen and other stimulants. Through binding to its receptors on plasma membrane, the secreted ADP serves as a positive feedback regulator and facilitates platelet activation, while ATP is antagonistic on ADP receptors. These nucleotides are also released from damaged vascular smooth muscles and endothelial cells and therefore have been suggested to play a role in regulating vascular tone. These two nucleotides are able to stimulate proliferation of vascular smooth muscle, implying a role of ADP in angiogenesis in vascular tissue repair or tumor progression. In addition, ADP stimulates phagocytic activity and chemotaxis of leukocytes. Thus, ADP is an important modulator in hemostasis and cardiovascular biology.

Although signaling of ADP has been studied extensively, little is known about the mitogenic effect of this nucleotide. Besides, the potential mechanisms responsible for ADP effect on proliferation, nor is the identity of receptor subtype coupled to proliferation elucidated. The studies proposed here are destined to answer these questions.

3. Results and Discussion

To test whether ADP stimulates proliferation, proliferation of HEL cells was determined by incorporation of [^{3}H]thymidine. Cells (1-2 × 10^5 cells/ml) were incubated overnight in six well plates in serum-free RPMI. Cells were then stimulated with various agents. Twenty-four hours later, cells were washed and the radioactivity associated with cells was analyzed by liquid scintillation counting. Of the agonists tested, ADP is the only agent that was able to evoke an increase of thymidine uptake to a level close to that of serum (Table 1). Thus these results indicate that ADP was able to induce mitogenesis in HEL cells.

ADP has also been shown to stimulate proliferation in aortic smooth muscle cells. However, ATP is mitogenic in smooth muscle cells, whereas it is without effect in this study. These findings imply that the receptor with which ADP interacts in HEL cells is different from that in vascular smooth muscle. Based on these findings, it may suggest that the ADP receptor in HEL cells is similar to those in platelets, i.e. P_{2Y1} and P_{2Y12} receptors. However, this hypothesis will require further studies. In addition to the receptor identity, the G proteins and the signaling mechanisms linking the ADP receptor to proliferation remains to be elucidated as well.

Table 1. Effect of various agents on DNA synthesis in HEL cells

Treatment	[^{3}H] Thymidine uptake, cpm (Mean ±S.E. or Range)	n
Control	11230 ±451	5
Fetal Bovine Serum	29516 ±4342	5
PGE_2	11857 ±2224	2
Thrombin	10966 ±883	2
ADP	25316 ±3756	5
ATP	10735 ±760	3
UTP	11178 ±1035	2

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