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嘉南藥理科技大學補助專題研究計畫成果報告

心血管細胞 ADP 受體之研究

計畫類別：個別型計畫

計畫編號：90-PH-04

執行期間：90/1/1－90/12/31

計畫主持人：吳烘

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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. Most of these events are thought to involve cell surface receptors called P_{2X} and P_{2Y} receptors. The P_{2Y} receptors are coupled to intracellular effectors via G proteins, whereas P_{2X} receptors are ligand-gated ion channels. Although earlier work has demonstrated that these receptors transduce signals via pathways such as phospholipid turnover, calcium mobilization, and inhibition of adenylyl cyclase, effect of ADP on mitogenesis in cardiovascular system is rarely documented and the underlying mechanisms remain to be determined. Using human erythroleukemia cells, the mitogenic effect of ADP was shown to be blocked by ATP. These results indicate that the receptors responsible for this phenomenon is likely to be the ADP receptors in platelets. Further studies indicate that ADP does not exert its effect via activation of ERK1/2.
Keywords: ADP, P2 receptor

2. Background and Significance

Prior to cloning of the ADP receptors, nucleotide receptors are classified as P_1 and P_2 receptors. The P_1 receptors respond to adenosine and P2 receptors interact with ATP, ADP and UTP. Radioligand binding and functional response studies have shown that ADP mediates a variety of effects on cardiovascular system and may interact with multiple receptors which some of them also respond to other nucleotides including ATP and UTP. Consistent with biochemical and pharmacological results, cloning of P_2 receptors has demonstrated that there are two types of P2 receptor: P_{2x} and P_{2Y} and both types are able to interact with ADP. The P_{2Y} receptors belong to the G protein coupled receptor superfamily, characterized by a single polypeptide with seven transmembrane domains, whereas P_{2X} receptors are ligand-gated channels for cations. Subtypes of P_{2X} and P_{2Y} receptors have been identified as well. The main differences between these receptor subtypes are characterized by the primary structure of the receptors and the binding affinity of subtype-selective ligands.

The ADP receptor in platelets, previously named as P_{2T} receptor, is unique because ATP blocks ADP-induced stimulation of phospholipase C and inhibition of cyclic AMP production. Utilization of more selective ligands in functional studies has suggested the occurrence of three subtypes of ADP receptor in platelets, i.e. P_{2X1}, P_{2Y1}, and P_{2TAC}.

The P_{2X1} receptor was first identified, followed by the cloning of P_{2Y1} receptor from platelets and human erythroleukemia cells. Although the P_{2X1} receptor induces rapid influx of Ca^{2+}, it does not involve in shape change of platelets. Studies using selective antagonists indicate that platelet aggregation is dependent on P_{2Y1} and P_{2TAC} receptors, which is coupled to stimulation of phospholipase C and inhibition of adenylyl cyclase respectively. In summary, isolation of ADP receptor subtypes and utilization of subtype-specific ligands not only confirms diversity of ADP receptors but also provides a basis accounting for various effects of ADP in cardiovascular system.

Although signaling of ADP has been studied extensively, little is known about the mitogenic effect of this nucleotide and the identity of the ADP receptors in megakaryocytic cells. 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 determine the receptor identity of ADP, I stimulated HEL cells with various agents and measured proliferation. Table 1 shows that ADP is able to evoke an increase of thymidine uptake to a level close to that of serum, indicating a functional coupling of ADP receptors with mitogenesis. In addition, it is interesting to note that ATP, although without effect by itself, blocked response of ADP. This antagonistic phenomenon was also observed on ADP-induced calcium mobilization 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 the antagonistic effect of ATP on thymidine uptake and calcium mobilization, it would suggest that the ADP receptor in HEL cells is similar to those in platelets, i.e. P_{2Y1} and P_{2Y12} receptors. Since ADP does not inhibit adenylyl cyclase in HEL cells, the P_{2Y1} receptor cloned from these cells may be responsible for ADP effect observed in this study.

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
ADP	25316 ±3756	5
ATP	10735 ±760	3
ATP+ADP	11352 ±212	2

To explore the mechanism responsible for the mitogenic effect of ADP, ERK1/2 activation was measured in HEL cells. The result indicates that ADP has no significant effect on ERK1/2 activation (Table 2). More studies are required to identify the G proteins and the signaling pathways coupled to ADP receptor.

Table 2. Effect of various agents on ERK1/2 phosphorylation in HEL cells

Treatment	Fold of Basal (Mean ±S.E.)	n
ADP	1.43 ±0.16	5
ATP	1.53 ±0.28	6
Fetal Bovine Serum*	5.73 ±1.31	3

4. References

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