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Roles of Prostanoids in the Regulation of Platelet Function

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Abstract

Prostanoids consisting of Prostaglandins (PGs) and Thromboxane (TX) exert a wide range of actions in the body through their respective receptors. Regulation of platelet function is one of the actions of prostanoids. Platelets participate critically in the pathogenesis of thrombotic diseases. Activated platelets aggregate and release various bioactive substances. Aggregation is the most notable criterion for evaluation of platelet activation. In this article, the effects of PGD_2, PGE_1, PGE_2, PGI_2 and TXA_2 on platelet aggregation are reviewed.

Keywords: Prostaglandin; Thromboxane; Platelet Aggregation; Hemostasis; Thrombosis

Abbreviations

ADP: Adenosine Diphosphate; cAMP: Cyclic Adenosine Monophosphate; CRTH2: Chemoattractant Receptor-Homologous Molecule Expressed on Th2 Cells; DP: Prostaglandin D_2 Receptor; EP: Prostaglandin E_2 Receptor; FP: Prostaglandin $F_2\alpha$ receptor; IP: Prostaglandin I_2 Receptor; PG: Prostaglandin; PLC: Phospholipase C; TP: Thromboxane A_2 receptor; TX: Thromboxane

Introduction

Platelets are involved not only in hemostasis but also in pathological thrombus formation. Activated platelets achieve their roles by both aggregating and releasing various bioactive substances such as growth factors, lysophospholipids and chemokines [1-3]. Accordingly, platelets play a critical role in several pathological conditions such as atherosclerosis, cerebral thrombosis and myocardial infarction [4-6].

Prostanoids consisting of Prostaglandins (PGs) and Thromboxane (TX) are lipid mediators that bind to cognate receptors named DP, EP, FP, IP and TP that are specific for PGD_2, PGE_2, $PGF_2\alpha$, PGI_2 (prostacyclin) and TXA_2, respectively [7]. There are four subtypes of EP: EP_1, EP_2, EP_3 and EP_4 [8-11]. In these four subtypes, EP_3 is unique and has several isoforms derived from alternative splicing [12,13]. In addition to these eight types and subtypes of prostanoid receptors, a novel PGD_2 receptor that has been isolated from type 2 T helper cells and named CRTH2 (DP_2) has no significant sequence homology of amino acids with DP (DP_1) and other prostanoid receptors [14].

Prostanoids exert a variety of actions in various tissues and cells [15] *via* their respective receptors. Regulation of platelet function is one of the most well-known actions of prostanoids [16,17].

Expression of prostanoid receptors in platelets

Several prostanoid receptors have been reported to be expressed in platelets. EP_2, EP_3, EP_4, IP and TP were shown to be expressed in murine platelets [18], and human platelets were shown to express DP1 along with EP_2, EP_3, EP_4, IP and TP [19,20].

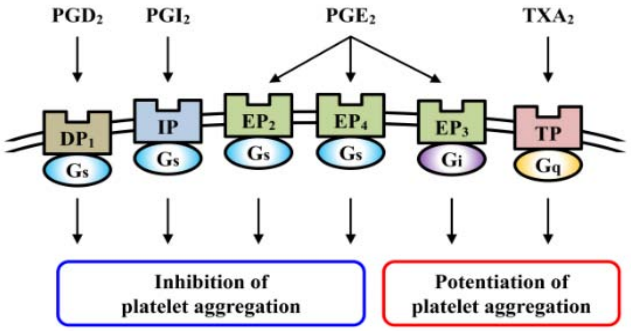

Figure 1: Effects of prostanoids on platelet aggregation.
Prostanoids play a role in the regulation of platelet aggregation *via* respective receptors. EP_3 and TP are stimulatory receptors, whereas DP_1, EP_2, EP_4 and IP are inhibitory receptors in aggregation.

Stimulatory effect of TXA_2 on platelet aggregation

TXA_2 is well known as a potent stimulator of platelets [4]. TP couples to G_q and activates phospholipase C (PLC), leading to elevation of intracellular Ca^{2+} concentrations. In human and rabbit platelets, a stable TXA_2 mimetic induced platelet aggregation and release of granule contents from platelets [21]. Platelets express TP constitutively and produce TXA_2 when activated with collagen, Adenosine Diphosphate (ADP), epinephrine, thrombin and TXA_2.

Therefore, TXA_2 plays an important role in the regulation of platelet function, working as a positive feedback regulator. In mice lacking TP, bleeding time was significantly prolonged compared with that in wild-type mice [22], suggesting that TXA_2 plays an important role in hemostasis.

Inhibitory effect of PGI_2 on platelet aggregation

In contrast to TXA_2, PGI_2 efficiently inhibits platelet aggregation [17]. The inhibitory potency of PGI_2 in platelet aggregation is higher than that of the other inhibitory prostanoids, PGD_2 and PGE_1 [23]. IP couples to G_s and increases intracellular Cyclic Adenosine Monophosphate (cAMP) concentrations, leading to activation of

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Table 1: Prostanoid receptor types and subtypes.

Prostanoid	Type	Subtype	G-protein	Main signal
PGD_2	DP (DP_1)		G_s	cAMP ↑
	CRTH2 (DP_2)		G_i	cAMP ↓
PGE_2	EP	EP_1	G_q	PLC ↑
		EP_2	G_s	cAMP ↑
		EP_3	G_i	cAMP ↓
		EP_4	G_s	cAMP ↑
$PGF_2\alpha$	FP		G_q	PLC ↑
PGI_2	IP		G_s	cAMP ↑
TXA_2	TP		G_q	PLC ↑

cAMP: Cyclic Adenosine Monophosphate; CRTH2: Chemoattractant Receptor-Homologous Molecule Expressed on Th2 Cells; DP: Prostaglandin D_2 Receptor; EP: Prostaglandin E_2 Receptor; FP: Prostaglandin $F_2\alpha$ Receptor; IP: Prostaglandin I_2 receptor; PG: Prostaglandin; PLC: Phospholipase C; TP: Thromboxane A_2 Receptor; TX: Thromboxane.

protein kinase A and inhibition of platelet aggregation. Bleeding time in mice lacking IP was not different from that in wild-type mice, but the susceptibility of mice lacking IP to thrombosis was increased, suggesting that PGI_2 is vital for the prevention of thrombus formation [24].

Inhibitory effect of PGD_2 on platelet aggregation

In addition to PGI_2, PGD_2 is also known as an inhibitor of platelet aggregation [25]. The inhibitory effect of PGD_2 on aggregation is observed in human and rabbit platelets but not in murine platelets due to the presence or absence of DP_1 coupling to G_s. In human platelets, the inhibitory potency of PGD_2 was two-times higher than that of PGE_1 but much less than that of PGI_2 [23,25].

Inhibitory effect of PGE_1 on platelet aggregation

Previous studies showed that PGE_1 stimulates cAMP synthesis and inhibits platelet aggregation [26,27]. In human platelets, PGE_1 can bind to IP as well as EPs [28]. The rank order of affinity of PGE_1 for murine EPs and IP was $EP_3 > EP_4 > EP_2 > EP_1$, IP [29]. However, the inhibitory effect of PGE_1 on human platelet aggregation was blocked by an IP antagonist but not by an EP_4 antagonist [30], suggesting that PGE_1 inhibits platelet aggregation *via* IP but not EP_4, the role of which will be described below.

Biphasic effect of PGE_2 on platelet aggregation

PGE_2 has been reported to have a biphasic effect on platelet aggregation; PGE_2 potentiates the aggregation at lower concentrations and inhibits it at higher concentrations [31,32]. However, PGE_2 alone could not induce platelet aggregation [18]. It has been thought that G_i- and G_q-mediated signaling activates platelets and that G_s-mediated signaling inhibits platelet activation. Accordingly, among the EP subtypes expressed in platelets, EP_3 (mainly G_i) is regarded as a stimulatory receptor, whereas EP_2 (G_s) and EP_4 (G_s) are regarded as inhibitory receptors in aggregation. Furthermore, EP_4 signaling has been reported to activate phosphatidylinositol 3-kinase, leading to activation of protein kinase B (Akt) [33].

Potentiating effect of PGE_2 at lower concentrations on platelet aggregation: First, the role of EP_3 in the regulation of platelet function was examined because the expression level of EP_3 mRNA was the highest among EP subtypes in platelets. In murine platelets lacking EP_3, the potentiating effect of PGE_2 at lower concentrations on platelet aggregation completely disappeared. In platelets prepared from wild-type mice, a specific EP_3 agonist enhanced aggregation induced by a TP agonist in a concentration-dependent manner [18]. These results indicate that EP_3 is involved in the potentiating effect of PGE_2 on platelet aggregation. In agreement with the potentiating effect of PGE_2 *via* EP_3, the bleeding time was significantly prolonged and the mortality after induction of arachidonic acid-induced acute thromboembolism was remarkably reduced in mice lacking EP_3 compared with those in wild-type mice. Moreover, the formation of thrombi in pulmonary arterioles and alveolar hemorrhage observed after injection of arachidonic acid were alleviated in mice lacking EP_3 [18]. Similarly, venous thrombosis induced by periadventitial application of arachidonic acid was almost completely abolished, although the bleeding time was not significantly prolonged in mice lacking EP_3 [34]. Furthermore, a previous study showed that atherosclerotic plaque-produced PGE_2 activated platelets through EP_3 and promoted atherothrombosis when the plaque was mechanically ruptured [35]. These results indicate that PGE_2 plays an important role in thromboembolism through activation of platelets *via* EP_3.

Inhibitory effect of PGE_2 at higher concentrations on platelet aggregation: It has been suggested that the inhibitory effect of PGE_2 on platelet aggregation is mediated by IP [36,37]. In fact, the inhibitory effect of PGE_2 was significantly blunted but was not entirely abolished in murine platelets lacking IP [38]. Meanwhile, specific agonists for EP_2 or EP_4 potently inhibited aggregation of murine and human platelets [38-41]. These results suggest that selective activation of EP_2 or EP_4 leads to inhibition of platelet aggregation. It is noteworthy that the inhibitory potency of an EP_4 agonist was two rank orders higher than that of an EP_2 agonist and was as high as that of an IP agonist in human platelets [38].

Conclusion

Anti-platelet agents having various mechanisms of action have been developed and used to prevent the recurrence of thrombotic diseases such as cerebral thrombosis and myocardial infarction, which have been major causes of death in developed countries [42,43]. The targets of these agents including aspirin, prasugrel and cilostazol are cyclooxygenase, ADP receptor $P2Y_{12}$ and phosphodiesterase, respectively. Although an IP agonist (PGI_2 or PGE_1 analogue) and a TX synthase inhibitor have been used for anti-platelet therapy, there are still no anti-platelet agents targeting EPs. Previous studies showed roles of EP_3 in thromboembolism [18,34,35] and higher inhibitory potency of an EP_4 agonist in platelet aggregation [38], suggesting a potential of EP_3 antagonists and EP_4 agonists as novel anti-platelet agents [41,44,45].

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