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The platelet P2 receptors in arterial thrombosis

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Abstract

ADP and ATP play a crucial role in hemostasis and thrombosis and their receptors are potential targets for antithrombotic drugs. The ATP-gated channel $P2X_1$ and the two G protein-coupled $P2Y_1$ and $P2Y_{12}$ ADP receptors selectively contribute to platelet aggregation. Due to its central role in the formation and stabilization of a thrombus, the $P2Y_{12}$ receptor is a well established target of antithrombotic drugs like clopidogrel which has proved efficacious in many clinical trials and experimental models of thrombosis. Competitive $P2Y_{12}$ antagonists have also been shown to be effective in experimental thrombosis as well as in several clinical trials. Studies in $P2Y_1$ and $P2X_1$ knock-out mice and experimental thrombosis models using selective $P2Y_1$ and $P2X_1$ antagonists have shown that, depending on the conditions, these receptors could also be potential targets for new antithrombotic drugs. Since both $P2X_1$ and $P2Y_1$ receptor inhibition result in milder prolongation of the bleeding time as compared to $P2Y_{12}$ inhibition, the idea is put forward that combination of P2 receptor antagonists could improve efficacy with diminished hemorrhagic risk. However, further studies are required to validate such a point of view.

Keywords: Haemostasis; Thrombosis; ADP; ATP; $P2Y_1$; $P2Y_{12}$; $P2X_1$; Antiplatelet drugs; Clopidogrel

Introduction

Forty-five years ago, ADP was identified as a factor derived from erythrocytes which influenced platelet adhesion to glass [1] and induced platelet aggregation [2]. Its presence in large amounts in platelets and its crucial roles in the physiological process of hemostasis and in the development and extension of arterial thrombosis were rapidly recognized [3,4], but the molecular identity of its receptors remained for a long time elusive. These receptors belong to the P2 family which consists of two classes of membrane receptors: P2X ligand-gated cation channels and G-protein-coupled P2Y receptors [5]. Starting from the concept of a unique P2T receptor (T for thrombocyte) originally postulated on the basis of pharmacological data [6], a model of three platelet P2 receptors progressively emerged [7]. These are the $P2X_1$ cation channel which is activated by ATP and two G-protein-coupled receptors, $P2Y_1$ and $P2Y_{12}$, both activated by ADP (Fig. 1).

The three platelet P2 receptors

The $P2Y_1$ receptor is widely distributed in many tissues including heart, blood vessels, smooth muscle cells, neural tissue, testis, prostate, and ovary. ADP is its preferred natural agonist, while ATP behaves as an antagonist in platelets [8]. About 150 $P2Y_1$ receptor binding sites are expressed per platelet [9], which is very low as compared for instance to the TP receptors or the thrombin receptor PAR-1 (1000 to 2000 sites/platelet). As it is coupled to Gαq, the $P2Y_1$ receptor triggers the mobilization of calcium from internal stores, which results in platelet shape change and weak, transient aggregation in response to ADP. Overall, the $P2Y_1$ receptor is a crucial factor in the initiation of the platelet activation induced by ADP or collagen [10,11].

The $P2Y_{12}$ receptor, despite its being well known and characterized on the basis of both pharmacological and genetic evidence, was the last to be cloned [12]. This receptor is deficient in patients with selective defects of ADP-induced platelet aggregation and is also the molecular target of the antiplatelet drug clopidogrel [13,14]. Its tissue distribution is very limited, although not entirely restricted to platelets as it is

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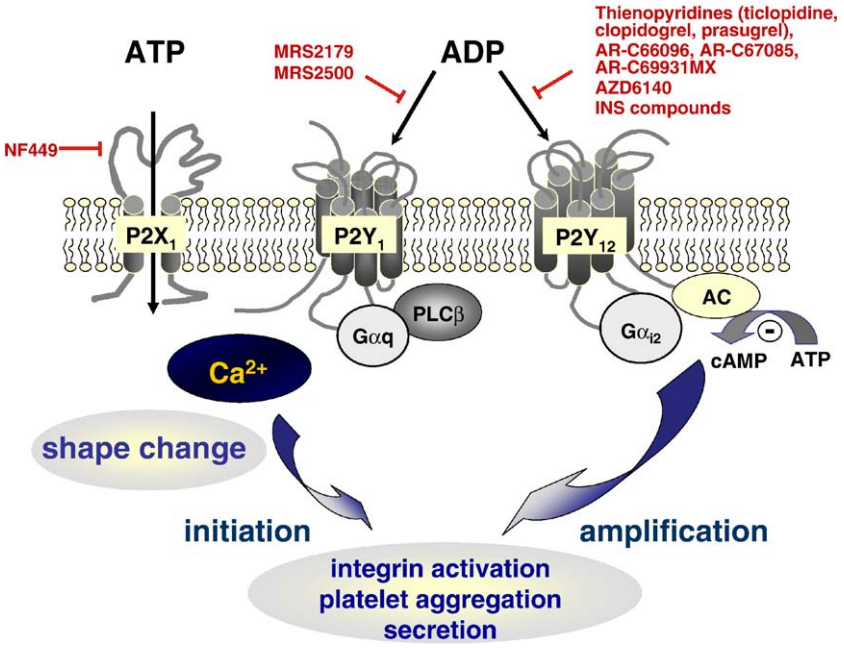

Fig. 1. Current model of three platelet P2 receptors: the $P2X_1$ receptor is responsible for rapid calcium influx and platelet shape change in response to ATP and contributes to the platelet activation induced by low concentrations of collagen. The $P2Y_1$ and $P2Y_{12}$ receptors are essential for normal aggregation in response to ADP: the Gq-coupled $P2Y_1$ receptor is responsible for intracellular calcium mobilization, shape change, and initiation of aggregation and the G_i-coupled $P2Y_{12}$ receptor for completion of the ADP-induced aggregation response and for potentiation of the aggregation and secretion induced by other agents through various intracellular pathways. Selective antagonists allow discrimination of the roles of the three receptors. $P2Y_{12}$ is the target of the antithrombotic drugs ticlopidine and clopidogrel while $P2Y_1$ and $P2X_1$ are potential targets for new antiplatelet compounds.

also present in brain, endothelial cells, glial cells, and smooth muscle cells [15]. Platelets express approximately 500 to 800 $P2Y_{12}$ receptors [9]. ADP is the natural agonist of this receptor, while ATP and its triphosphate analogues are antagonists [16,17]. The $P2Y_{12}$ receptor is coupled to $G\alpha_{i2}$ and is responsible for completion of the platelet aggregation response to ADP. It plays a central role in amplification of the aggregation induced by all known platelet agonists whatever their signaling pathway, including collagen, thrombin, immune complexes, TXA_2, adrenaline, and serotonin [18,19]. The $P2Y_{12}$ receptor is also involved in the potentiation of platelet secretion [20]. All these features make this receptor a pivotal factor in sustaining platelet aggregation and hence in promoting thrombus growth and stabilization.

Co-activation of the $P2Y_1$ and $P2Y_{12}$ receptors is necessary for normal ADP-induced platelet aggregation [21–23]. They are differentially involved in the procoagulant activity of platelets. While both receptors are indirectly involved through their role in platelet P-selectin exposure and in the formation of platelet–leukocyte conjugates leading to leukocyte tissue factor exposure [24,25], the $P2Y_{12}$ receptor is also directly implicated in the exposure of phosphatidylserine at the surface of platelets [24,26,27].

The third component of the platelet P2 receptors is $P2X_1$, the ligand-gated cation channel responsible for the fast calcium entry induced by ATP [28]. One characteristic of the $P2X_1$ receptor is that it is very quickly desensitized, which hampers study of its function in platelet activation in vitro. However, when desensitization is prevented by addition of a high concentration of apyrase (ATP-diphosphohydrolase E. C.3.6.1.5.), the selective $P2X_1$ agonist alpha, beta-methylene-ATP ($\alpha\beta$MeATP) induces a rapid calcium influx accompanied by a transient shape change in human platelets [29]. Although unable to trigger platelet aggregation by itself, the $P2X_1$ receptor has been shown to participate in collagen- and shear-induced aggregation [30–32]. Synergistic interplay between $P2X_1$ and $P2Y_1$ has been reported, suggesting that $P2X_1$ might act as a primer in the subsequent activation of $P2Y_1$ [33,34].

The platelet P2 receptors as targets for antithrombotic drugs

The $P2Y_{12}$ receptor

Long before its molecular cloning, the pharmacological importance of this receptor in hemostasis and thrombosis was well recognized. This was due to the fact that the potent antithrombotic thienopyridine compounds ticlopidine and clopidogrel, of which an active liver metabolite selectively targets the $P2Y_{12}$ receptor [12,35], were used as molecular tools to characterize platelet responses to ADP and the role of the latter in thrombosis [4,13]. Conversely, patients with a congenital defect of ADP-induced platelet aggregation were shown to display a “clopidogrel-like” syndrome and later found to carry $P2Y_{12}$ mutations [12,14,36]. Clopidogrel treatment leads to inhibition of platelet aggregation in response to ADP with conserved shape change but blockade of the ability of ADP to inhibit cyclic AMP production. Platelet aggregation in response to other agents is also affected through the effect on