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The platelet P2 receptors as molecular targets for old and new antiplatelet drugs

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Abstract

Platelet activation by ADP and ATP plays a crucial role in haemostasis and thrombosis, and their so-called P2 receptors are potential targets for antithrombotic drugs. The ATP-gated channel $P2X_1$ and the 2 G protein-coupled $P2Y_1$ and $P2Y_{12}$ ADP receptors selectively contribute to platelet aggregation. The $P2Y_1$ receptor is responsible for ADP-induced shape change and weak and transient aggregation, while the $P2Y_{12}$ receptor is responsible for the completion and amplification of the response to ADP and to all platelet agonists, including thromboxane A_2 (TXA_2), thrombin, and collagen. The $P2X_1$ receptor is involved in platelet shape change and in activation by collagen under shear conditions. 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 ticlopidine or clopidogrel, which have proved efficacy 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 $P2X_1$ knockout 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.

Keywords: ADP; ATP; P2Y; P2X; Haemostasis; Thrombosis

Contents

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1. Introduction

The main role of blood platelets is haemostasis. Under normal conditions, platelets circulate freely in the blood and do not adhere to each other. Following vessel wall injury, platelets adhere to the exposed subendothelium and become activated. Platelet activation includes a series of rapid positive feedback loops such as synthesis of TXA_2 or release of nucleotides, which greatly amplify the activation signals and enable robust platelet recruitment at the site of vascular injury leading to the formation of the so-called haemostatic plug. The same mechanisms are involved when platelets are activated at the site of an atherosclerotic plaque rupture, leading to vessel occlusion and, depending on the vascular bed involved, ischemic diseases such as myocardial infarction, stroke, or peripheral artery disease (Ruggeri, 2002). Forty years ago, ADP was identified as a factor released from erythrocytes, which influenced platelet adhesiveness to glass (Hellem, 1960; Gaarder et al., 1961) and induced platelet aggregation (Ollgard, 1961; Born, 1962). The crucial role of ADP as a mediator of platelet activation was rapidly recognized in the physiological process of haemostasis and in the development and extension of arterial thrombosis (Born, 1985; Maffrand et al., 1988). Adenine nucleotides are present at very high concentrations in platelet dense granules and are released when platelets are exposed to thrombin, collagen, or thromboxane A_2 (TXA_2), thus reinforcing their aggregation (Kinlough-Rathbone et al., 1977; Reimers, 1985). Inhibitors of ADP-induced platelet aggregation are effective antithrombotic drugs in animal models and in clinical trials (Cusack & Hourani, 2000; Humphries, 2000; Herbert & Savi, 2003). ADP removing enzymes are antithrombotic in experimental models (Zawilska et al., 1982; Enjyoji et al., 1999; Marcus et al., 2003), while patients with defects of ADP receptors or lacking ADP in their platelet dense granules experience bleeding (Cattaneo & Gachet, 1999; Cattaneo, 2005).

Direct stimulation of platelets by ADP results in shape change, reversible aggregation at physiological concentrations of calcium, and finally, desensitization. Transduction of the ADP signal involves a transient rise in free cytoplasmic calcium, due to the mobilization of internal stores and secondary store-mediated influx, and a concomitant inhibition of adenylyl cyclase activity. ATP induces an extremely rapid influx of calcium from the extracellular medium associated to platelet shape change (Gachet, 2001; Rolf et al., 2001; Mahaut-Smith et al., 2004). Since the time where ADP was recognized as a key agonist of platelets, its receptors have been identified, and the role of each of the nucleotide receptors, or P2 receptors, in thrombosis could now be described. The P2 receptor family consists of 2 classes of membrane receptors: P2X ligand-gated cation channels and G protein-coupled P2Y receptors (Ralevic & Burnstock, 1998). To date, 7 subtypes of mammalian P2X receptor ($P2X_{1-7}$; Khakh et al., 2001) and 8 subtypes of P2Y receptor ($P2Y_{1, 2, 4, 6, 11, 12, 13, 14}$; Communi et al., 2000; Shaver, 2001; Abbracchio et al., 2003) have been cloned and characterized. Other sequences have been tentatively included in the P2Y family, which are, in fact, species orthologues ($P2Y_3$, $P2Y_5$) or which have been found eventually not to be genuine P2Y receptors ($P2Y_7$, $P2Y_{15}$; Abbracchio et al., 2005). Platelets express 3 separate nucleotide receptors. These are the $P2X_1$ cation channels that is activated by ATP and 2 G protein-coupled receptors, $P2Y_1$ and $P2Y_{12}$, both activated by ADP. Each of these receptors has a selective role during platelet activation (Hechler, Cattaneo, & Gachet, 2005), which should have implications for their role in thrombosis (Gachet & Hechler, 2005).

2. The respective roles of the P2 receptors in platelet function

2.1. The $P2Y_1$ receptor initiates platelet activation and aggregation

The presence and the role of the $P2Y_1$ receptor in platelets, initially suggested by the detection of $P2Y_1$ mRNA in both megakaryoblastic cells and platelets (Léon et al., 1997), was confirmed by pharmacological studies using selective $P2Y_1$ antagonists (Léon et al., 1997; Hechler et al., 1998b) and by studies of $P2Y_1$ receptor-deficient mice (Fabre et al., 1999; Léon et al., 1999a). The human $P2Y_1$ receptor contains 373 amino acid residues, has a classical structure of a G protein-coupled receptor, and is widely distributed in many tissues, including the heart, blood vessels, smooth muscle cells, neural tissue, testis, prostate, and ovary (Léon et al., 1996; Ralevic & Burnstock, 1998; Communi et al., 2000). ADP is the preferred natural agonist of the $P2Y_1$ receptor, while ATP behaves as an antagonist in platelets (Léon et al., 1997; Hechler et al., 1998c) or as a poor partial agonist in heterologous transfected or reconstituted systems, depending on the receptor density (Palmer et al., 1998; Waldo & Harden, 2004). Around 150 $P2Y_1$ receptor binding sites are expressed per platelet (Baurand et al., 2001), which is very low as compared, for instance, with the thromboxane prostanoid (TP) receptors or to the thrombin receptor PAR-1 (1000 to 2000 receptors/platelet). Several selective antagonists of this receptor have been described, namely, A2P5P, A3P5P, or A3P5PS (Boyer et al., 1996) and, more recently, MRS2179 (Boyer et al., 1998; Baurand et al., 2001; Baurand & Gachet, 2003), MRS2279 (Boyer et al., 2002), or MRS2500 (Cattaneo et al., 2004), which constitute valuable tools to investigate the role of the $P2Y_1$ receptor in platelet function (see Table 1). This receptor, coupled to Gαq, triggers calcium mobilization from internal stores, which results in platelet shape change and weak and transient aggregation in response to ADP (Hechler et al., 1998a, 1998b; Savi et al., 1998). In addition to its key role in the initiation of platelet activation by ADP, the $P2Y_1$ receptor participates to aggregation induced by