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The P2Y$_1$ Receptor Antagonist Adenosine-2',5'-Diphosphate Non-selectively Antagonizes the Platelet P2X$_1$ Ion Channel

Dear Sir,

Extracellular adenine nucleotides regulate a broad range of physiological cellular responses through G-protein-coupled P2Y receptors and ligand-gated P2X ion channels, operating on different scales of time and distances (1). Platelet P2 receptors have been identified to consist of three representatives, namely P2Y$_1$, P2Y$_{12}$, and P2X$_1$ (2).

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ADP, acting at P2Y$_1$ and P2Y$_{12}$, has been recognized as an important mediator of primary haemostasis (2). Coactivation of both P2Y$_1$ and P2Y$_{12}$ is necessary for normal ADP-induced platelet aggregation. The Gq protein-coupled P2Y$_1$ receptor is responsible for platelet shape change through mobilization of Ca^{2+} from intracellular stores and for the initiation of platelet aggregation, whereas P2Y$_{12}$, targeted by specific antithrombotic drugs, leads to adenylate cyclase inhibition through a Gi protein, completing and amplifying platelet responses to ADP.

In contrast, ATP has commonly been regarded as a platelet inhibitor due to its antagonistic action at the platelet P2Y receptors for ADP. However, because ATP, which is co-released with ADP from platelet dense granules during platelet activation, activates the P2X$_1$ ion channel allowing entry of extracellular Ca^{2+} (3), the purely antagonistic
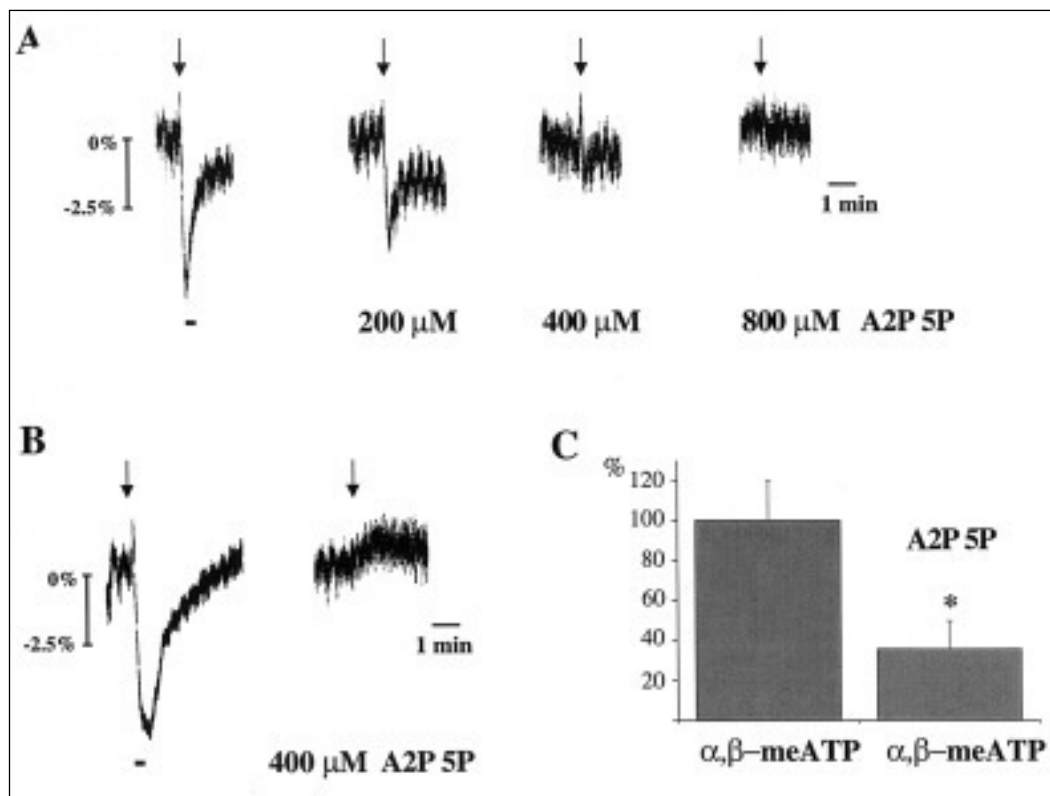

Fig. 1 Light transmission recordings in hirudinized PRP containing 1U/ml apyrase. Platelet shape change was induced by 0.5 μM HPLC-purified α,β-meATP (A) or 1 μM HPLC-purified ADP (B), in the presence of increasing concentrations of A2P5P, as indicated. Arrows show the time of addition of agonists. Percentage light transmission as well as time bars are specified. The curves are representative of at least 3 independent experiments performed on different individuals. Aggregation is prevented by the simultaneous presence of the neutralizing monoclonal anti-GPIIb/IIIa antibody MA-16N7C2 (50 μg/ml) (11) C. Averaged peak currents measured in P2X$_1$-expressing *Xenopus* oocytes stimulated with 100 μM α,β-meATP (n = 13) alone or in the presence of 400 μM A2P5P (n = 11). Data are represented as the mean ± SEM and are expressed as percentage of the peak current induced by α,β-meATP within the same batch of oocytes (*: p< 0.05)

view of ATP in haemostasis has been questioned. Recent findings indeed indicate that $P2X_1$ mediates reversible platelet shape change (4), but also is involved in platelet shape change and aggregation induced by collagen (5). The concept of P2 receptor activation in human platelets would thus rely both on ATP-mediated fast signaling through the $P2X_1$ channel, and on ADP activation of the $P2Y_1$ and $P2Y_{12}$ receptors.

Pharmacological classification of P2Y and P2X receptors and unambigous definition of their physiological roles have long been problematic due to lack of selective receptor antagonists. Although selective antagonists now exist that discriminate between $P2Y_1$ and $P2Y_{12}$, selective blockade of platelet $P2X_1$ is still complicated. Notably, the $P2Y_1$ antagonist, N^6-methyl-2'-deoxyadenosine-3',5'-biphosphate (MRS 2179) (6), acting as an antiplatelet agent, has also been reported to be an antagonist of $P2X_1$ *in vitro* (7). The adenine nucleotide derivatives, adenosine 3'-phosphate 5'-phosphosulfate (A3P5PS), adenosine 3',5'-diphosphate (A3P5P), and adenosine 2',5'-diphosphate (A2P5P) are competitive antagonists at the human $P2Y_1$ receptor stably expressed in 1321N1 astrocytoma cells (8). Because these compounds are inactive at Gi protein-coupled P2Y receptors, they are widely used for the analysis of the Gq protein-coupled $P2Y_1$ receptor function in platelet activation without affecting $P2Y_{12}$ (2). For instance, the involvement of $P2Y_1$ in the initiation of platelet aggregation elicited by low concentrations of U46619 or thrombin, known to depend on ADP release, has been demonstrated through the inhibitory effect of A2P5P (9). However, whether these molecules affect the platelet $P2X_1$ receptor function is unknown.

We have investigated the effect of A2P5P on the quickly reversible platelet shape change induced by the selective $P2X_1$ agonist, α,β-methylene adenosine 5'-triphosphate (α,β-meATP). It appeared that a 1 min-pre-incubation of the platelets with A2P5P dose-dependently inhibited platelet shape change evoked by 0.5 μM α,β-meATP, inhibition being almost complete at 400 μM A2P5P (Fig. 1A). This concentration of A2P5P fully prevents the ADP-induced platelet shape change through $P2Y_1$ (Fig. 1B). Accordingly, upon heterologous expression of $P2X_1$ in *Xenopus* oocytes (10), 400 μM A2P5P could reduce the peak amplitude current evoked by 100 μM α,β-meATP to $35,74 \pm 14$ % (p = 0.019) of its initial value (mean peak currents = 0.805 $\pm$ 0.162 μA, n = 13 *versus* 0.2877 $\pm$ 0.1129 μA, n = 11 in the presence of A2P5P) (Fig. 1C). These data thus indicate that the $P2Y_1$ antagonist A2P5P, previously described to be specific, exhibits non-selective antagonism at recombinant and human platelet $P2X_1$ receptors.

The non-selective inhibitory effect of A2P5P on $P2X_1$ activity has probably hampered correct evaluation of the contribution of the $P2X_1$ ion channel to platelet activation by many authors. This phenomenon, added to the difficulties to preserve fast-desensitizing $P2X_1$ responses during platelet preparation, may partly explain why these authors have often considered the role of this receptor in platelets to be elusive.

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Genetic Polymorphisms of Angiotensin Converting Enzyme (I/D) and Endothelial Nitric Oxide Synthase (T(-788)C) Genes in Japanese Patients with Myocardial Infarction

Dear Sir,

Genetic polymorphisms of angiotensin converting enzyme (I/D) (1, 2) and endothelial nitric oxide synthase (T(-788)C) (3, 4) have

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been proposed as potential genetic factor for myocardial infarction (MI).

Despite numerous studies, the roles of these polymorphisms as a clinically relevant, inherited risk factor for MI are still controversial. The purpose of the present study is to assess the significance of these polymorphisms by employing a large epidemiological cohort as a control population. The study population consisted of 454 subjects with MI recruited from the National Cardiovascular Center, and 3918 subjects