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<sub>1</sub>, P2Y<sub>12</sub>, and P2X<sub>1</sub> (2).

ADP, acting at P2Y<sub>1</sub> and P2Y<sub>12</sub>, has been recognized as an important mediator of primary haemostasis (2). Coactivation of both P2Y<sub>1</sub> and P2Y<sub>12</sub> is necessary for normal ADP-induced platelet aggregation. The Gq protein-coupled P2Y<sub>1</sub> receptor is responsible for platelet shape change through mobilization of Ca<sup>2+</sup> from intracellular stores and for the initiation of platelet aggregation, whereas P2Y<sub>12</sub>, 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<sub>1</sub> ion channel allowing entry of extracellular Ca<sup>2+</sup> (3), the purely antagonistic

Thromb Haemost 2001; 86: 1338–9

**The P2Y<sub>1</sub> Receptor Antagonist Adenosine-2’,5’-Diphosphate Non-selectively Antagonizes the Platelet P2X<sub>1</sub> Ion Channel**


Received June 18, 2001 Accepted June 26, 2001

---

**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<sub>1</sub>-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 indicate that P2X1 mediates reversible platelet shape change (4), but is also 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 P2X1 channel, and on ADP activation of the P2Y1 and P2Y12 receptors.

Pharmacological classification of P2Y and P2X receptors and unambiguous definition of their physiological roles have long been problematic due to lack of selective receptor antagonists. Although selective antagonists now exist that discriminate between P2Y1 and P2Y12, selective blockade of platelet P2X1 is still controversial. Notably, the P2Y1 antagonist, Nα-methyl-2'-deoxynoadenosine-3',5'-biphosphate (MRS 2179) (6), acting as an antiplatelet agent, has also been reported to be an antagonist of P2X1, 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 P2Y1 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 P2Y1 receptor function in platelet activation without affecting P2Y12 (2). For instance, the involvement of P2Y1, 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 P2X1 receptor function is unknown.

We have investigated the effect of A2P5P on the quickly reversible platelet shape change induced by the selective P2X1 agonist, α,β-methylene adenosine 5'-triphosphate (α,β-meATP). It appeared that a 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 P2Y1 (Fig. 1B). Accordingly, upon heterologous expression of P2X1 in Xenopus oocytes (10), 400 μM A2P5P could reduce the peak amplitude current evoked by 100 μM α,β-meATP to 35.74 ± 14 % (p = 0.019) of its initial value (mean peak currents = 0.805 ± 0.162 μA, n = 13 versus 0.2877 ± 0.1129 μA, n = 11 in the absence of A2P5P) (Fig. 1C). These data thus indicate that the P2Y1 antagonist A2P5P, previously described to be specific, exhibits non-selective antagonistic activity at recombinant and human platelet P2X1 receptors.

The non-selective inhibitory effect of A2P5P on P2X1 activity has probably hampered correct evaluation of the contribution of the P2X1 ion channel to platelet activation by many authors. This phenomenon, added to the difficulties to preserve fast-desensitizing P2X1 responses during platelet preparation, may partly explain why these authors have often considered the role of this receptor in platelets to be elusive.

E. Toth-Zsamboki1, C. Oury, J. Tytgat1, J. Vermeylen, M. F. Hoyaerts Center for Molecular and Vascular Biology, University of Leuven, Leuven, Belgium; 1Laboratory of Toxicology, University of Leuven, Belgium

References

Received June 25, 2001 Accepte June 26, 2001

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 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...