Platelets From a Patient Heterozygous for the Defect of P2\textsubscript{CYC} Receptors for ADP Have a Secretion Defect Despite Normal Thromboxane A\textsubscript{2} Production and Normal Granule Stores

Further Evidence That Some Cases of Platelet ‘Primary Secretion Defect’ Are Heterozygous for a Defect of P2\textsubscript{CYC} Receptors

Marco Cattaneo, Anna Lecchi, Rossana Lombardi, Christian Gachet, Maddalena L. Zighetti

Abstract—Two unrelated patients with a congenital bleeding diathesis associated with a severe defect of the platelet ADP receptor coupled to adenylate cyclase (P2\textsubscript{CYC}) have been described so far. In one of them, platelet secretion was shown to be abnormal. We recently showed that platelets with the primary secretion defect (PSD; characterized by abnormal secretion but normal granule stores, thromboxane A\textsubscript{2} production, and ADP-induced primary wave of aggregation) have a moderate defect of P2\textsubscript{CYC}. Therefore, the interaction of ADP with the full complement of its receptors seems to be essential for normal platelet secretion, and PSD patients may be heterozygotes for the congenital severe defect of P2\textsubscript{CYC}.

In this study, we describe 2 new related patients with a severe defect of P2\textsubscript{CYC} and the son of one of them, who is to be considered an obligate heterozygote for the defect. The 2 patients with the severe defect had lifelong histories of abnormal bleeding, prolonged bleeding times, abnormalities of platelet aggregation and secretion, lack of inhibition of adenylate cyclase by ADP, and a deficiency of platelet-binding sites for [\textsuperscript{33}P]2 MeS-ADP (240 and 225 sites per platelet; normal range, 530 to 1102). The son of one of them had a mildly prolonged bleeding time and abnormalities of platelet aggregation and secretion similar to those found in patients with PSD. In addition, his platelets showed a moderate defect of binding sites for [\textsuperscript{33}P]2 MeS-ADP (430 sites per platelet) and of adenylate cyclase inhibition by ADP. This study of a family with the platelet disorder characterized by a defect of the platelet P2\textsubscript{CYC} receptor supports our hypothesis that the full complement of the platelet ADP receptors is essential for normal platelet secretion and that some patients with the common, ill-defined diagnosis of PSD are actually heterozygous for the defect. (Arterioscler Thromb Vasc Biol. 2000;20:e101-e106.)

Key Words: ADP ■ purinoceptors ■ platelet secretion ■ congenital disorders of platelet function ■ thromboxane A\textsubscript{2}

Platelet secretion defects are the most common congenital abnormalities of platelet function.\textsuperscript{1,2} They include storage pool deficiency (characterized by a diminished content of platelet granule constituents), defective arachidonate metabolism (aspirin-like defects), and an ill-defined and heterogeneous group of abnormalities characterized by abnormal secretion but a normal content of platelet granule constituents and arachidonate metabolism (sometimes referred to as primary secretion defects, or PSD).\textsuperscript{1-3}

We recently showed that released ADP potentiates platelet secretion induced by the thromboxane mimetic U46619 independently of the formation of large aggregates and thromboxane A\textsubscript{2} production, and that at least some of the PSD patients have a partial reduction of platelet-binding sites for [\textsuperscript{33}P]2 MeS-ADP.\textsuperscript{3} This finding suggested to us that some PSD patients have a partial defect of platelet ADP receptors, which is responsible for their secretion defect. Because the inhibition of adenylate cyclase in PSD platelets was partially impaired, we hypothesized that these individuals may be heterozygotes for the severe defect of platelet ADP receptors coupled to adenylate cyclase and termed P2T\textsubscript{AC} or P2\textsubscript{CYC}.\textsuperscript{4-11} Two unrelated patients with a congenital, severe defect of P2\textsubscript{CYC} have been described so far.\textsuperscript{12,13} Their platelets, when exposed to ADP, change shape normally but undergo no or only very slight and rapidly reversible aggregation and do not exhibit the normal inhibition of prostaglandin (PG) E\textsubscript{1}–stimulated adenylyl cyclase. In addition, they have a severely reduced number of binding sites for the ADP analogue 2 MeS-ADP.\textsuperscript{12,13} Platelet secretion induced by several agonists was studied in 1 of the 2 patients and found to be severely impaired.\textsuperscript{12}

Received March 14, 2000; revision accepted June 14, 2000.

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Arterioscler Thromb Vasc Biol. is available at http://www.atvbaha.org
In the present study, we describe 2 sisters with a severe defect of platelet-binding sites for 2 MeS-ADP and abnormalities of platelet function identical to those of the 2 previously described patients, suggesting that they also have a severe defect of P2\textsubscript{Cyc}. The son of one of them had a moderate deficiency of platelet-binding sites for 2 MeS-ADP, and his platelets had a secretion defect and partial impairment of inhibition of adenylate cyclase by ADP, similar to that found in platelets with PSD. The results of this study, therefore, support our initial hypothesis that some patients with PSD are heterozygous for the congenital, severe defect of the platelet P2\textsubscript{Cyc} receptors for ADP and confirm our previous demonstration that released ADP, by interacting with its platelet P2\textsubscript{Cyc} receptor, potentiates platelet secretion.

**Methods**

**Patients**

The proband (II-4 in Figure I), a 57-year-old woman born from first cousins and living in southern Italy, was referred to our center for a lifelong history of easy bruising, menorrhagia, and severe bleeding complications after dental extractions or major surgery. Of her family members, only her 48-year-old sister (II-6), who was living in Milan, could be studied. She had a lifelong history of easy bruising, epistaxis, menorrhagia, and moderate bleeding complications after dental extractions or major surgery. Her 13-year-old son (III-1) and 45-year-old husband (II-7), who had never suffered abnormal bleeding episodes, also accepted to be investigated. All studied subjects had normal prothrombin time and normal von Willebrand factor plasma levels. None of the other family members could be investigated or directly interviewed. Patients II-4 and II-6 reported that their mother (I-1) and 1 of their brothers (II-1), who died of Parkinson’s disease at 60 years of age had a mild bleeding tendency. The nature of the study was explained to the proband and her family members and to 55 healthy controls (male/female 25:30; median age, 38 years; range, 25 to 52), who gave their informed consent.

**Materials**

2 MeS-ADP was from Boehringer. \([^{33}P]2\) MeS-ADP was from New England Nuclear. ADP, adenosine 2-phosphate 5’-phosphate (A23P3), epinephrine, collagen, the thromboxane/prostaglandin endoperoxide analogue 9,11-dideoxy-11,9-epoxymethano-prostaglandin \(F_1\) (U66619), platelet-activating factor (PAF-acether), \(\alpha\)-thromboxane/prostaglandin \(I_3\) (PGI\_3), prostaglandin \(I_2\) (PGI\_2), prostaglandin \(E_1\) (PGE\_1), and acetylsalicylic acid (ASA) were from Sigma. AR-C69931 MX, a P2\textsubscript{Cyc} antagonist, was a kind gift of AstraZeneca R&D (Charnwood, UK). Fura 2 AM was from Calbiochem. Apyrase was a kind gift of Dr R.L. Klinough-Rathbone (McMaster University, Hamilton, Ontario, Canada). All other products were of reagent grade or better. Commercial preparations of luciferin/luciferase reagent were used to measure the platelet ATP and ADP contents (ATP assay kit, BioOrbit Oy) and platelet secretion concurrently.

**Preparation of Platelet-Rich Plasma (PRP) and Washed Platelet Suspensions**

Nine volumes of blood was drawn into 1 volume of 129 mmol/L trisodium citrate for preparation of PRP, and 6 volumes was drawn into 1 volume of acid-citrate-dextrose anticoagulant for preparation of washed platelet suspensions. Twice, washed platelet suspensions were prepared according to the method described by Mustard et al,\textsuperscript{15} with the exception that 500 mmol/L PGI\_2 was added during the first and second wash.\textsuperscript{14} Platelet counts were adjusted to 300\(\times\)10\(^{9}\)/L in PRP and to 400\(\times\)10\(^{9}\)/L in washed platelet suspensions. For measurement of cytoplasmic concentrations of ionized calcium (\([Ca^{++}]_i\)), platelets were loaded with 2 mmol/L fura 2 AM for 45 minutes at 37°C as previously described.\textsuperscript{16}

**Platelet Aggregation and Secretion Studies**

Samples of PRP (0.45 mL) were incubated with 50 \(\mu\)L of luciferin/luciferase reagent at 37°C for 30 seconds and stirred at 1000 rpm in a lumiglo aggregometer (Lumi-aggregometer, Chrono-log Corp). After incubation, 10 \(\mu\)L of an aggregating agent was added, and the aggregation and ATP secretion tracings were recorded for 3 minutes.

**Measurement of Platelet Shape Change**

Platelet shape change induced by 0.1 or 1 \(\mu\)mol/L ADP was measured in the aggregometer by using citrated PRP to which 2 mmol/L EDTA had been added to prevent platelet aggregation. Decreases in oscillations of the basal tracings and increases in optical density were interpreted as being caused by platelet shape change.

**Measurement of [Ca\textsuperscript{++}]\textsubscript{i}**

Aliquots of fura 2 AM–loaded platelets were transferred to quartz cuvettes maintained at 37°C. Fluorescence was monitored continuously before and after stimulation with ADP by using a spectrofluorometer (LS50B, Perkin-Elmer Co). The excitation wavelength was alternatively fixed at 340 and 380 nm, and fluorescence emission was determined at 510 nm.

**Measurement of Platelet Granule Content**

Total platelet ADP and ATP content was measured by the firefly luciferin/luciferase method\textsuperscript{17} in a luminometer (luminometer 1250, LKB, BioOrbit Oy). Platelet serotonin (5-HT) content was measured with the \(\alpha\)-thromboxaldehyde method.\textsuperscript{18} Fibrinogen was measured in washed platelet lysates by an ELISA with a polyclonal antifibrinogen antibody (Atlantic Antibodies) conjugated with peroxidase (type I, Boehringer Mannheim).\textsuperscript{19} The sensitivity of the method was 0.02 \(\mu\)g/mL.\textsuperscript{18}

**Measurement of Platelet Thromboxane \(B_2\) Production**

Thromboxane \(B_2\) (Tx\(B_2\)) was measured in supernatant serum of blood samples collected in glass tubes containing no anticoagulant and clotted at 37°C for 2 hours.

**Binding of \([^{33}P]2\) to MeS-ADP to Washed Platelets**

Binding of \([^{33}P]2\) MeS-ADP (872 to 1044 Ci/mmol) to washed platelets was measured as described.\textsuperscript{14} \([^{33}P]2\) MeS-ADP (0.1 mmol/L) with increasing concentrations of unlabeled ligand (0 to 49.9 mmol/L) was incubated with washed platelet suspensions (1 mL final volume) at 37°C for 5 minutes. The ligand bound to platelets was separated from free ligand by filtration through Whatman GF/C glass filters under vacuum. Radioactivity bound to the platelets on the filters was measured by scintillation counting (Minibeta 1211, LKB). Nonspecific binding, determined by incubation in the presence of 1 mmol/L 2 MeS-ADP, amounted to 1% to 3% of total binding. Data were analyzed by a computer program (LIGAND).\textsuperscript{19}
Measurement of Platelet cAMP

Platelet cAMP was measured by a radioisotopic assay with a commercially available kit (Amersham International). Duplicate samples of 1 mL of citrated PRP containing 1 mmol/L theophylline were incubated with Tyrode’s solution, PGE1 (1 μmol/L) plus Tyrode’s solution, or PGE1 plus ADP (0.1, 1, and 10 μmol/L). After incubation at 37°C for 2 minutes, 1 mL of 5% trichloroacetic acid was added, and the samples were snap-frozen in dry ice and methanol, thawed at room temperature, and then shaken at 4°C for 45 minutes. After centrifugation at 4°C for 30 minutes, the supernatant was extracted 3 times with 5 mL of water-saturated ether, dried under a stream of N2 at 60°C, and stored at −20°C. Before assay, the samples were reconstituted with 0.05 mol/L Tris buffer containing 4 mmol/L EDTA.

Results

The bleeding time was prolonged in the proposita (II-4), her sister (II-6), and her nephew (III-1) and normal in II-7, her brother-in-law (II-6 in Table I). The platelet count and contents of ADP, ATP, 5-HT, and fibrinogen were normal in all subjects except in II-7, whose platelet content of 5-HT and ADP was slightly lower than normal (Table I), suggesting that he had a mild δ-storage-pool deficiency.

Platelet Aggregation and ATP Secretion

ADP at concentrations ranging from 2 to 20 μmol/L induced a very small and rapidly reversible wave of platelet aggregation in PRP of II-4 and II-6 (Figure II). Platelet aggregation induced by 2 or 4 μmol/L ADP was reversible in III-1 (son of II-6 and II-7) and comparable to that of normal platelets that had been incubated in vitro with 0.5 mmol/L ASA for 30 minutes (Figure II). Higher concentrations of ADP (up to 20 μmol/L) induced normal platelet aggregation in PRP of III-1 (Figure II). The P2Y1 antagonist A2P5P (1 mmol/L), which was tested in II-6 (Figure III) and III-1 (not shown), completely abolished platelet aggregation induced by 2 μmol/L ADP. Platelet aggregation induced by 0.2 μmol/L PAF-acether or 0.5 μmol/L U46619 was reversible in II-4, II-6, and III-1, whereas it was irreversible in normal controls (not shown). The extent of platelet aggregation induced by collagen (2 μg/mL) was normal in PRP of III-1 and ~50% of normal in that of II-4 and II-6 (not shown). Platelet aggregation induced by any agonist tested was absent or severely impaired in the proposita (II-4) and her sister (II-6) (Table II). In III-1 (son of II-6 and II-7), it was absent or severely reduced when induced by ADP or U46619 and borderline-low when induced by PAF-acether or collagen (Table II). It was borderline-low with any tested agonist in II-7 (Table II), the husband of II-6 and father of III-1, whose platelet concentration of ADP and 5-HT was slightly reduced.

Platelet Shape Change

ADP (0.1 and 1 μmol/L) caused normal shape change of platelets from II-6 (Figure III) and III-1 (not shown), which was completely inhibited by 1 mmol/L A2P5P. Platelet shape change was not studied in II-4 and II-7.

ADP-Induced Increase in [Ca2+]

In the presence of 2 mmol/L external CaCl2, stimulation with 5 μmol/L ADP caused a rapid increase in [Ca2+], which was

![Figure II](http://atvb.ahajournals.org/ Downloaded from)

**Table I. Routine Parameters of Platelet (plt) Function in the 4 Patients Studied**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>II-4</th>
<th>II-6</th>
<th>III-1</th>
<th>II-7</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding time, min</td>
<td>&gt;30</td>
<td>20</td>
<td>13</td>
<td>3.5</td>
<td>2–8</td>
</tr>
<tr>
<td>Platelet count, ×10⁹/L</td>
<td>335</td>
<td>147</td>
<td>174</td>
<td>263</td>
<td>150–347</td>
</tr>
<tr>
<td>Platelet 5-HT, nmol/10⁹ plt</td>
<td>2.6</td>
<td>3.5</td>
<td>4.5</td>
<td>1.6</td>
<td>2.1–6.7</td>
</tr>
<tr>
<td>Platelet ADP, nmol/10⁹ plt</td>
<td>18.6</td>
<td>28.1</td>
<td>50.4</td>
<td>12.0</td>
<td>13.5–33.9</td>
</tr>
<tr>
<td>Platelet ATP, nmol/10⁹ plt</td>
<td>38.9</td>
<td>64.2</td>
<td>67.3</td>
<td>49.5</td>
<td>35.7–80.5</td>
</tr>
<tr>
<td>Platelet fibrinogen, nmol/10⁹ plt</td>
<td>0.35</td>
<td>0.23</td>
<td>0.18</td>
<td>0.17</td>
<td>0.1–0.6</td>
</tr>
<tr>
<td>Serum TxB₂, nmol/10⁹ plt</td>
<td>ND</td>
<td>13.5</td>
<td>14.1</td>
<td>3.4</td>
<td>1.8–26</td>
</tr>
</tbody>
</table>

Patient values are the mean of at least 2 different determinations. Normal ranges are set to the 2.5th and 97.5th percentiles of the distribution of values obtained in 55 healthy subjects.
similar in platelets from II-6 and a normal control; in the absence of external Ca²⁺ (ie, with 0.2 mmol/L EGTA), the mobilization of [Ca²⁺]ᵢ was lower, but again it was similar in II-6 and a normal control; the ADP-induced increases in [Ca²⁺]ᵢ were completely abolished by 0.5 mmol/L A2P5P in both II-6 and normal platelets (data not shown). The mobilization of [Ca²⁺]ᵢ was not studied in the other patients.

Inhibition of PGE₁-Induced Increase in Platelet cAMP

The basal levels of platelet cAMP in the 4 patients studied were normal (5.9 pmol/10⁹ in II-4, 12.5 in II-6, 9.1 in III-1, and 12.5 in II-7; normal range, 6 to 14.2 pmol/10⁹; n = 18) and increased normally after stimulation with PGE₁ (32.3 in II-4, 54.8 in II-6, 35.6 in III-1, and 44.3 in II-7; normal range, 22.5 to 68.6). ADP (0.1 to 10 µmol/L) did not affect the PGE₁-induced increase in cAMP levels in platelets from patients II-4 and II-6, whereas it inhibited the cAMP increase in a concentration-dependent manner in platelets from healthy subjects and patients II-7 and III-1 (Figure IV). However, although the degree of inhibition of the PGE₁-induced increase in platelet cAMP was very similar to that seen in healthy subjects for patient II-7, it was much less for patient III-1 at all ADP concentrations tested (Figure III). AR-C69931 MX (0.1 mmol/L), a P2 CYC antagonist, completely abolished the residual inhibitory effect of ADP on the PGE₁-induced increase in the platelet cAMP of patient III-1, whereas A2P5P (1 mmol/L), a P2Y₁ antagonist, had no effects (not shown).

Binding experiments were performed in 29 healthy subjects and in at least 2 different sessions in the 4 patients studied. Specific binding of [³²P]2 MeS-ADP to washed normal and patient platelets was saturable and the Scatchard plot was linear. For all patients, the Kᵦ values were comparable to those calculated for normal volunteers (Table III). In contrast, the number of binding sites was severely decreased in the proposita II-4 and her sister II-6, moderately decreased in III-1, and normal in II-7 (Table III).

Discussion

Three purinergic receptors are currently believed to contribute separately to the complex platelet responses to ADP: the P2X₁ ionotropic receptor, responsible for rapid influx of ionized calcium into the cytosol; the P2Y₁ metabotropic receptor, responsible for mobilization of ionized calcium from internal stores, platelet shape change, and initial aggregation; and an as-yet-unidentified receptor coupled to Gαᵢ₂, which is essential for the full aggregation response to ADP and is termed P2CYC or P2TAC. The elusive P2 CYC receptor is probably the molecular target of thienopyridine compounds and other selective inhibitors of ADP-induced platelet aggregation. In addition, it is probably defective in patients C69931 MX (0.1 mmol/L), a P2CYC antagonist, completely abolished the residual inhibitory effect of ADP on the PGE₁-induced increase in the platelet cAMP of patient III-1, whereas A2P5P (1 mmol/L), a P2Y₁ antagonist, had no effects (not shown).

<table>
<thead>
<tr>
<th>Agonist</th>
<th>ATP Secreted, nmol/10⁹ plt</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agonist</td>
<td>II-4</td>
<td>II-6</td>
</tr>
<tr>
<td>ADP, 2 µmol/L</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ADP, 4 µmol/L</td>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td>ADP, 20 µmol/L</td>
<td>0</td>
<td>0.6</td>
</tr>
<tr>
<td>PAF-acether, 0.2 µmol/L</td>
<td>0.2</td>
<td>0</td>
</tr>
<tr>
<td>U46619, 0.5 µmol/L</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Collagen, 2 µg/mL</td>
<td>0.7</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Patient values are the mean of at least 2 different determinations. Normal ranges are set to the 2.5th and 97.5th percentiles of the distribution of values obtained in 18 to 50 healthy subjects.

<table>
<thead>
<tr>
<th>Binding sites/platelet</th>
<th>II-4</th>
<th>II-6</th>
<th>III-1</th>
<th>II-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binding sites/platelet</td>
<td>240</td>
<td>225</td>
<td>430</td>
<td>1020</td>
</tr>
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<td>240</td>
<td>225</td>
<td>430</td>
<td>1020</td>
</tr>
</tbody>
</table>

Patient values are the mean of at least 2 different determinations. Normal ranges are set to the 2.5th and 97.5th percentiles of the distribution of values obtained in 29 healthy subjects.
with a congenital bleeding diathesis characterized by a severe defect of platelet responses to ADP. Two such patients have been described so far by our group\(^\text{12}\) and by Nurden et al\(^\text{13}\): the many similarities between the 2 patients suggest that they are affected by the same type of abnormality of platelet ADP receptors.\(^\text{26}\)

In this study, we describe 2 sisters with a congenital bleeding tendency characterized by a platelet function defect that is similar to that of the 2 previously described patients: (1) severe impairment of aggregation but normal shape change induced by ADP; (2) lack of adenylate cyclase inhibition by ADP; (3) normal mobilization of Ca\(^{2+}\) from internal stores induced by ADP, which was completely inhibited by the P2Y\(_1\) receptor antagonist A2P5P; and (4) decreased number of binding sites for \(^{[\text{33}}\text{P}\)2 MeS-ADP. In addition, similar to those of the first patient described by our group,\(^\text{12}\) the patients’ platelet secretion of δ-granule constituents induced by several agonists was severely impaired, confirming our previous observation that ADP plays an important role in the potentiation of platelet secretion stimulated by release-inducing agonists.\(^\text{3}\)

Another similarity between the 2 patients described in this study and those previously described\(^\text{12,13}\) is that all were born from consanguineous parents, indicating that their platelet defect is most likely inherited as a recessive trait. As expected, the platelets of the son of 1 of them, who is an obligate heterozygote, had a number of binding sites for 2 MeS-ADP that was intermediate between normal and that of his mother and aunt. ADP induced a normal primary wave of aggregation of his platelets and partially inhibited adenylate cyclase. In addition, a variety of agonists, including the TXA\(_2\) mimetic U46619, PAF-acether, and collagen, induced impaired or borderline-low secretion of ATP from his platelets. This secretion defect was not caused by impaired production of TXA\(_2\) or low concentrations of platelet granule contents and is therefore, very similar to that described in patients with an ill-defined and probably heterogeneous group of congenital defects of platelet secretion, sometimes referred to by the general term “primary secretion defect” (PSD).\(^\text{3}\) This defect, which is the most common congenital abnormality of platelet secretion, is characterized by abnormal/borderline-low platelet secretion induced by different agonists, a normal primary wave of aggregation induced by ADP, normal granule stores, and normal arachidonate metabolism.\(^\text{1}\) The results of this study therefore confirm our previous hypothesis that some patients with PSD are heterozygous for the severe defect of P2\(_{\text{Cyc}}\), the platelet ADP receptor that is coupled to adenylate cyclase.\(^\text{3}\) The important role of ADP interaction with this receptor in primary hemostasis is emphasized by the finding that patient III-1, like others with PSD, has, despite the mild defect of P2\(_{\text{Cyc}}\), a mild prolongation of the bleeding time. The bleeding history, which is usually mild in PSD, was negative in patient III-1, probably because he had never been exposed to situations at risk for excessive bleeding owing to his young age.

ADP is a weak agonist because by itself it does not trigger platelet secretion directly.\(^\text{27}\) When exogenous ADP is added to a normal platelet suspension, it causes shape change and aggregation. It is the close platelet-to-platelet contact brought about by the aggregation process that, in a minority of individuals, triggers the formation of trace amounts of TXA\(_2\), which stimulates the secretion of small amounts of δ-granule constituents and reinforces aggregation.\(^\text{10,28–30}\) This effect is greatly enhanced and can be observed in most individuals when the concentration of ionized calcium in the extracellular medium is artificially decreased to the micromolar level, such as in citrated PRP.\(^\text{28,30}\) On the other hand, the mechanism that is responsible for the potentiation of platelet secretion by ADP released from the platelet δ-granules, which was demonstrated for the first time by our group in 1997,\(^\text{3}\) is quite different from that triggered by exogenous ADP because it is independent of TXA\(_2\) synthesis and the formation of large platelet aggregates.\(^\text{3}\) It is likely that although ADP by itself cannot directly stimulate the secretion of δ-granule constituents, it does potentiate platelet secretion induced by other agonists. The results of the present study and of our previous one\(^\text{3}\) indicate that the full complement of platelet ADP receptors is necessary for the potentiation of platelet secretion by ADP, whereas it is not essential for normal aggregation.

Based on current knowledge, it is unknown whether the potentiation of platelet secretion by ADP is mediated solely by its interaction with P2\(_{\text{Cyc}}\) or also by the other platelet purinoreceptors. In 2 preliminary reports of congenital defects of platelet P2Y\(_2\),\(^\text{31}\) and P2X\(_1\)\(^\text{32}\) receptors, both associated with abnormal platelet aggregation and a mild bleeding diathesis, platelet secretion was not studied. However, recent unpublished data in P2Y1 knockout mice\(^\text{33}\) (Gachet et al, 2000) and in human platelets incubated with selective P2Y\(_1\) and P2\(_{\text{Cyc}}\) antagonists (Cattaneo et al, 2000) indicate that the P2\(_{\text{Cyc}}\) receptor is the main receptor responsible for the potentiation of platelet secretion, suggesting that the G\(_i\) pathway cross-talks with other pathways of platelet activation. The molecular mechanisms for this are still unknown but could involve phosphoinositide 3-kinase activation,\(^\text{35}\) tyrosine kinases specifically linked to this receptor and/or the vasodilator-stimulated phosphoprotein.\(^\text{36,37}\) Thus, the patients described here should help in better defining the selective role of the P2\(_{\text{Cyc}}\) receptor in primary hemostasis.

In conclusion, our study describes 2 new patients with a congenital bleeding diathesis associated with a severe defect of platelet P2\(_{\text{Cyc}}\) receptors. In addition, the possibility of studying the son of 1 of the 2 patients, who is an obligate heterozygote for the disease, allowed us to confirm our previous hypothesis that some patients with the common PSD are heterozygotes for the disease and that the interaction of released ADP with the full complement of its platelet receptors is essential for normal platelet secretion.

References


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Platelets From a Patient Heterozygous for the Defect of P2\textsubscript{\text{CYC}} Receptors for ADP Have a Secretion Defect Despite Normal Thromboxane A\textsubscript{2} Production and Normal Granule Stores: Further Evidence That Some Cases of Platelet 'Primary Secretion Defect' Are Heterozygous for a Defect of P2\text{CYC} Receptors

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Arterioscler Thromb Vasc Biol. 2000;20:e101-e106
doi: 10.1161/01.ATV.20.11.e101

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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