High Plasma Serotonin Levels in Primary Pulmonary Hypertension

Effect of Long-Term Epoprostenol (Prostacyclin) Therapy


Abstract—Elevated plasma serotonin is associated with primary pulmonary hypertension (PPH). To test whether this elevation could be related to platelet activation, the 2 pools of blood serotonin (platelets and plasma) and plasma 5-hydroxyindoleacetic acid (5-HIAA) as well as markers of platelet activation (α_tβ_3, CD36, P-selectin, and CD63 membrane epitopes) were measured in 16 patients with severe PPH (group 1) before and at days 10 and 40 of treatment with a continuous infusion of epoprostenol (prostacyclin). The same biological parameters were also measured in 19 healthy subjects (group 2) and in 10 patients after cardiovascular surgery with extracorporeal circulation (group 3), a condition known to profoundly activate the platelets. Twelve PPH patients showed hemodynamic and clinical improvement, 3 remained stable, and 1 had the treatment stopped because of clinical aggravation. At day 0, mean plasma serotonin (5-hydroxytryptamine [5-HT]) concentration was much higher in PPH patients than in normal subjects (34.4 ± 21.2 vs 9.1 ± 6.0 nmol/L, respectively; P < 0.001) and positively correlated with total pulmonary resistance. The mean platelet 5-HT content was not significantly different in PPH compared with normal individuals. Mean plasma 5-HIAA concentrations were much higher in PPH than in normal patients (162 ± 57 vs 61 ± 7 nmol/L, respectively; P < 0.001). These parameters did not significantly change during epoprostenol treatment. There was no correlation between the changes in plasma 5-HT during treatment and clinical or hemodynamic improvement. In PPH patients, the mean platelet volume significantly decreased (ANOVA, P < 0.01) during treatment. Positive correlations were evidenced between the size of platelets and the number of α_tβ_3 and CD36 epitopes. When compared with control platelets, the number of α_tβ_3 epitopes detected on PPH platelets at day 0 tended to be higher, but this difference did not reach a statistical significance (41 300 ± 7140 for PPH patients versus 36 010 ± 3930 for control subjects, P = 0.069). The number of CD36 epitopes, in the range of controls at day 0 (11 590 ± 5080 for PPH patients versus 11 900 ± 1790 for control subjects), decreased during treatment (ANOVA, P = 0.038) and became significantly low at day 40 (8660 ± 3520, P < 0.001). The number of CD63 epitopes was not elevated, and P-selectin was never detected at any time point on PPH platelets. This glycoprotein profile indicates that the platelets of PPH patients were not highly activated but had an accelerated turnover and returned to normal under epoprostenol treatment without change of the elevated plasma serotonin, characteristic of PPH. In conclusion, neither platelet activation nor a significant alteration of the 5-HT endothelial metabolism explains the high level of plasma 5-HT in PPH patients. The 5-HT plasma concentration is not a predictive marker of the severity of PPH, and its evolution is independent of the clinical and hemodynamic status. Treatment by a potent antiaggregating agent, epoprostenol, does not affect the increase of plasma 5-HT, despite a therapeutic benefit. (Arterioscler Thromb Vasc Biol. 2000;20:2233-2239.)

Key Words: primary pulmonary hypertension • serotonin • platelet activation • prostacyclin • epoprostenol

Pulmonary hypertension (PPH) occurs as a familial1 and a sporadic disease, with an increased incidence in users of appetite suppressants.2-5 The features of PPH include a mean pulmonary artery pressure > 25 mm Hg and histological abnormalities of the vascular wall with medial hypertrophy, proliferation of smooth muscle cells,6 and the frequent presence of arterial microthrombi.7 These vascular lesions could result from an impaired balance of vasoactive, prothrombotic, and growth-promoting substances.8 Among these substances, serotonin (5-hydroxytryptamine [5-HT]) has been suggested as a candidate (see Reference 9 for review). In the circulation, 5-HT, synthesized by the intestinal enterochromaffin cells, is actively incorporated in platelets and stored in platelet β-storage granules.10 5-HT circulates mainly as a

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reserve pool stored in platelets and minimally in plasma (which is the interactive pool). 5-HT is mostly metabolized into 5-hydroxyindoleacetic acid (5-HIAA) by monoamine oxidase in hepatic and lung endothelial cells. 5-HT induces smooth muscle cell contraction and proliferation but stimulates endothelial cells to release vasodilating substances and acts as a “helper agonist” of platelet aggregation in humans. All these effects are transduced through specific cell membrane receptors.

In a case report describing a patient affected by a rare hereditary thrombocytopenia (a platelet -storage pool disease) who developed PPH, an increase of plasma 5-HT associated with pulmonary hypertension was first described. Indeed, 5-HT was found to be increased in plasma obtained from patients with PPH. In these patients with PPH, the increased plasma 5-HT could result from a platelet activation, which induces the release of the storage granule content to the extracellular (plasma) space. The hypothesis of a platelet activation in PPH patients was also supported by the clinical efficacy of treatment for PPH with prostacyclin or epoprostenol, a potent vasodilator participating in the expression of these parameters in parallel with plasma 5-HT levels in patients treated with continuous epoprostenol infusion, giving an insight into the potential mechanisms of the disease. An alternative explanation for high plasma 5-HT levels would be an impaired metabolism of 5-HT. Pulmonary endothelial cells, which play an important role in 5-HT clearance, present indirect signs of lesions in PPH patients. However, it is not yet clear whether PPH disease results from endothelial lesions or induces them. This metabolic implication can be evaluated in PPH patients treated with epoprostenol by the sequential measure of plasma 5-HIAA, because 60% to 80% of 5-HT is metabolized as 5-HIAA (see Reference 20 for review), whereas 10% is eliminated unchanged as 5-HT, and the remaining amount is eliminated as sulfonic or glycuronic conjugates.

**Methods**

**Patients: Sample Preparations**

Sixteen patients with severe PPH (group 1) referred to the Antoine Béclère Hospital between June 96 and May 97 were treated with a continuous infusion of epoprostenol. Patients were characterized by risk factors for PPH (6 patients had taken appetite suppressants, and 1 patient had a familial PPH) and by clinical and hemodynamic parameters (echocardiography, right heart catheterization, and a 6-minute walking test). Mean pulmonary arterial pressure (mPAP), cardiac index (CI), and total pulmonary resistance (TPR = mPAP/CI) are given in Table 1. Patients had their 5-HT and platelet parameters measured before treatment and at days 10 and 40 after continuous intravenous infusion of epoprostenol was started (group 1). The same biological parameters were measured in parallel for 19 healthy subjects (group 2) aged 32.4 ± 8.7 years and for 10 patients (group 3) aged 64.4 ± 9.1 years at the end of cardiovascular surgery that required extracorporeal circulation (ECC), a condition known to profoundly activate the platelets. Five milliliters of blood was drawn by venipuncture from a peripheral vein in EDTA, and 3 × 4.5 mL was drawn in 0.5 mL of 0.129 mol/L trisodium citrate. This protocol was submitted to the Ethics Committee of Hôpital Bicêtre (December 1995) and received approval.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Risk Factors and Relevant Clinical Information</th>
<th>mPAP, mm Hg (Day 0/Day 40)</th>
<th>CI, L · min⁻¹ · m⁻² (Day 0/Day 40)</th>
<th>TPR (mPAP/CI), U/m² (Day 0/Day 40)</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>Appetite suppressant, systemic hypertension</td>
<td>77/52</td>
<td>1.70/1.92</td>
<td>45.3/27.1</td>
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<tr>
<td>2</td>
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<td>65/61</td>
<td>1.80/2.70</td>
<td>36.1/22.6</td>
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<tr>
<td>3</td>
<td>...</td>
<td>84/70</td>
<td>1.02/1.90</td>
<td>82.3/36.8</td>
</tr>
<tr>
<td>4</td>
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<td>72/...</td>
<td>2.54/...</td>
<td>28.3/...</td>
</tr>
<tr>
<td>5</td>
<td>...</td>
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<td>2.35/2.39</td>
<td>22.6/22.6</td>
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<tr>
<td>6</td>
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<td>58/58</td>
<td>2.01/2.50</td>
<td>28.4/23.2</td>
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<tr>
<td>7</td>
<td>HIV, HCV</td>
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<td>2.06/1.82</td>
<td>24.8/26.4</td>
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<td>8</td>
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<td>1.81/1.82</td>
<td>39.8/34.1</td>
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<tr>
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<td>Scleroderma</td>
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<td>1.70/1.80</td>
<td>51.8/41.7</td>
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<tr>
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<td>24.4/18.4</td>
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<td>25.4/21.3</td>
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<td>2.50/3.0</td>
<td>20.4/17.0</td>
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<tr>
<td>15</td>
<td>...</td>
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<td>...</td>
<td>76/66</td>
<td>1.70/1.99</td>
<td>44.7/33.2</td>
</tr>
<tr>
<td>Mean</td>
<td>Appetite suppressant (6 of 16)</td>
<td>67.9/58.6</td>
<td>2.10/2.33</td>
<td>35.3/26.3</td>
</tr>
</tbody>
</table>

HCV indicates hepatitis C virus; PAP, pulmonary arterial pressure.

*Sex ratio.
Platelet count and volume were measured (STKS Coultronic) in citrated and EDTA blood, respectively. Platelet rich plasma was prepared by centrifugation of citrated blood at 120g for 10 minutes. Platelets were counted, and platelet-rich plasma was further centrifuged at 2000g for 10 minutes to harvest separately the platelet pellets and the platelet-poor plasma (also referred to as plasma). Blood, plasma, and platelet aliquots were immediately frozen at −80°C until measurement (<1 month).

5-HT and 5-HIAA Measurements
5-HT concentrations were determined in citrated whole blood, platelet-poor plasma, and the platelet pellet. The mean platelet concentration was then calculated according to the platelet count in the pellet. 5-HIAA concentration was determined in citrated plasma.

Flow Cytometric Analyses
Flow cytometric analyses were accomplished by use of the following: mouse monoclonal antibodies, consisting of P-selectin–FITC (CLBThromb/6), αIXaβ2 (P2), CD36, and CD63 (Immunotech); goat anti-mouse (GAM), consisting of anti–Fe–FITC (Nordic Immunology); calibration beads (Biocytex); red cell lysing buffer (FACS Lysis Solution, Becton Dickinson); and BSA (Sigma Chemical Co). Within 15 minutes after the venipuncture, 50 μL whole blood anticoagulated with EDTA was diluted to avoid platelet aggregation in 450 μL buffer A, pH 6.50 (36 mmol/L citric acid, 5 mmol/L glucose, 5 mmol/L KCl, 2 mmol/L CaCl2·2H2O, 1 mmol/L MgCl2·6H2O, and 103 mmol/L NaCl) containing 0.1% BSA. Fifty microliters of diluted blood was added to 2.5 μL of each monoclonal antibody (final concentration 5 μg/μL) and incubated for 30 minutes at room temperature protected from any light. Red blood cells were lysed by adding 1 mL lysing buffer. Platelets were washed in 2 mL PBS, collected by centrifugation for 5 minutes at 250g, and resuspended in 100 μL GAM-FITC diluted 1:30. After 30 minutes of incubation, the specimens were fixed with 1% paraformaldehyde, and 200 μL of PBS was added before data acquisition. The fluorescence intensity was determined by use of a flow cytometer (FACScan, Becton Dickinson) and Cell Quest software. Calibration beads are latex beads coated with mouse immunoglobulins (3-μm diameter; 350, 7800, 22,000, and 53,000 immunoglobulins per bead). Five-microfilter bead suspensions were added to 100 μL GAM-FITC diluted 1:30 and incubated for 30 minutes. Flow cytometry defined 4 peaks of fluorescence intensity, corresponding to the 4 immunoglobulin densities, and allowed a calibration curve to be drawn (fluorescence intensity as a function of the number of epitopes for the monoclonal antibody). For platelet analyses, all fluorescence intensities were expressed as the number of epitopes per platelet.

Statistical Analysis
Data are given as mean±SD. Comparisons between 2 groups were performed by using the nonparametric Mann-Whitney test (Statview SE, Abacus Concepts). Kinetic studies were tested by ANOVA. There were great discrepancies in the changes of 5-HT levels between days 0 and 40: 5 patients had an increase of 50% above baseline, and 7 patients had a decrease of ≥50%, whereas 4 patients had stable plasma 5-HT levels. Three patients returned to normal values after 40 days of treatment.

5-HT and 5-HIAA Measurements
5-HT and 5-HIAA measurements can be found on Table I (which can be accessed online at www.ahajournals.org). At day 0, the mean plasma 5-HT concentration was much higher in PPH patients (highest baseline concentration 61.4 nmol/L, Figure 1) than in normal subjects (34.4±21.2 versus 9.1±6.0 nmol/L, respectively; P<0.001). There was no difference between patients who had a history of appetite suppressant (31.1±24.9 nmol/L) and other patients (36.5±19.9 nmol/L). Patients 2, 5, and 12 had a baseline value in the “normal” range (<15 nmol/L), but subsequent determinations of plasma 5-HT levels showed elevated values (26, 27, and 16 nmol/L, respectively) after epoprostenol therapy was started. Mean plasma 5-HT concentration did not significantly change during epoprostenol treatment (day 10, 38.4±23.4 nmol/L; day 40, 48.3±49.4 nmol/L; ANOVA, P>0.3), but there were great discrepancies in the changes of 5-HT levels between days 0 and 40: 5 patients had an increase of 50% above baseline, and 7 patients had a decrease of ≥50%, whereas 4 patients had stable plasma 5-HT levels. Three patients returned to normal values after 40 days of treatment.

Mean plasma 5-HIAA concentrations were also much higher in PPH patients (162±57 versus 61±7 nmol/L in normal subjects, P<0.001; Figure 2) and remained unchanged during treatment (ANOVA, P>0.5). Nevertheless, there was no correlation (P>0.4) between plasma 5-HT and 5-HIAA levels. The mean platelet 5-HT content was not significantly different in PPH patients compared with normal subjects (4.3±3.6 attomoles per platelet [amol/plt] versus 2.1±0.5 amol/plt, respectively; P=0.12) and did not significantly change during treatment (ANOVA, P=0.07). In 2

Results
Clinical Data
Sixteen patients with PPH, 11 women and 5 men (aged 43.3±11.9 years), were studied before and 10 and 40 days after the onset of continuous epoprostenol infusion. At day 40, most patients showed hemodynamic and clinical improvement according to the measurements of pulmonary arterial pressure (mPAP −9.3±9.1 mm Hg, CI 0.23±0.33 L·min⁻¹·m⁻², and TPR −9.07±11.4 U/m²) and functional tests (6-minute walk 145±125 m), except patients 5, 7, and 9, who had stable hemodynamic parameters (no change of the TPR), and patient 4, in whom the treatment was interrupted because of clinical aggravation (Table 1).
patients (Nos. 2 and 7), the platelet 5-HT level was low at day 0 (0.2 and 1.2 amol/plt, respectively) but was in the normal range at day 40 (Figure 3). Mean whole blood 5-HT concentration was within the normal range and remained unchanged at days 10 and 40. As in the control subjects, there was a positive correlation between platelet and whole blood 5-HT in PPH patients ($r=0.53$, $P<0.005$).

Because of the destruction by cardiopulmonary bypass circuit, the ECC patients had a lower platelet count (105±39×10⁹ platelets per liter [plt/L], $P<0.05$) than did the PPH patients at day 0 (140±63×10⁹ plt/L, $P<0.05$ versus controls, because of 8 thrombopenic patients, ie, patients with a platelet count <150×10⁹ plt/L) and the control subjects (232±51×10⁹ plt/L). ECC patients had normal plasma 5-HT levels (7.2±2.9 nmol/L for ECC patients versus 9.1±6.0 nmol/L for control subjects), normal platelet 5-HT contents (2.2±0.8 amol/plt for ECC patients versus 2.1±0.5 amol/plt for control subjects), and normal plasma 5-HIAA concentrations (50±11 nmol/L for ECC patients versus 61±7 nmol/L for control subjects). Whole blood 5-HT concentration was lower in ECC patients than in healthy individuals (236±116 versus 424±223 nmol/L, respectively; $P<0.01$) in relation to the decrease in platelet count.

**Correlations Between Clinical Data and Plasma 5-HT Levels**

At day 0, plasma concentrations of 5-HT did correlate with TPR (Figure 4; $r=0.60$, $P<0.05$) but not with mPAP (Figure I, which can be accessed online at www.ahajournals.org; $r=0.4$, $P=0.12$). There was also no correlation between the changes in plasma 5-HT during treatment and the decrease of mPAP (Figure II, which can be accessed online at www.ahajournals.org) or TPR (Figure III, which can be accessed online at www.ahajournals.org); eg, a good hemodynamic response to epoprostenol, determined by mPAP or TPR, was associated with a decrease of plasma 5-HT in 5 patients and with an increase of plasma 5-HT in 7 patients.

**Quantification of Platelet Membrane Glycoproteins as Markers of Activation**

Quantification of platelet membrane glycoproteins can be seen on Table 2. The platelet count was slightly reduced (see above) and remained so during epoprostenol treatment in PPH patients. The mean platelet volume was normal in PPH patients and decreased (ANOVA, $P<0.01$) during treatment. There was a positive correlation between the size of the platelets and the number of $\alpha_{\text{IIb}}\beta_3$ ($r=0.49, P<0.001$) and CD36 ($r=0.36, P=0.01$) epitopes. The number of $\alpha_{\text{IIb}}\beta_3$ epitopes detected on PPH platelets compared with control platelets at day 0 did not reach a statistical significance ($41300±7140$ versus $36010±3930$, respectively; $P=0.069$). The number of CD36 epitopes, normal at day 0 (11 590±5080 for PPH platelets versus 11 900±1790 for control platelets), decreased during treatment (ANOVA, $P=0.038$) and became significantly low at day 40 (8660±3520, $P<0.001$; Figure IV, which can be accessed online at www.ahajournals.org). The number of CD63 epitopes was not elevated at any time point on PPH platelets (Figure V, which can be accessed online at www.ahajournals.org), and P-selectin was never detected. This glycoprotein profile indicates that the platelets of PPH patients were not highly activated but had an accelerated turnover and returned to normal under epoprostenol treatment without change of the elevated plasma serotonin, which is characteristic of PPH.

Conversely, compared with control platelets, ECC platelets exposed significantly more $\alpha_{\text{IIb}}\beta_3$, more CD36, and much more CD63 epitopes ($P<0.01$ for all), which corresponds with the phenotype of activated platelets. The platelet size was smaller in ECC patients than in control subjects ($P<0.01$). P-selectin was not detected at the surface of the ECC platelets.

**Discussion**

Several biological abnormalities have been reported to characterize PPH patients; these abnormalities could affect endothelial integrity or functions (endothelin-1, NO, prostacyclin, thromboxane A₂, serotonin, thrombomodulin, and plasminogen activator inhibitor type 1), platelet functions (NO, prostacyclin, thromboxane A₂, and serotonin), and antithrombotic or fibrinolytic balance (NO, prostacyclin, thromboxane A₂, serotonin, thrombomodulin, and plasminogen activator inhibitor type 1). However, it remains unclear whether these abnormalities play a role in the genesis of the disease or are a simple consequence of the PPH. If these biological changes are a consequence of PPH, they should tend to return to normal values when patients are successfully treated. The lack of effect on the biological parameters of such an efficient treatment would not help to distinguish predisposing factors from markers of a consequence not significantly affected by the treatment. We and others have found convergent data suggesting that plasma 5-HT was elevated in patients with
various types of pulmonary hypertension. The development of a pulmonary hypertension in a patient with a congenital thrombocytopenia, characterized by a defect in the platelet 5-HT storage capacities and the consequent increase of plasma 5-HT, argued for a potential involvement of plasma 5-HT in the development of pulmonary hypertension. Elevated plasma 5-HT associated with a decreased platelet content in patients with PPH was not corrected by cardiopulmonary transplantation, suggesting that elevated plasma 5-HT was not the mere consequence of elevated pulmonary pressures. This hypothesis makes sense, inasmuch as serotonin, normally concentrated in the platelet-dense granules and almost absent in plasma, could be increased in its plasma pool in PPH (by defective platelet uptake or by active release from activated platelets) and stimulate pulmonary arterial vasoconstriction and the proliferation of arterial wall smooth muscle cells (see Reference 25 for review).

Alternatively, high levels of 5-HT could result from an impaired endothelial metabolism, but that would lead to a decrease of 5-HIAA. To pursue this line of investigation, we conducted the present study in PPH patients treated with continuous epoprostenol infusion, a treatment for PPH that promotes vasodilatation of the pulmonary arteries and prevents platelet activation. Twelve patients of the 16 included in the present study showed clinical and hemodynamic improvement after 1 month of therapy, but 1 patient markedly deteriorated, and her treatment was discontinued.

Because plasma 5-HT results from an equilibrium among 5-HT synthesis, platelet uptake and storage, and metabolism, we focused our attention on 5-HT and its main metabolite (5-HIAA) and on markers of platelet activation. Therefore, we measured 5-HT levels in whole blood, plasma, and platelets as well as 5-HIAA in plasma before epoprostenol treatment, which is an argument in favor of a normal or even increased 5-HT metabolism.

An impaired metabolism of circulating 5-HT can be hypothesized, considering that lung endothelial cells control the serotonin clearance through their metabolism of 5-HT to 5-HIAA. Histological and biochemical studies have indicated that the arterial endothelium suffers damage in PPH, which results in modifications of several aspects of endothelial metabolism: impaired coagulation and fibrinolysis, impaired regulation of vascular tonus with an increase of vasoactive substances such as endothelin-1 or thromboxane, and a decrease of vasoactive compounds such as NO and prostacyclin. The monoamine oxidase enzymatic system could also be damaged, which would lead to a decreased 5-HT metabolism. However, this hypothesis is not sustained by the present measurements of plasma 5-HIAA. Despite the absence of correlation between plasma 5-HT and 5-HIAA concentrations, the mean plasma 5-HIAA was above normal values in PPH patients before and during epoprostenol treatment, which is an argument in favor of a normal or even increased 5-HT metabolism.

The second hypothesis that could explain the elevated levels of plasma 5-HT is that 5-HT is insufficiently taken up and stored by the platelets or abnormally released from dense granules of activated platelets. Our previous finding of a low 5-HT platelet content could sustain the hypothesis of an abnormal platelet function, but this has been confirmed in only 2 of the 16 present patients. We must draw attention to the large individual variability in the 5-HT platelet content in PPH patients. This variability is unusual in control subjects and could reflect in PPH patients a reduced kinetics of uptake of plasma 5-HT, an ability of the platelets to release their 5-HT content, or a slowly reacting endothelial metabolism. A theoretical calculation shows that the release of only 10% of

### TABLE 2. Quantification of Several Glycoprotein Epitopes on Platelets

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (PPH)</th>
<th>Group 2 (Control)</th>
<th>Group 3 (ECC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 10</td>
<td>Day 40</td>
</tr>
<tr>
<td>Platelet volume, fl</td>
<td>9.3±1.1</td>
<td>8.9±1.3</td>
<td>8.4±1.2</td>
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<tr>
<td>αIIβ3, epitope/plt</td>
<td>41 300±7 140</td>
<td>42 350±12 360</td>
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<tr>
<td>CD36/Gp IV, epitope/plt</td>
<td>11 590±3 080</td>
<td>10 160±4 880</td>
<td>8 660±3 520</td>
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<tr>
<td>CD63, epitope/plt</td>
<td>2040±1 400</td>
<td>1470±8 50</td>
<td>1990±11 20</td>
</tr>
</tbody>
</table>

Gp indicates glycoprotein; plt, platelet.

*Significantly different compared with group 2 (P<0.05).
circulating platelets of their 5-HT content (in the absence of immediate uptake by the platelets or metabolism by the endothelium) would be sufficient to increase plasma 5-HT by 40 nmol/L without significantly diminishing the mean platelet content (40 nmol/L corresponds to the total 5-HT storage in $20 \times 10^9$ pt/L). This $\approx 10\%$ of activated and degranulated platelets could be detected ex vivo by flow cytometry.\(^{35}\) Therefore, to investigate the hypothesis of a platelet activation (either a slow level of activation affecting the whole platelet population or a stronger activation of a subpopulation of platelets with positive activation markers), we used a flow cytometric technique and followed the evolution of the parameters during a long-term epoprostenol infusion.

When intense platelet stimulation occurs, $\alpha_{IIb} \beta_3$ and CD36 are redistributed, and surface expression increases, corresponding to the membrane exposition of the intracellular granular pool.\(^{16,17}\) Two additional proteins normally absent from the platelet surface, P-selectin (GMP-140 and CD62P), abundant in $\alpha$-granules, and CD63, a lysosomal component, are exposed on the plasma membrane after secretion of granular contents.\(^{18,19}\) At day 0, normal CD36 and $\alpha_{IIb} \beta_3$ epitopes were both correlated with the platelet size. Neither P-selectin nor CD63 was expressed at the membrane. There were neither global signs of platelet activation nor the presence of a subset population of activated platelets. Moreover, the number of CD36 epitopes decreased when the patients received epoprostenol, indicating that this drug effectively prevented platelet activation.

When these measurements were performed in patients undergoing cardiopulmonary bypass (a condition known to dramatically induce platelet activation), $\alpha_{IIb} \beta_3$ and CD36 were significantly elevated, and CD63 was very high in the whole platelet population. P-selectin was not detected, but it is also known that platelets exposing this epitope rapidly adhere to monocytes and endothelial cells and are removed from the circulation. Additionally, this membrane adhesive protein is rapidly cleaved in vivo from the platelet surface and becomes soluble, which explains the absence of labeling by specific antibodies.\(^{36}\)

5-HT was in the normal range in plasma and in platelets from ECC patients, even though it is well known and confirmed in the present study that the platelets are massively activated and consumed during cardiopulmonary bypass. In ECC patients, a rapid adaptability of the remaining platelets and of the endothelial metabolism of 5-HT appears sufficient to obtain a rapid clearance of the plasma 5-HT released from the destroyed platelets. That also indicates that the mechanisms leading to elevated plasma 5-HT are not the simple consequence of platelet activation and that the abnormality in PPH patients could be related to a 5-HT defective uptake of the platelets or a defective endothelial metabolism. A severe defect of platelet 5-HT uptake is disregarded because of the normal platelet 5-HT content found in 14 of the present 16 patients, the absence of any mutation detected in the coding regions of the 5-HT transporter gene\(^{25}\) of fawn-hooded rats, and the reported induction of the 5-HT transporter by hypoxia in rat pulmonary vascular smooth muscle cells.\(^{38}\) A generalized defect of 5-HT metabolism is disregarded because of the elevated 5-HIAA level. The reasons for high levels of plasma 5-HT in PPH patients could be a slow uptake of 5-HT by platelets (as could occur in the kinetic change in 5-HT transport mechanisms), an easy release of 5-HT from platelets, or the slow kinetics of endothelial metabolism.

Furthermore, the severity of PPH and the beneficial effect of treatment by epoprostenol cannot be predicted by the level or the change in the level of plasma 5-HT. This suggests that raised concentration of 5-HT in plasma could participate to the initiation and the evolution of the mechanisms leading to increased pulmonary arterial pressure during PPH and that it is not a direct consequence of these circulatory changes. Investigations of the kinetics of 5-HT uptake by platelets and of 5-HT metabolism by endothelial cells and the determination of smooth muscle cell sensitivity to 5-HT are necessary to proceed further in the understanding of the increase of plasma 5-HT in PPH patients. Our data suggest that inhibition of platelet function does not play a pivotal role in PPH patients treated with continuous epoprostenol therapy and that epoprostenol acts mainly as a potent vasodilator in this setting.\(^{39}\)

In conclusion, neither platelet activation nor a generalized deficit of the 5-HT endothelial metabolism explains the high level of plasma 5-HT encountered during PPH. The 5-HT plasma concentration is not a predictive marker of the severity of PPH, and its evolution is independent of the clinical and hemodynamic status. Treatment by a potent antiaggregating agent, epoprostenol, does not prevent further increases of plasma 5-HT, despite a therapeutic benefit. Because of the key role of platelets in determining the distribution of 5-HT among the various circulating pools, we favor the hypothesis that kinetic change in 5-HT uptake by platelets may explain the raised 5-HT plasma level in PPH patients. Alternatively or together, an altered pulmonary clearance of 5-HT would also raise plasma 5-HT. This increase could predispose patients to environmental exposures, such as appetite suppressants. Why PPH patients display elevated 5-HT plasma levels remains unclear but might be due to a genetic predisposition of these individuals. Finally, the increased concentration of plasma 5-HT might be a genetically linked but inactive marker of the etiologic mechanism(s) of PPH.

References


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