High Plasma Serotonin Levels in Primary Pulmonary Hypertension
Effect of Long-Term Epoprostenol (Prostacyclin) Therapy

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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 ($\alpha_{IIb}\beta_3$, 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 versus 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 versus 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 $\alpha_{IIb}\beta_3$ and CD36 epitopes. When compared with control platelets, the number of $\alpha_{IIb}\beta_3$ epitopes detected on PPH platelets at day 0 tended to be higher, but this difference did not reach a statistical significance ($41\,300\pm7140$ for PPH patients versus $36\,010\pm3930$ for control subjects, $P=0.069$). The number of CD36 epitopes, in the range of controls at day 0 ($11\,590\pm5080$ for PPH patients versus $11\,900\pm1790$ 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

Primary pulmonary hypertension (PPH) occurs as a familial[1] 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 δ-storage granules.[10] 5-HT circulates mainly as a

TABLE 1. Clinical Data for Group 1

Patient	Sex/Age	Risk Factors and Relevant Clinical Information	mPAP, mm Hg (Day 0/Day 40)	CI, L · min^{-1} · m^{-2} (Day 0/Day 40)	TPR (PAP/CI), U/m^2 (Day 0/Day 40)
1	M/45 y	Appetite suppressant, systemic hypertension	77/52	1.70/1.92	45.3/27.1
2	F/55 y	Appetite suppressant	65/61	1.80/2.70	36.1/22.6
3	F/35 y	. . .	84/70	1.02/1.90	82.3/36.8
4	F/38 y	Appetite suppressant	72/. . .	2.54/. . .	28.3/. . .
5	F/30 y	. . .	53/54	2.35/2.39	22.6/22.6
6	F/59 y	Appetite suppressant	58/58	2.01/2.50	28.4/23.2
7	M/42 y	HIV, HCV	51/48	2.06/1.82	24.8/26.4
8	F/30 y	Appetite suppressant	72/62	1.81/1.82	39.8/34.1
9	F/64 y	Scleroderma	57/52	2.75/2.7	20.7/19.3
10	M/33 y	. . .	88/75	1.70/1.80	51.8/41.7
11	F/60 y	Systemic hypertension	50/42	2.05/2.28	24.4/18.4
12	F/48 y	Appetite suppressant, systemic hypertension	64/57	1.99/1.94	32.2/29.8
13	F/36 y	. . .	71/61	2.80/2.87	25.4/21.3
14	F/33 y	Familial PPH	51/51	2.50/3.0	20.4/17.0
15	M/30 y	. . .	102/70	3.28/3.39	31.1/20.6
16	M/55 y	. . .	76/66	1.70/1.99	44.7/33.2
Mean	0.45*/43.3 y	Appetite suppressant (6 of 16)	67.9/58.6	2.10/2.33	35.3/26.3

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

*Sex ratio.

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.[11] All these effects are transduced through specific cell membrane receptors.

In a case report describing a patient affected by a rare hereditary thrombocytopathy (a platelet δ-storage pool disease) who developed PPH, an increase of plasma 5-HT associated with pulmonary hypertension was first described.[12] Indeed, 5-HT was found to be increased in plasma obtained from patients with PPH.[13] 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.[14] The hypothesis of a platelet activation in PPH patients was also supported by the clinical efficacy of treatment for PPH with prostacyclin or epoprostenol,[15] a potent vasodilator participating in the decrease of pulmonary arterial pressure. Because prostacyclin is also a potent inhibitor of platelet activation, epoprostenol treatment might reduce platelet release of 5-HT and, consequently, the plasma 5-HT level. Therefore, we evaluated the degree of platelet activation by measurement of the number of $\alpha_{IIb}\beta_3$ and CD36 (2 glycoproteins implicated in platelet aggregation) epitopes as well as those of CD63 and P-selectin (2 proteins normally absent from the platelet surface but exposed after secretion of granular contents) expressed at the surface membrane of the platelet,[16–19] and we determined the evolution of 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.

Platelet count and volume were measured (STKS Coultronics) in citrated and EDTA blood, respectively. Platelet rich plasma was prepared by centrifugation of citrated blood at 120*g* for 10 minutes. Platelets were counted, and platelet-rich plasma was further centrifuged at 2000*g* 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. 5-HT and 5-HIAA levels were measured by radioenzymology[21] and high-performance liquid chromatography,[22] as previously described.

Flow Cytometric Analyses

Flow cytometric analyses were accomplished by use of the following: mouse monoclonal antibodies, consisting of P-selectin–FITC (CLBThromb/6), $\alpha_{IIb}\beta_3$ (P2), CD36, and CD63 (Immunotech); goat anti-mouse (GAM), consisting of anti–Fc-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 CaCl$_2$-2H$_2$O, 1 mmol/L MgCl$_2$-6H$_2$O, 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/mL) 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 250*g*, 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-microliter 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.

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/m^2) 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 I).
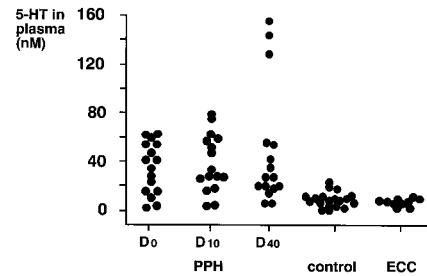

Figure 1. Individual plasma 5-HT concentrations in PPH patients (group 1) before (day 0 [D0]) and at days 10 and 40 (D10 and D40, respectively) of a continuous intravenous infusion of epoprostenol compared with concentrations in control subjects (group 2) and in patients studied after ECC (group 3).

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
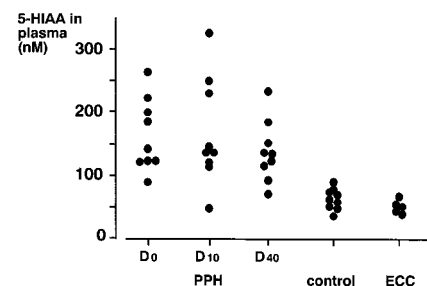

Figure 2. Individual plasma 5-HIAA concentrations in PPH patients (group 1) before (D0) and at D10 and D40 of a continuous intravenous infusion of epoprostenol compared with concentrations in control subjects (group 2) and in patients studied after ECC (group 3).

Figure 3. Individual platelet 5-HT concentrations in PPH patients (group 1) before (D0) and at D10 and D40 of a continuous intravenous infusion of epoprostenol compared with concentrations control subjects (group 2) and in patients studied after ECC (group 3).
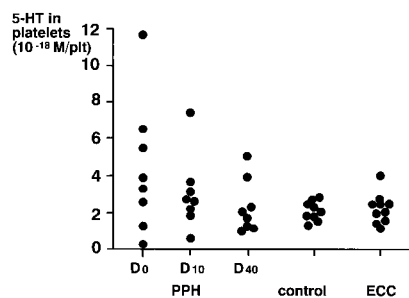
patients (Nos. 2 and 7), the platelet 5-HT level was low at day 0 (0.2 and 1.2 amol/plt, respectively) but was in the normal range at day 40 (Figure 3). Mean whole blood 5-HT concentration was within the normal range and remained unchanged at days 10 and 40. As in the control subjects, there was a positive 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 ($105\pm39\times10^9$ platelets per liter [plt/L], $P<0.05$) than did the PPH patients at day 0 ($140\pm63\times10^9$ plt/L, $P<0.05$ versus controls, because of 8 thrombopenic patients, ie, patients with a platelet count $<150\times10^9$ plt/L) and the control subjects ($232\pm51\times10^9$ 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
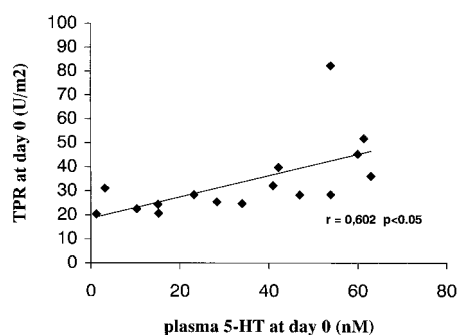

Figure 4. Significant correlation between plasma 5-HT concentrations and TPR in PPH patients (group 1) before (D0) continuous intravenous infusion of epoprostenol.

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 $\alpha_{IIb}\beta_3$ ($r=0.49$, $P<0.001$) and CD36 ($r=0.36$, $P=0.01$) epitopes. The number of $\alpha_{IIb}\beta_3$ epitopes detected on PPH platelets compared with control platelets at day 0 did not reach a statistical significance ($41\,300\pm7140$ versus $36\,010\pm3930$, respectively; $P=0.069$). The number of CD36 epitopes, normal at day 0 ($11\,590\pm5080$ for PPH platelets versus $11\,900\pm1790$ 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[23] 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 $\alpha_{IIb}\beta_3$, 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 A_2, serotonin, thrombomodulin, and plasminogen activator inhibitor type 1), platelet functions (NO, prostacyclin, thromboxane A_2, and serotonin), and antithrombotic or fibrinolytic balance (NO, prostacyclin, thromboxane A_2, 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

TABLE 2. Quantification of Several Glycoprotein Epitopes on Platelets

	Group 1 (PPH)			Group 2 (Control)	Group 3 (ECC)
	Day 0	Day 10	Day 40		
Platelet volume, fL	9.3±1.1	8.9±1.3	8.4±1.2	8.5±0.7	7.3±0.8*
$\alpha_{II}\beta_3$, epitope/plt	41 300±7140	42 350±12 360	37 880±8360	36 010±3930	43 130±5480*
CD36/Gp IV, epitope/plt	11 590±5080	10 160±4880	8660±3520*	11 900±1790	17 410±2850*
CD63, epitope/plt	2040±1400	1470±850*	1990±1120*	2190±370	8680±2200*

Gp indicates glycoprotein; plt, platelet.

*Significantly different compared with group 2 ($P<0.05$).

various types of pulmonary hypertension. The development of a pulmonary hypertension in a patient with a congenital thrombocytopathy, 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.[13] This hypothesis makes sense, inasmuch as serotonin, normally concentrated in the platelet-dense granules and almost absent in plasma,[24] 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[14] 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.[15] 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 was started and 10 and 40 days after the onset of treatment.

We confirmed previous results in this new group of PPH patients, including elevated plasma 5-HT levels in 13 of the 16 studied PPH patients with a whole blood 5-HT content in the normal range, but contrary to our previous results, a decreased platelet 5-HT content was observed in only 2 patients. This increase in plasma 5-HT was characteristic of PPH patients, with the mean plasma 5-HT concentration remaining significantly elevated during epoprostenol therapy, but there were large individual variations. Among the 12 patients who underwent a beneficial hemodynamic response to epoprostenol, 7 increased their plasma 5-HT levels, and in 3 patients, the plasma 5-HT levels returned to normal range. The therapeutic benefit was thus not associated with normalization of plasma 5-HT, and we conclude that plasma 5-HT is independent of the clinical improvement. Furthermore, in the

absence of treatment, the mPAP did not correlate with the plasma 5-HT level. By contrast, TPRs were positively correlated with plasma 5-HT levels. The exact meaning of such a correlation remains unclear. On one hand, this may reflect a direct effect of 5-HT on human small muscular pulmonary arteries through 5-HT receptors, possibly of the 1B[26] and/or of the 2B[27] subtype(s), as recently suggested. On another hand, this TPR–plasma 5-HT correlation disappeared when patients were treated either with heart-lung transplantation[13] or continuous intravenous epoprostenol therapy (the present study). Obviously, additional studies are needed to firmly assert the positive correlation observed between plasma 5-HT levels and hemodynamic parameters of PPH severity, such as TPR. In the genesis of this elevated plasma 5-HT in PPH patients, an abnormal metabolism by the endothelium or a decreased uptake and storage or an increased release by the platelets have to be explored.

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,[28] which results in modifications of several aspects of endothelial metabolism: impaired coagulation[29] and fibrinolysis,[30] impaired regulation of vascular tone with an increase of vasoactive substances such as endothelin-1 or thromboxane, and a decrease of vasorelaxing compounds such as NO and prostacyclin.[31–34] 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

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 20×10^9 plt/L). This $\leq 10\%$ 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 all 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 α-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[37] 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 case of 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.

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Arteriosclerosis, Thrombosis, and Vascular Biology

High Plasma Serotonin Levels in Primary Pulmonary Hypertension: Effect of Long-Term Epoprostenol (Prostacyclin) Therapy

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