Increased Plasma Serotonin in Primary Pulmonary Hypertension

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PURPOSE: Pulmonary hypertension can occur in patients who have disorders associated with altered platelet serotonin storage, including collagen vascular disease and platelet storage pool disease. We tested the hypothesis that primary pulmonary hypertension (PPH) may be also associated with impaired handling of serotonin by platelets, resulting in increased plasma serotonin levels.

PATIENTS AND METHODS: We used radioenzymatic assays to measure serotonin in platelets and plasma and serotonin released during in vitro platelet aggregation in 16 patients with PPH, and in 16 normal controls matched for age and sex. Six patients were restudied after heart-lung transplantation to determine whether serotonin abnormalities persisted after pulmonary arterial pressure returned to normal.

RESULTS: Patients had decreased platelet serotonin concentration ($1.8 \pm 0.6 \times 10^{-18}$ mol/platelet versus $3.2 \pm 0.2 \times 10^{-18}$ mol/platelet in controls; $P < 0.01$) and increased plasma serotonin concentration ($30.1 \pm 9.2 \times 10^{-9}$ mol/L versus $0.6 \pm 0.1 \times 10^{-9}$ mol/L in controls; $P < 0.001$). Serotonin released during in vitro platelet aggregation was higher in patients than in controls. After heart-lung transplantation, platelet serotonin concentrations remained decreased and plasma levels remained increased.

CONCLUSIONS: Abnormal handling of serotonin by platelets leading to an increase in plasma serotonin occurs in PPH. The persistent decrease in platelet storage of serotonin after heart-lung transplantation suggests that this platelet abnormality is not secondary to PPH.

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The causes of primary pulmonary hypertension (PPH) are unknown. Numerous pathological conditions have been described in association with PPH.[1] Analysis of these disorders may shed light on cellular and/or humoral mechanisms involved in the development of PPH. Abnormalities in platelet-vascular wall interaction are among these mechanisms.[2,3] Platelets may release vasoactive substances that promote constriction of small pulmonary arteries and factors that stimulate cellular proliferation within the pulmonary arterial wall, which are both characteristic features of PPH. Among these substances, serotonin is the predominant mediator of pulmonary vasoconstriction caused by aggregating platelets,[4] and has been shown to increase pulmonary vascular smooth muscle proliferation.[5,6] Under normal conditions, the pulmonary vascular bed is not exposed to excessive plasma serotonin because of the ability of platelets to store large amounts of serotonin. However, altered platelet serotonin storage has been reported in various disorders that are occasionally associated with pulmonary hypertension, including Raynaud's phenomenon,[7] collagen vascular disease,[8] use of the appetite suppressant fenfluramine,[9,10] and platelet storage pool disease.[11]

These observations prompted us to investigate whether abnormalities in platelet storage of serotonin occur in patients with PPH. We measured (1) serotonin concentrations in whole blood, platelets, and plasma and (2) serotonin release by platelets during in vitro aggregation, in patients with PPH and normal controls matched for sex and age. Six patients who underwent heart-lung transplantation for severe PPH were studied again after transplantation in order to determine whether serotonin abnormalities persisted after pulmonary arterial pressure returned to normal.

PATIENTS AND METHODS

Patients

Sixteen patients with suspected PPH were referred to the Hospital Antoine Béclère between January 1985 and December 1988. Pulmonary hypertension was defined as a mean pulmonary arterial pressure >25 mm Hg at rest. The diagnosis of PPH was accepted only when no cause was demonstrated by the chest roentgenogram, pulmonary function studies, blood gas measurements, echocardiography, perfusion lung scan, cardiac catheterization, and biplane selective pulmonary angiography. No patient had

TABLE

Concentrations of Serotonin in Plasma in Patients With Primary Pulmonary Hypertension

Patient No.	Age (y)	Sex	Associated Disease	Mean PAP (mm Hg)	Plasma Serotonin ($\times 10^{-9}$ mol/L)
1*	27	F		58	11
2*	39	M	Familial	61	15
3	24	M	Familial	88	12
4	28	M	Fenfluramine	110	7
5*	45	F	Raynaud's phenomenon	75	40
6*	35	F		65	3
7	53	F	Antinuclear antibody >1/100	48	2
8	35	F	Fenfluramine	50	21
9	54	M		50	14
10*	46	M		46	40
11	49	F	Fenfluramine	66	50
12*	36	F		47	139
13	36	F	Antinuclear antibody >1/200	55	11
14	50	F	Raynaud's phenomenon	45	71
15	54	M	Antinuclear antibody >1/300	47	18
16	63	M		47	8

*Patient studied before and after heart-lung transplantation.
PAP = pulmonary arterial pressure.

clinical evidence of collagen vascular disease. Clinical characteristics and mean pulmonary arterial pressure values in each patient are given in the **Table**. All were nonsmokers.

Six of the 16 patients with PPH were studied before and after heart-lung transplantation. The mean time from transplantation to reevaluation was 350 ± 30 days. Immunosuppressive treatment in transplant recipients consisted of prednisone (0.2 ± 0.03 mg/kg per day), cyclosporin (8 mg/kg per day) and azathioprine (1.5 mg/kg per day). Sixteen normal nonsmokers matched for age and sex comprised the control group.

Hemodynamic Studies

All 16 patients with PPH underwent right-sided cardiac catheterization. All vasodilators were discontinued prior to the study. A floating triple-lumen thermodilution catheter was advanced into the pulmonary artery to record pulmonary arterial and occlusion pressures. Cardiac output was estimated by averaging three successive thermodilution determinations, and converted to cardiac index (L/min/m^2). Pulmonary arterial resistance was calculated as the difference between mean pulmonary arterial and pulmonary occlusion pressures divided by cardiac index (mm Hg/L/min/m^2).

Platelet Aggregation and Serotonin Measurements

Patients refrained from taking aspirin or any vasodilator for 10 days preceding the study. Blood was collected after an overnight fast following a 48-hour diet free of the main foods containing serotonin or tryptophan (banana, grapefruit, tomato, chocolate, nuts, etc.). Blood was drawn with minimal venostasis from a peripheral vein into a plastic tube. One blood sample was drawn on heparin (25 U/mL) for determination of whole blood serotonin, and one on sodium citrate (3.8%) for all other tests. Blood samples were brought to the laboratory for analysis within 2 hours of collection. Platelet-rich plasma and platelet-poor plasma were prepared by centrifugation for 15 minutes at 300*g* and 2,000*g*, respectively, at 18°C. For all aggregation experiments, platelet counts in platelet-rich plasma were adjusted to 3×10^5 platelets/μL. Platelet counts were done using a Coulter counter S6 (Coultronic, Margency, France).

Platelet aggregation which was stimulated by epinephrine (2.4×10^{-6} mol), adenosine diphosphate (ADP) (1.2×10^{-6} mol), collagen (12.5 mg/L), and arachidonic acid (5×10^{-4} mol) was studied in platelet-rich plasma (turbidimetric technique) using a chronolog dual-channel aggregometer (Coultronic).

After an ethanol-acetone extraction, serotonin levels were determined in whole blood (expressed in 10^{-6} mol/L), platelet-rich plasma (expressed in 10^{-18} mol/platelet), and platelet-poor plasma (expressed in 10^{-9} mol/L) using a radioenzymatic assay.[12] This method was based on the conversion of serotonin to tritiated melatonin by a two-step reaction: (1) N-methylation of serotonin to form N-acetylserotonin by acetyl coenzyme A in the presence of partially purified serotonin-N-acetyltransferase from Drosophila, and (2) transfer of a tritiated methyl group from ^{3}H-Same, 11.1 Ci/mmol (NEN, Boston, Massachusetts) to the hydroxyl group of N-acetylserotonin by semi-

purified hydroxynolole-O-methyltransferase from bovine pineal gland (Collectorgane, Paris, France). The residues were carefully dissolved in HCl 10^{-3} mol ethanol (1:4, v/v) and spotted on predried 250 µm-thick Whatman silica-gel plates (LK 5DF, Poly Labo, Strasbourg, France).

After developing the chromatogram in toluene/ethyl acetate/glacial acetic acid (27:68:5, v/v/v), the radioactivity of the melatonin spot was quantified using a thin-layer chromatography radioactivity scanner (LB 2832, Berthold, Wildblad, Germany). Samples were analyzed in duplicate with ^{14}C-serotonin binoxalate as an internal calibrator. The detection limit was 0.02 ng/mL, and inter-assay and intra-assay coefficients of variation were 5.8% ± 1.4% (n = 12) and 4.3% ± 1.2% (n = 34), respectively. Accuracy was evaluated by supplementing to a platelet-poor plasma sample with a known amount of serotonin, up to 100 nmol (recovery 98% ± 3%; n = 8). Linear correlation of disintegrations per minute and serotonin concentrations was highly significant (r = .99; P <0.01).

Amounts of serotonin released by platelets (expressed in 10^{-8} mol/platelet) during aggregation induced by epinephrine (2.4×10^{-6} mol), ADP (1.2×10^{-6} mol), and collagen (12.5 mg/L) were measured using the same radioenzymatic assay. Briefly, at peak platelet aggregation, the platelet-rich plasma was centrifuged at 12,000g for 1 minute and the supernatant was then analyzed for serotonin release. Serotonin release was studied in 10 patients with PPH and 10 matched controls.

Analysis of Results

All results are expressed as mean ± standard error of the mean. Comparisons between patients with PPH and controls were done using the nonparametric Mann-Whitney U test[13] with a statistical package for personal computers (SPSS Inc., Chicago, Illinois). Comparisons between pretransplantation and posttransplantation values were done using the nonparametric Wilcoxon signed rank test.[13] Correlations between data were evaluated using the nonparametric Spearman's rank order correlation test.[13]

RESULTS

Clinical and Hemodynamic Profiles

Clinical characteristics of the 16 patients with PPH are shown in the Table. They were 7 men and 9 women with an age range of 24 to 63 years. An associated disorder was found in 8 patients: primary Raynaud's phenomenon (2); chronic use of the appetite suppressant fenfluramine (3); positive antinuclear-antibody titers without overt symptoms of collagen vascular disease (3). Two patients had familial histories of pulmonary hypertension. Mean pulmonary arterial pressure was 59 ± 4 mm Hg, mean cardiac index 2.2 ± 0.1 L/min/m^2, and mean pulmonary arterial resistance 25 ± 3 mm Hg/L/min/m^2. Vital capacity was 90% ± 5%, and forced expired volume in 1 second (FEV_1) 94% ± 6% of the predicted value. Determination of arterial blood gas values while patients breathed room air disclosed a mean PaO_2 of 67 ± 2 mm Hg, a mean $PaCO_2$ of 29 ± 2 mm Hg, and a mean pH of 7.47 ± 0.04.

In the 6 patients tested after heart-lung transplantation, pulmonary hemodynamic values were normal. The mean pulmonary arterial pressure was 14 ± 2 mm Hg, mean cardiac index 3.5 ± 0.2 L/min/m^2, and mean pulmonary arterial resistance 2.8 ± 0.5 mm Hg/L/min/m^2. At the time of reevaluation, all transplant recipients had normal lung histologic findings in transbronchial biopsy specimen. Mean vital capacity was 75% ± 7%, and mean FEV_1 78% ± 5% of the predicted value. Blood gas determinations while the patient breathed room air disclosed a mean PaO_2 of 78 ± 3 mm Hg, a mean $PaCO_2$ of 32 ± 2 mm Hg, and a mean pH of 7.46 ± 0.03.

Platelet Study

All patients had blood platelet counts in the normal range with a mean value of 290 ± 25 × 10^9/L. Platelet aggregation in response to epinephrine, ADP, collagen, and arachidonic acid were similar in patients with PPH and controls (not shown).

Serotonin Measurements

In the patients with PPH, whole blood concentration of serotonin was not different from control subjects (0.40 ± 0.07 × 10^{-6} mol/L versus 0.48 ± 0.02 × 10^{-6} mol/L). Serotonin concentration in platelets was lower in patients with PPH as compared to controls (1.8 ± 0.6 × 10^{-18} mol/platelet versus 3.2 ± 0.2 × 10^{-18} mol/platelet; P <0.005) (**Figure 1**). The serotonin level in patients' plasma was higher than in controls' (30.1 ± 9.2 × 10^{-9} mol/L versus 0.6 ± 0.1 10^{-9} mol/L, P <0.001) (**Figure 2**, Table I). In the 11 patients with pulmonary hypertension without associated collagen vascular disease or history of drug ingestion, serotonin concentrations in platelet and plasma were not different from the 5 patients with PPH and Raynaud's phenomenon or fenfluramine intake (2.0 ± 0.6 × 10^{-18} mol/platelet versus 1.32 ± 0.2 × 10^{-18} mol/platelet, and 26.6 ± 15.0 × 10^{-9} mol/L versus 37.8 ± 9.4 × 10^{-9} mol/L, respectively).

In all patients, serotonin concentration in plasma was inversely correlated to serotonin concentration in platelet (P <0.05). The amount of serotonin released during platelet aggregation in response to epinephrine, ADP, and collagen was greater in patients than in controls (P <0.02, P <0.05, P <0.001, respectively) (**Figure 3**).

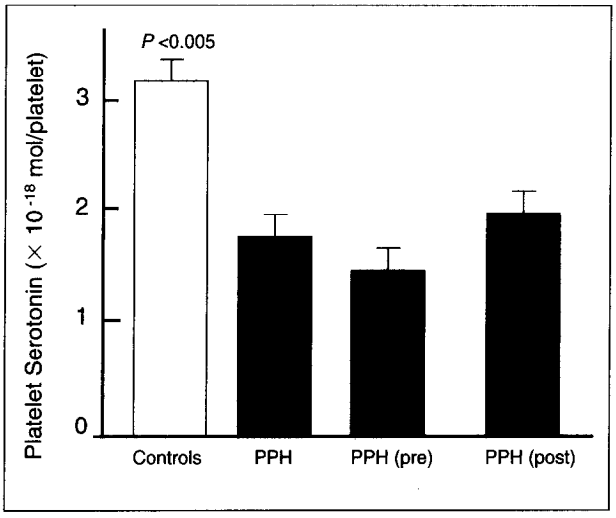

Figure 1. Mean (± standard error of the mean) concentration of serotonin in platelet in 16 controls (**open bar**) and 16 patients with primary pulmonary hypertension (PPH) (**closed bars**), of whom 6 were studied before (pre) and after (post) heart-lung transplantation.

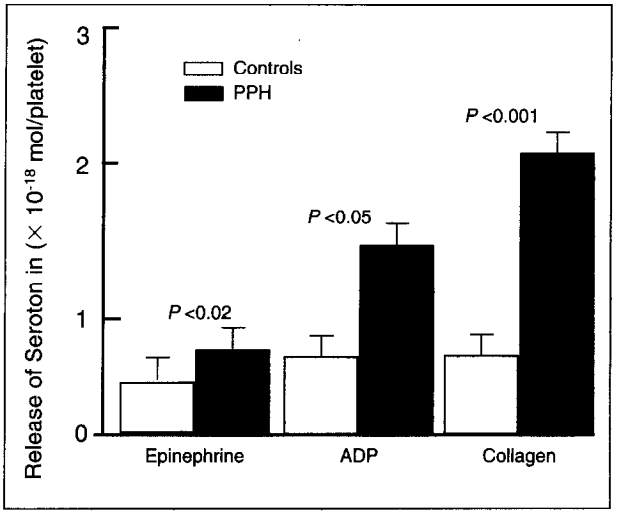

Figure 3. Mean (± standard error of the mean) platelet release of serotonin (expressed in 10^{-18}mol/ platelet) during in vitro aggregation triggered by epinephrine, adenosine diphosphate, or collagen in 10 controls (**open bars**) and 10 patients with PPH (**closed bars**).

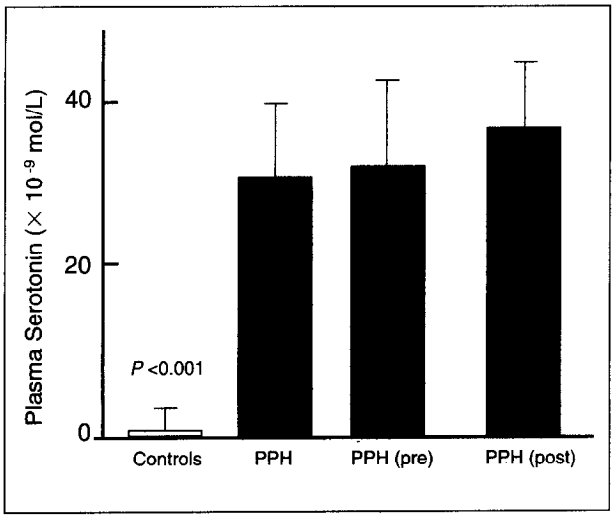

Figure 2. Mean (± standard error of the mean) concentration of serotonin in plasma in 16 controls (**open bar**) and 16 patients with primary pulmonary hypertension (PPH) (**closed bars**), of whom 6 were studied before (pre) and after (post) heart-lung transplantation.

In the patients with PPH studied before and after heart-lung transplantation, platelet and plasma serotonin concentrations after transplantation (1.9 ± 0.7 × 10^{-18} mol/platelet and 36.7 ± 10.1 × 10^{-9} mol/L) were not different from pretransplant values (1.5 ± 0.6 × 10^{-18} mol/platelet and 32.3 ± 13.0 × 10^{-9} mol/L) (Figures 1 and 2).

DISCUSSION

Our study shows that patients with PPH had a large increase in plasma serotonin concentrations. This finding raises questions concerning the mechanisms of the increase in plasma serotonin. Blood serotonin is produced in the enterochromaffin cells of the intestine. Virtually all blood serotonin is stored in the platelets. Free serotonin in plasma is rapidly metabolized by the endothelial monoamine oxidase enzymatic system in the liver and lung.[14] Under normal conditions, however, the pulmonary vascular bed is not exposed to excessive serotonin levels, because of its position as a secondary filter located downstream from the liver, and because of the ability of platelets to store large amounts of serotonin.

Increased concentrations of free serotonin in plasma might result from excessive production by the gut and/or decreased lung endothelial metabolism and/or impaired ability of the platelets to store serotonin.[15] As to the first hypothesis, increased gut production of serotonin was unlikely in our patients because the concentration of serotonin in whole blood was not increased.[15] As to the second, pulmonary serotonin metabolism has been shown to be normal or increased in humans with pulmonary hypertension.[16] The third explanation, abnormal handling of serotonin by the platelets, appears to be consistent with our finding of a decrease in the concentrations of serotonin in platelets in association with correspondingly higher concentrations in plasma.[16] The fact that whole blood serotonin was not increased suggests that the very high plasma serotonin was offset by the reduction in platelet serotonin. The possible mechanisms include a diminished platelet uptake of serotonin and/or increased platelet release. Indeed, release of serotonin during in vitro platelet aggregation was increased in patients as compared to controls.

The platelet uptake of serotonin was not investigated in this study. However, the impairment in serotonin storage was not associated with an alteration in platelet aggregation, suggesting that other important platelet functions remained unaltered. Such a dissociation in the alterations in platelet function has

been previously reported in patients with PPH. A recent study[17] reported normal platelet factor 4, ß-thromboglobulin, and fibrin formation and dissolution, whereas another study found an increase in urinary excretion of the platelet-derived thromboxane metabolite 11-dehydro-thromboxane B_2.[18] In patients with pulmonary hypertension, it is difficult to differentiate platelet changes with a pathogenetic role from platelet changes secondary to the increased pulmonary pressure. However, several findings from this study and from the literature suggest that abnormalities in platelet serotonin storage may develop independently from pulmonary hypertension.

First, plasma serotonin was still elevated and platelet serotonin remained decreased in our heart-lung transplant recipients with normal lung histology and normal pulmonary arterial pressure. Since experimental studies have found normal serotonin metabolism in lung transplants,[19] this continued increase in plasma serotonin was most likely related to a persistent abnormal handling of serotonin by the platelets rather than to a decrease in serotonin metabolism in the transplanted lung. The immunosuppressive treatment is not a likely explanation of this platelet dysfunction, since patients with emphysema treated by heart-lung transplantation and renal transplant patients receiving the same immunosuppressive regimen as our patients had plasma serotonin concentrations close to the values measured in controls $2.1 \pm 2.5 \times 10^{-9}$ mol/L (n = 4) and $2.7 \pm 0.8 \times 10^{-9}$ mol/L (n = 5), respectively; (JM Launay, PhD, personal communication, 1993).

Second, before the onset of pulmonary hypertension, 5 of our 16 patients had a disorder possibly associated with alterations in platelet storage of serotonin,[7,9] including Raynaud's phenomenon and exposure to the appetite suppressant fenfluramine (Table).

- We recently reported a case of pulmonary hypertension in a patient with a familial platelet serotonin storage disorder leading to high plasma serotonin concentration.[11] In this patient, the platelet disease preceded the development of pulmonary hypertension by more than 20 years.
- Fawn Hooded rats, that have a similar inherited platelet disorder with an increase in plasma serotonin,[20] have a genetically determined idiopathic form of pulmonary hypertension.[21] Conversely, in rats with normal platelet function, monocrotaline-induced pulmonary hypertension failed to alter platelet serotonin metabolism or serotonin levels in plasma and platelets.[22]

Whether altered platelet handling of serotonin contributes to the progressive vascular narrowing in PPH and the attendant increase in pulmonary resistance remains unclear. Studies have suggested that increased circulating free serotonin levels may contribute to pulmonary vascular remodeling by promoting vascular smooth muscle proliferation,[5,6] and to pulmonary arterial vasoconstriction by stimulating 5-HT2 serotonin receptors of the smooth muscle.[4] Furthermore, platelets may release other vasoactive factors and other substances stimulating cellular proliferation together with serotonin.[11,17]

Although platelet dysfunction persisted after heart-lung transplantation, pulmonary arterial pressure remained normal in our transplant recipients. This finding does not exclude the hypothesis that pulmonary hypertension may recur in the long term. Alternatively, it may suggest that the pretransplant pulmonary vascular endothelium and/or underlying smooth muscle were dysfunctional and played a part in the development of PPH, very possibly in combination with serotonin abnormalities. Indeed, in a preliminary study, Brink et al[23] reported that isolated pulmonary artery muscle preparations from 4 of our PPH patients treated by heart-lung transplantation showed increased sensitivity to serotonin as compared with tissue from normal subjects. Furthermore, rats that have a similar platelet serotonin storage disorder exhibit increased pulmonary artery smooth muscle sensitivity to serotonin as compared to normal rats.[24]

In conclusion, our data demonstrate impaired handling of serotonin by platelets responsible for elevated plasma serotonin levels in patients with PPH. The persistent decrease in platelet storage of serotonin after heart-lung transplantation suggests that this platelet dysfunction may develop independently of the pulmonary hypertension. We speculate that this platelet abnormality may contribute to the development of pulmonary hypertension in some patients with dysfunctional pulmonary endothelium and/or underlying smooth muscle.

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