A Role for Potassium Channels in Smooth Muscle Cells and Platelets in the Etiology of Primary Pulmonary Hypertension*

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Plasma serotonin levels are markedly elevated in patients with primary pulmonary hypertension (PPH) and platelet levels of serotonin are low. Furthermore, plasma serotonin levels remain elevated after bilateral lung transplantation, in the absence of any pulmonary hypertension. Dexfenfluramine can cause the anorexigen-induced form of PPH that is clinically and histologically indistinguishable from PPH. We find that dexfenfluramine releases serotonin from platelets and inhibits its reuptake. These observations suggest that serotonin might be involved in, or be a marker for, the mechanism responsible for both forms of PPH. Dexfenfluramine causes inhibition of voltage-sensitive potassium (Kv) channels, membrane depolarization, and calcium entry in pulmonary artery smooth muscle cells and vasoconstriction in isolated perfused rat lungs. We have recently found that dexfenfluramine also inhibits Kv channels in megakaryocytes, the stem cell for platelets. In smooth muscle cells, taken from the pulmonary arteries of PPH patients, Kv channels appear to be dysfunctional. The underlying defect in PPH is likely to be an abnormality of one or more Kv channels in both pulmonary artery smooth muscle cells and platelets. Relatively few patients exposed to dexfenfluramine develop PPH. The factors responsible for susceptibility might be a difference in expression of potassium channels and/or a decrease in the endogenous production of nitric oxide. *(CHEST 1998; 114:2005-2048)*

Primary pulmonary hypertension (PPH) is caused by a combination of vasoconstriction, cellular proliferation, and thrombosis in small vessels. It is not apparent whether endothelial or smooth muscle cell dysfunction occurs first. However, abnormalities of smooth muscle function have recently been described in PPH.1 Smooth muscle cells taken from small pulmonary arteries of patients with PPH were found to be depolarized, relative to cells from patients with secondary pulmonary hypertension, and to have higher cytosolic calcium levels. A blocker of voltage-gated potassium (Kv) channels, 4-aminopyridine, failed to increase calcium in the PPH cells but did so in the secondary pulmonary hypertension cells. These observations focus attention on the possibility that some Kv channels are absent or down-regulated in pulmonary vascular smooth muscle cells of some PPH patients. There is a precedent for the concept that a gene defect can result in the loss of potassium channels and cause human disease. Persistent hyperinsulinemic hypoglycemia of infancy is a condition that occurs because a loss of functional adenosine triphosphate (ATP)-sensitive potassium channels results in increased insulin secretion.2,3

It is important to remember that the mechanisms that determine insulin release by the β-cells of the pancreas and that control smooth muscle tone in the pulmonary artery smooth muscle cell are strikingly similar. In both cells, resting membrane potential is largely regulated by an outward potassium current. Inhibition of this current results in membrane depolarization and calcium entry through voltage-gated L-type calcium channels. In the case of the pancreatic β-cell, glucose metabolism increases ATP, which inhibits ATP-sensitive potassium channels and results in insulin secretion.4 In the case of the pulmonary artery smooth muscle cell, inhibition of a potassium channel by hypoxia,5-7 or a drug such as dexfenfluramine,8 causes pulmonary vasoconstric-
tion. It was the elucidation of the means by which insulin secretion is controlled that provided clues to the mechanism of hypoxic pulmonary vasoconstriction.9 Now, the recognition that the loss of potassium channels in the pancreatic β-cell can lead to dysfunction may point to a similar problem in PPH.

Susceptibility to Developing PPH

Complications related to the use of appetite-suppressant drugs may indicate mechanisms important in the etiology of PPH. Between 1967 and 1972, there was an outbreak of pulmonary hypertension in Austria, Germany, and Switzerland. The condition was clinically and histologically indistinguishable from PPH but seemed to be related to the use of the anorectic agent, aminorex, in those countries during those years. In one study of 582 patients who developed PPH, 61% reported that they had taken aminorex.10 It was estimated that about 0.1% of those who took aminorex developed clinically significant PPH. The conclusion is that those who develop PPH must have some genetic susceptibility to the condition.

A similar epidemic of “primary” pulmonary hypertension has followed the use of another anorectic agent, fenfluramine and its d-isomer, dexfenfluramine. A series of case reports, starting in 1981,11 led to a description of 15 patients in 199312 and finally to an epidemiologic study from 1992 to 1994, which identified 22 patients with PPH who had used fenfluramine or dexfenfluramine.13 The latter study was conducted in France, Belgium, United Kingdom, and the Netherlands, where about 7.3% of the control (without PPH) patients (mean [±SD] age, 45±13 years) had been exposed to a derivative of fenfluramine. Although use of appetite suppressants for >3 months was associated with a marked increase in the risk of developing PPH (odds ratio, 23), the annual incidence of PPH in the population as a whole remained very low (1.7 per million in Belgium). These numbers again indicate that the small number of people who develop PPH on exposure to anorectic agents must have a genetic predisposition to do so. This predisposition could include a variety of factors, such as decreased ability to metabolize the drug, altered expression of potassium or calcium channels, decreased production of endogenous vasodilators (eg, nitric oxide [NO], prostacyclin, and endothelium-derived hyperpolarizing factor), or increased production of endogenous substances promoting vasoconstriction and proliferation (eg, endothelin, serotonin, and thromboxane A2).

Aminorex, fenfluramine, and dexfenfluramine cause consistent slight vasoconstriction in the isolated perfused rat lung, but only at high doses and in the presence of the cyclooxygenase inhibitor, meclofenamate.8 However, the addition of the NO synthase inhibitor, L-NAME, dramatically increases the vasoconstrictor response to dexfenfluramine and a significant rise in pressure is seen at doses as low as 10⁻⁵ M. This level is comparable to plasma concentrations of fenfluramine measured in patients. The observation that the inhibition of endogenous vasodilators makes the lung much more susceptible to the pulmonary hypertensive effects of dexfenfluramine raises the possibility that, in some patients, low NO production might be a factor predisposing to PPH. In fact, while patients with PPH have elevated levels of breath NO,14 these levels are not elevated in patients with fenfluramine-associated pulmonary hypertension, and they are the same as in control subjects.15 It may be that patients with fenfluramine-associated pulmonary hypertension lack the ability to increase endogenous NO in the face of a vasoconstrictor challenge. This would be similar to the impaired synthesis of NO recently reported in some patients with “essential” systemic hypertension.16 The factor(s) predisposing to PPH may not always be the same. A small percentage of HIV-positive patients develop PPH. In this instance, the virus might cause endothelial dysfunction and reduce NO synthesis, while the genetic susceptibility might be a difference in the expression of potassium channels. It is known that there is a difference in the distribution of cells predominated by calcium-sensitive (KCa) and Kv potassium channels between conduit and resistance pulmonary arteries in the rat.17 Consequently, it is not difficult to consider that loss of functional potassium channels or a shift in the distribution of channels in the resistance arteries might predispose to PPH. This would be compatible with the observations of Yuan et al discussed previously. It is also important to remember that chronic membrane depolarization can selectively reduce the expression of some Kv channels.18

Mechanism of Fenfluramine-Induced Pulmonary Vasoconstriction

Fenfluramine induces serotonin release from neurons and inhibits reuptake. As serotonin causes pulmonary vasoconstriction and proliferation of pulmonary vascular smooth muscle,19-22 it is reasonable to consider that serotonin might mediate fenfluramine-induced PPH. This is supported by the observation that plasma serotonin levels are elevated in PPH patients that is unrelated to anorectic agents (30.1±9.2 [SEM] ×10⁻⁹ mol/L), compared with control subjects (0.6±0.1×10⁻⁹ mol/L).23 In the
latter study, plasma serotonin levels were still elevated after heart-lung transplantation, indicating that the high serotonin levels were not merely secondary to the vascular changes present in pulmonary hypertension. The potential importance of serotonin is reinforced by the case report of a patient (age, 46 years) who had platelet storage pool disease since infancy. He developed PPH in adult life and was found to have a 15-fold increase in plasma serotonin level compared with control subjects. The pulmonary hypertension was, in part, reversible by the serotonin antagonist, ketanserin.

Before it is concluded that serotonin is the mediator responsible for fenfluramine-induced PPH, other data should be considered. Although dexfenfluramine is usually thought to cause anorexia by the release of serotonin in the brain, effective inhibition of serotonin synthesis does not prevent the anorectic effect of dexfenfluramine. Furthermore, a dose of the metabolite D-norfenfluramine, which did not cause a detectable rise in extracellular serotonin, produced almost total anorexia. These observations raise the possibility that the anorectic action, and maybe other actions, of fenfluramine may involve mechanisms in addition to those mediated by serotonin. As it has been shown previously that hypoxia causes pulmonary vasoconstriction by an action that includes inhibition of a potassium current and membrane depolarization, we examined the hypothesis that fenfluramine might be a potassium channel blocker.

Whole cell potassium current was studied in pulmonary vascular smooth muscle cells dispersed from resistance pulmonary arteries of the rat. Fenfluramine reduced the potassium current as much as an equimolar dose of the classic Kv channel blocker, 4-aminopyridine. Dexfenfluramine caused a dose-dependent inhibition of the current and membrane depolarization ($13 \pm 2 \text{ mV}$ in response to $100 \text{ mmol/L}$). Aminorex also reduced the potassium current. Thus, the two anorectic agents that have been associated with PPH cause potassium channel inhibition in smooth muscle cells. It could be that comparable membrane depolarization in neurons and platelets is responsible for serotonin release and that serotonin causes the PPH. Alternatively, the depolarization might lead to an influx of extracellular calcium through the voltage-sensitive calcium channels. The subsequent rise in cytosolic calcium would cause vasoconstriction and could also lead to cellular proliferation. In this model, the serotonin could be a co-factor in stimulating proliferation, or it might be secondary to the membrane depolarization and relatively unimportant.

Cardiac valvular heart disease has been reported in patients taking fenfluramine and phentermine and in patients taking dexfenfluramine alone. Morphologically, the valvular disease looks like that seen in carcinoid syndrome, but it predominantly affects the mitral and aortic valves. In patients with carcinoid heart disease, the plasma serotonin level is markedly elevated; $1.1 \times 10^{-6} \text{ mol/L}$. It seems likely that the anorexigen-associated valvular heart disease is caused by the high plasma serotonin levels. As greatly increased pulmonary artery pressures, out of keeping with the severity of the valvular regurgitation, have been recorded in only a minority of the patients with valve disease, it is possible that the etiology of anorexigen-associated PPH involves other factors. If the etiologic mechanism was the same in patients with both PPH and valve disease, valve lesions would probably have been reported in the many anorexigen-associated PPH patients in Europe. Vulnerability to PPH might involve a reduced capacity to generate NO, as discussed earlier, or an unusual expression of potassium channels increasing susceptibility to block by anorexigen.

**ROLE OF PLATELETS**

One of the major storage sites for serotonin is the dense granule of the platelet. The importance of platelets in controlling the plasma concentration of serotonin is illustrated by platelet delta storage pool disease. In this inherited condition, there is a deficiency in the number and content of the dense granules that contain adenosine nucleotides and calcium, as well as serotonin. In one patient, who developed PPH, the plasma concentration of serotonin was increased 15-fold. Fawn hooded rats, a strain that has a spontaneous tendency to develop pulmonary hypertension, have a similar inherited platelet disorder. In addition, fawn hooded rats have impaired pulmonary vascular endothelial function, shown by diminished vasodilatation in response to adenosine diphosphate and in response to acetylcholine. Dexfenfluramine inhibits Kv channels in megakaryocytes (Fig 1), the cell of origin for platelets. This may help to explain the observation that dexfenfluramine (100 mmol/L) both causes release of serotonin from platelets (35±3% released at 2 h; control, 2% release) and markedly reduces serotonin reuptake (to 19±3% of control at 2 h). It is also known that phentermine inhibits the metabolism of serotonin in the lung and thus, given in combination with dexfenfluramine, would further increase plasma serotonin levels. In this respect, dexfenfluramine plus phentermine makes patients like fawn hooded rats.

The high plasma serotonin level reported in patients with PPH unrelated to anorectic agents
makes it likely that a platelet defect is a primary cause of PPH in these patients. This, combined with the observations that dexfenfluramine inhibits Kv channels in smooth muscle cells and megakaryocytes (as a model for platelets) and releases serotonin from platelets, together with the finding of dysfunctional Kv channels in the pulmonary artery smooth muscle cells of patients with PPH, suggests that PPH is the result of the same dysfunction of Kv channels in platelets and pulmonary vascular smooth muscle cells.

Given the similarity of the cardiac valve abnormality in carcinoid syndrome, and the abnormality seen in association with exposure to fenfluramine/phentermine, it seems likely that serotonin may be responsible. In the case of carcinoid, the tricuspid and pulmonary valves are usually involved, as serotonin and other bioamines are released from secondaries in the liver and pass directly in the blood to the right side of the heart. Presumably, metabolism of these substances by the pulmonary vascular endothelium explains why mitral and aortic valve damage is less common. Release of serotonin from platelets, such as that caused by dexfenfluramine, might account for involvement of left-sided heart valves and possible systemic vascular complications, when patients take anorectic agents. Greater turbulence of the blood on the left side of the heart, increasing platelet activation, might also contribute to the involvement of the mitral and aortic valves. PPH is not seen in carcinoid syndrome, in which serotonin levels are very high, suggesting that the pathophysiology of anorectic-induced PPH is not caused by serotonin alone. It may require elevation of cytosolic calcium, secondary to potassium current inhibition and membrane depolarization and possibly secondary to calcium release from the sarcoplasmic reticulum. Whether the increased serotonin is involved in the etiology of the pulmonary hypertension or is merely a marker of the potassium channel inhibition in the platelet remains to be determined. The efficacy of ketanserin, a serotonin blocker, in reducing pulmonary hypertension in the patient with platelet storage disease makes an etiologic role likely. We suggest that PPH and anorexigen-induced pulmonary hypertension both involve a decrease in current through the Kv channels of platelets and pulmonary arterial smooth muscle cells. The associated increase in plasma serotonin, combined with membrane depolarization and increased cytosolic

Figure 1. Whole cell potassium current in a rat megakaryocyte, measured using patch-clamp technique. Top, A: Family of potassium currents elicited by voltage steps (20 mV) from a holding potential of −70 to +50 mV. Single steps every 10 s from −70 to +50 mV, demonstrating the rapid and progressive inhibition by 100 μM dexfenfluramine addition, after 1 min of control recordings. Family of potassium currents (−70 to +50 mV) 1.5 min after run-off of dexfenfluramine. Bottom, B: Current/voltage relationship before, during, and after run-off of dexfenfluramine. Also, effect of 1 mM 4-aminopyridine (4AP) on the current after the dexfenfluramine was washed off.
calcium in the pulmonary artery smooth muscle cells, may cause vasoconstriction and proliferation.

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