Pulmonary Hypertension and Acidemia: Activation of Pressor Substances from Whole Blood Fractions[1] (35867)

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The causes of the pulmonary arterial pressor response to increasing hydrogen ion concentrations induced by infusion of acids are poorly understood. Previous studies have suggested that the magnitude of the increase in pulmonary arterial pressure is directly related to the hydrogen ion concentration of the blood perfusing the lung (1). However, this direct relationship has not always been identified (2, 3). Indeed, recent studies in this laboratory suggested that vasopressor substances were activated by the production of acidemia, since, in those studies, the intensity of this pressor response was more closely related to the localized change in blood pH at the site of acid infusion, than to the changes in the pH of blood actually perfusing pulmonary vessels (4). In those studies, the pressor response was not modified by restoration of the pH of the acidified blood to control levels before this blood actually perfused these vessels. Additionally, during perfusion of a hemodynamically separated lung lobe with acidified low molecular weight dextran, the pH of the perfusate was usually decreased to 6.5–5.5 before a lobar vasopressor response occurred.

The present investigation was designed to further investigate the nature of the vasopressor substance by studying the response of the hemodynamically separated lobar vessels to perfusion with various acidified whole blood fractions. Transseptal and right heart catheterization techniques were used in intact dogs to pump-perfuse the lobar vessels at a constant volume flow and thus to avoid the circulatory and respiratory changes that accompany thoracotomy and cannulation of pulmonary vessels.

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Methods. Adult mongrel dogs (19.5–25.9 kg) were anesthetized with urethane (1.0 g/kg, iv) and were strapped in the supine position to a fluoroscopic table. The animals spontaneously breathed 35% oxygen in room air through an endotracheal tube. A yellow Kifa[2] catheter with four side holes near the tip was introduced through a peripheral vein and positioned transseptally in the left atrium just above the mitral valve. Teflon catheters (0.9-mm o.d.) with two distal side holes and a closed tip were then placed in the main pulmonary artery just above the pulmonary valve, in the right atrium, and in the descending thoracic aorta. Pressures were recorded by Statham P23D transducers[3] connected to an oscilloscopic recorder.[4]

A specially designed 23F balloon catheter[5] was introduced fluoroscopically from the external jugular vein into the pulmonary arterial branch to the left lower lobe. A Teflon catheter (0.9-mm o.d.), affixed to the balloon catheter, was used to measure pressures in that left lower lobar artery 1–2 cm distal to the perfusion catheter. A 14F polyethylene catheter was passed transseptally and positioned in the vein draining the left lower lobe. The dogs then received heparin, 150 units, iv. After all catheters were positioned, the balloon was distended with 2-4 ml of sodium diatrizoate (Hypaque), until pressure in the lobar artery distal to the catheter tip decreased to 1–3 mm Hg of the left atrial pressure. A Sarns roller-pump,[6] calibrated

[2] Odman-Ledin, No. 17, 887 Shick X-ray, Chicago, Ill.

[3] Statham Physiological Division, Oxnard, Calif.

[4] Electronic for Medicine, White Plains, N.Y.

[5] U.S. Catheter and Instrument Co., Glens Falls, N.Y.

[6] Model 4600, Sarns, Inc., Ann Arbor, Mich.

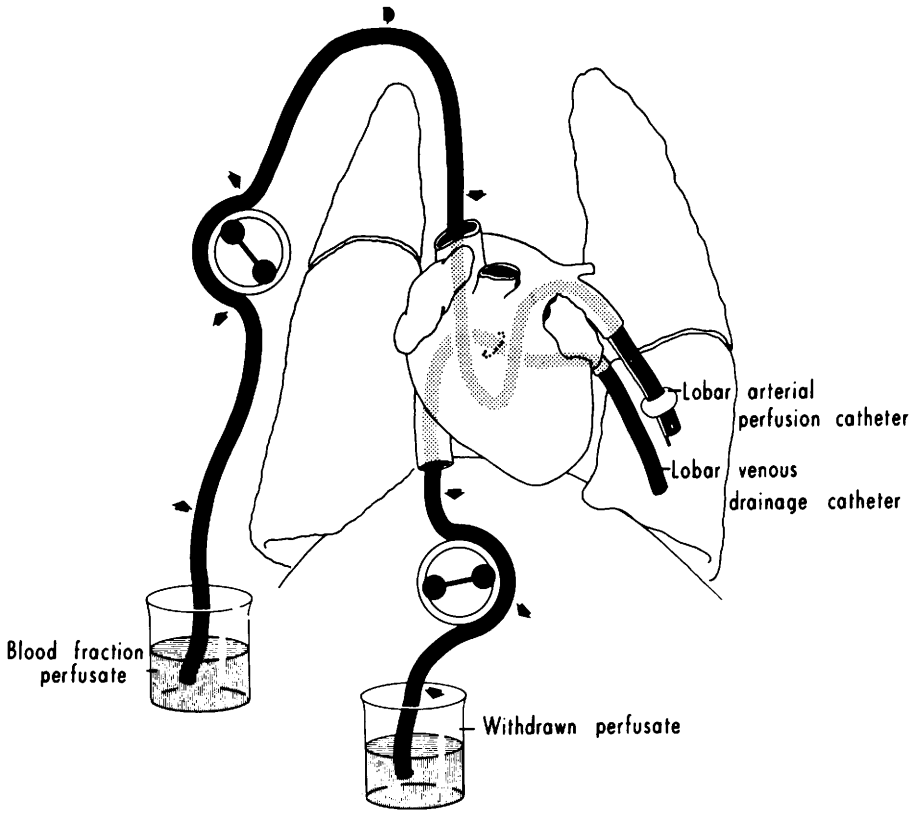

FIG. 1. Schematic representation of experimental preparation used in these studies.

with an electromagnetic flowmeter and with a graduated cylinder, was used to deliver the acidified dextran perfusates at a constant rate from an open polyvinyl reservoir. After perfusing the lobe, the dextran was, for the most part, drained by the lobar venous catheter into another reservoir, with a second roller-pump at a rate equal to the perfusion rate. The effects of hypervolemia, hemodilution, and systemically mediated pulmonary responses were thus largely avoided. This experimental preparation (Fig. 1) has been detailed elsewhere (4, 5). The lobar artery was initially perfused with 4% LMD in normal saline[7] (pH 7.3–7.4) at gradually increasing rates until pressure in that vessel approximated pressure in the main pulmonary artery before balloon distention. Perfusion was continued at this rate and was maintained for 6–8 min to ensure stability before measuring the lobar arterial responses to perfusion with whole blood fractions. Three different fractions were investigated: platelet-poor plasma, 11 studies; platelets suspended in LMD, 11 studies; and red blood cells suspended in LMD, 6 studies. These were prepared from dog blood shed within 1 hr, and collected in 500-ml plastic bags containing 75 ml of USP acid-citric-dextrose solution. The blood was centrifuged at 1500 rpm for 10 min at 4°. The platelet-rich plasma was transferred to another 500-ml plastic bag and centrifuged at 3000 rpm for 30 min at 4°. The platelet-poor plasma then was drained into an open polyvinyl container, placed in a water bath regulated at 37°, and adjusted to a pH of 7.3–7.4 by the addition of sodium bicarbonate. The platelet residue was resuspended in 4% LMD in normal saline prepared by mixing 750 ml of normal saline and 500 ml of 10% LMD in normal saline. This solution is

[7] Ten percent Rheomacrodex in normal saline; kindly supplied by Pharmacia Laboratories, Atlanta, Ga., and by Abbott Laboratories, Chicago, Ill.

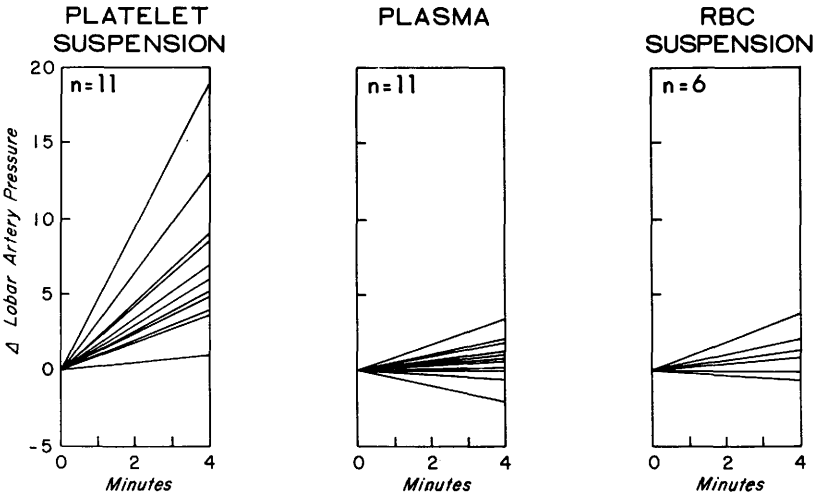

FIG. 2. Pressor response in the hemodynamically separated lobar artery to perfusion with acidified plasma and suspensions of red blood cells and platelets (n = number of experiments).

approximately isotonic and iso-osmolar with blood. The platelet suspension was transferred to an open polyvinyl container, placed in water bath regulated at 37°, and adjusted to a pH of 7.3–7.4 with sodium bicarbonate. The red blood cells were washed three times in normal saline, all gross evidence of buffy coat being removed with each washing, and were suspended in 4% LMD in normal saline. This suspension was then transferred to an open polyvinyl container, placed in a water bath regulated to 37°, and its pH was adjusted to 7.3–7.4 by the addition of sodium bicarbonate. Before beginning each perfusion, samples were taken from the perfusate reservoir and analyzed for platelet content using the direct counting method with phase microscopy; in four experiments in each group of perfusates, white blood cell concentrations were also determined. In four studies with red blood cell suspensions, the erythrocyte packed cell volume was measured.

These whole blood fractions were randomly substituted for the dextran perfusate and pumped into the lobar artery at the previously selected rate for 2–3 min to ensure the stability of the vascular pressures. Hydrochloric acid, 0.3 M, was then infused at 1.12 ml/min into the extracorporeal circuit near the point of withdrawal from the perfusate reservoir. Three-tenths molar solutions of sodium bicarbonate and tromethamine (THAM) were also infused into the perfusate as it reached the catheter perfusing the lobar artery. These solutions were infused at a rate of 1.92 ml/min beginning 20–30 sec after the acid was commenced. Initial studies in 3 dogs in this laboratory indicated that infusions of 0.3 M sodium bicarbonate into the dextran perfusate caused a 1.2 to 2.5 mm Hg fall in lobar arterial pressure; infusions of 0.3 M tromethamine into the dextran perfusate caused no change in the lobar arterial pressure. Samples of each perfusate were withdrawn from the lobar artery for pH determinations before and 3–4 min after extracorporeal acidification and alkalinization was begun. Perfusion was continued at a constant rate throughout each experiment and was maintained for at least 4 min during the test period, a time interval which allowed the appearance of maximal vasopressor response (4). At the conclusion of each experiment, the dog was killed; and the lungs were examined for gross pathologic changes and for catheter position.

Results. Constant flow perfusion of an extracorporeally acidified suspension of platelets in 4% LMD into the lobar artery of dogs significantly ($p<0.01$) increased the lobar arterial pressure despite extracorporeal restoration of the pH of the perfusate to control levels before it reached the pump-perfused vessels (Fig. 2). This pressor response gener-

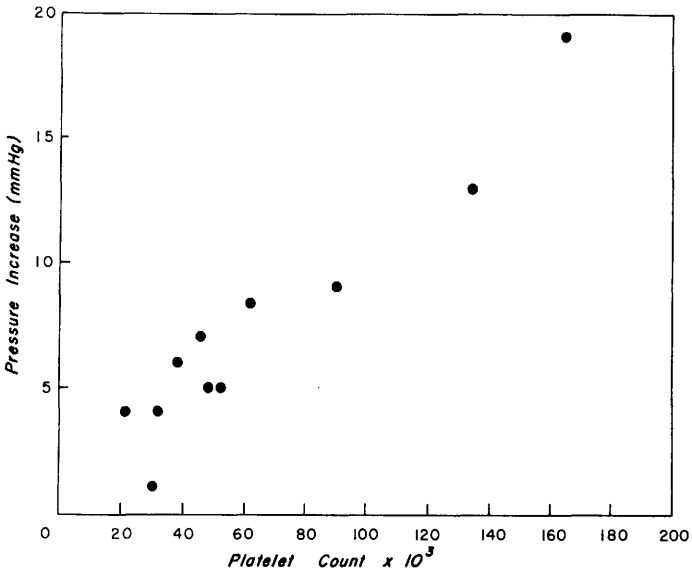

FIG. 3. Relationship of changes in mean lobar arterial pressure to platelet concentration during lobar arterial perfusion with platelets suspended in low molecular weight dextran. The pressor response to the acidified perfusate is directly related to the platelet concentration of the perfusate.

ally began within 1 min after the perfusate reached the pump-perfused lobar artery. Maximal response appeared 2–3 min later and persisted for 3 to 5 min after infusions were stopped. The pressor response observed during perfusion with platelet suspensions was directly related ($r = 0.9538$, $p<0.01$) to the platelet concentration of the perfusate (Fig. 3).

Perfusion of the lobar artery with red blood cell suspensions or platelet poor plasma which had similarly been extracorporeally acidified and then returned to control pH did not significantly ($p>0.50$) increase pressure in that vessel (Fig. 2). Additionally, in the experiments in which small pressor responses did occur, the platelet concentration of the plasma or the red blood cell suspensions did not correlate with the pressor response ($r = 0.4000$, $p > 0.20$; $r = 0.1703$, $p>0.07$, respectively).

Pressure in the main pulmonary artery, aorta, and left and right atria did not significantly change during any of the perfusions. The mean pH values of the lobar artery perfusate also did not change significantly in any of the experiments (7.43 ± 0.04 SE to 7.41 ± 0.07 for the platelet suspensions; 7.38 ± 0.06 to 7.45 ± 0.06 for the platelet-poor plasma; and 7.36 ± 0.01 to 7.36 ± 0.03 for the red blood cell suspensions). White blood cell counts of each of the perfusates ranged between 325 and 780 cells/mm^3 for platelet suspensions, between 565 and 1600 cells/mm^3 for plasma, and between 3850 and 7000 cells/mm^3 for red blood cell suspensions. Packed red blood cell volumes of the erythrocyte suspensions ranged from 24 to 30%. The pressor response observed in the hemodynamically separated lobar vessels during extracorporeal acidification and pH restoration was not related to the white blood cell concentration of the perfusate.

At autopsy, the hemodynamically separated lobes perfused with LMD, plasma, or platelet suspensions appeared pale and asanguinous. Microscopical examination of the lungs was not done in these investigations; however, previous studies in this laboratory with light and electron microscopy of lung biopsies obtained at the peak pressor response during similar perfusions with aortic blood similarly acidified did not show agglutination of formed elements in either the hemodynamically separated lobe or the normally perfused pulmonary vessels (4). Addition-

ally, in those studies, the platelet concentration of the acidified blood was not altered by perfusion through the lung (4).

Discussion. In these experiments, the lobar arterial pressor response to pump-perfusion of acidified fractions of whole blood was directly related to the platelet concentration of the perfusate. Since flow in the pump-perfused lobar artery and pressure in the normally perfused main pulmonary artery, in the left and right atria and in the aorta did not change, the data additionally suggest that this pressor response resulted largely from active constriction of lobar vessels. The possibility that localized bronchoconstriction might have contributed to this pressor response is unlikely since similar studies have shown that perfusions of acidified aortic blood increased lobar arterial pressure even during periods of induced apnea (4). It is also unlikely that conglutination of formed elements contributed to the pressor response since, in similar previous studies (4), examination by light and electron microscopy of lobar biopsies obtained during perfusions with acidified blood failed to reveal any evidence of blood cell aggregations. Moreover, the absence of a significant lobar arterial pressor response during pump-perfusion with platelet-poor plasma and with washed red blood cell suspensions suggests that these blood fractions probably did not contribute directly to the pulmonary hypertension caused by acidification of whole blood. The absence of a correlation between the platelet content of the plasma and red cell suspensions and the lobar arterial pressor response might, however, have been due to inaccuracies in counting techniques at low platelet levels. Similarly, the absence of correlation between white blood cell concentration and lobar arterial pressor response suggests that these blood cellular elements did not participate significantly in this response. Since the pressor response occurred despite extracorporeal restoration of the pH of the acidified platelet suspensions to control values, these data indicate that this lobar pressor response was not dependent on local vascular effects of changes in pH of the perfusate alone. These experiments, as well as earlier studies (4), suggest that the pulmonary vasopressor response to acid infusion is mediated at the site of acid infusion by activation of vasoactive materials, and that these materials are largely derived from platelets.

The possibility that the 4% LMD in normal saline in which the platelets and red blood cells were suspended might have altered the vascular response to acidification seems unlikely. No pressure changes occurred in the lobar vessels during perfusion with LMD for 15–20 min or with the whole blood fractions in LMD before acidification. Lloyd (6) similarly found no alteration in pulmonary pressure during LMD perfusion within the time limits of these experiments. Slight, undetected admixture of whole blood from the normally perfused lung or the bronchial circulation with the perfusate is unlikely to have influenced the results because the pH of the perfusate reaching the lobar vessels was at control levels. Furthermore, admixture of whole blood with the perfusate should have caused similar pressor responses in all groups. The possibility that the low oxygen carrying capacity of the plasma and platelet suspensions contributed to the pressor responses observed is remote since perfusion with similar perfusates or with blood containing little oxygen does not increase pulmonary arterial pressure without concurrent ventilatory hypoxia (6–8). Although some changes in responsiveness of the lobar vessels might have resulted from perfusion with LMD rather than blood, the ability of these vessels to response to infusions of serotonin during perfusion with LMD at a pH level of 7.40–7.35 units was similar to that previously observed during pump-perfusion with whole blood at similar pH levels (4, 9).

The mechanism by which platelets mediate the pulmonary arterial pressor response to acid infusion is not determined from the present data. However, since these cell fragments contain vasoactive materials which are capable of producing a pressor response in pulmonary vessels, liberation of these materials either directly by the effect of acid on platelets or indirectly by induction of the platelet release reaction (10–12) might have

produced the increase in lobar arterial pressure. Aggregation of platelets during the release reaction might have further accentuated this pressor response (13). However, previous studies with acidified whole blood perfusates failed to demonstrate blood cellular aggregates within the pump-perfused lobar vessels (4). Additionally, the platelet count of lobar venous blood did not decrease during acidification of the lobar arterial perfusate in those studies. Furthermore, Markwardt (14) has shown that the platelet release reaction largely involves metabolically active transport mechanisms which require glucose and physiologic concentrations of hydrogen and calcium ions. Since glucose was absent and an excess of hydrogen ions and probably very few calcium ions were present in the platelet-rich dextran perfusate at the point of acidification, it is unlikely that this mechanism was primarily involved. These considerations, moreover, suggest that release of vasoactive materials by a direct effect of acid on platelets was principally responsible for the lobar arterial pressor response observed.

Previous studies have indicated that serotonin is stored within platelets in ionic combination with ATP (15–17). Thus, infusion of a strong acid might have produced an alteration in the physicochemical state of this complex causing liberation of these vasoactive materials. Such a mechanism has been suggested for the release of ATP-bound catecholamines in the adrenal medulla during alterations in environmental pH (18) and if further suggested by additional similarities between the storage and release of catecholamines from sympathetic nerve endings (19) and adrenal medulla (14) and the behavior of serotonin in platelets. Alternatively, acidification might have activated lysosomal enzymes which in turn induced the platelet release reaction (14) or might themselves have directly constricted pulmonary vessels.

Previous investigations have shown that vasoactive substances may be activated in plasma by acidification (20–23). Since, during perfusion with acidified plasma, the pressor responses were small and statistically insignificant, the present data suggest that the direct contribution of vasopressor agents produced by acidifying plasma to the pulmonary pressor responses to acidified blood is small.

Summary. These data show that the pulmonary vasopressor response to perfusates of acidified plasma or whole blood fractions suspended in LMD is directly related to the platelet concentration of the perfusate. The pressor response is not directly related to local vascular effects of lowered perfusate pH within the range 7.40–6.35. This response appears to be mediated by the release of vasoactive substances from platelets at the site of acid infusion. It is suggested that the effect of an increase in hydrogen ion concentration on platelets causes direct physicochemical alterations which activate and release pulmonary vasopressor materials.

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