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Effect of Blood pH on Pulmonary Artery Pressure, Left Atrial Pressure and Fluid Filtration Rate in Isolated Rabbit Lung*

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To determine the effects of pH changes on Pulmonary Artery Pressure (PAP), 18 isolated rabbit lung preparations, perfused with autologous blood mixture and constant $PaCO_2$ have been studied. Each preparation was studied under 3 conditions: Baseline: 30 minutes equilibration period. Acidosis: pH was decreased by 0.2 N HCl infusion, the ventilatory rate was changed and different CO_2 mixtures were used to maintain the PCO_2 within the initial parameters. Compensated Acidosis (CA): pH was returned to normal values by 0.7 N $NaHCO_3$ infusion maintaining PCO_2 in its initial values. The decrease in pH (acidosis) from 7.36 ± 0.05 to 7.18 ± 0.06 at constant PCO_2, generated a significant increase in PAP (13.6 ± 3.2 cm H_2O to 18.8 ± 5.2 cm H_2O, $p < 0.01$). The pH increase (CA) from 7.18 ± 0.06 to 7.40 ± 0.09 caused the PAP to decrease (18.8 ± 5.2 cm H_2O to 15.9 ± 4.2 cm H_2O); the fluid filtration rate remained unchanged during the whole experiment. It is concluded that blood pH changes at constant PCO_2 result in significant changes of PAP. Acidemia produces pulmonary vasoconstriction, which may be a contributing factor in the genesis of pulmonary hypertension in clinical conditions with increased hydrogen ion concentration [H^+].

Key words: Pulmonary circulation, Acidosis, Fluid filtration rate, Pulmonary vasoconstriction.

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Abbreviations: A, Acidosis; B, Baseline; CA, Compensated Acidosis; COP, Colloid osmotic pressure; COPD, Chronic Obstructive Pulmonary Disease; FFR, Fluid Filtration Rate; HPV, Hypoxic pulmonary vasoconstriction; LAP, Left Atrial Pressure; PAP, Pulmonary Artery Pressure; Paw, Airway pressure; PEEP, Positive end- expiratory pressure; PO_2, PCO_2, O_2 and CO_2 partial pressure; PVR, Pulmonary vasoconstriction response; P.V.R, Pulmonary vascular resistance.

The effect of blood hydrogen ion concentration [H^+] on the pulmonary vasculature has been widely studied by several authors (1-23). LILJESTRAND (18) suggested that increases of [H^+] in blood could be the chemical stimulus which generates pulmonary vasoconstriction during hypoxia, but subsequent experiments both in animals (3-6, 19-22) and humans (9, 11, 14, 15, 39), reported contradictory results. Most of these studies have shown that a decrease in pH results in an increase in PAP (4, 6, 7, 9, 11, 12, 16, 17) especially in the presence of alveolar hypoxia. However, it is not clear whether PAP increases as a result of pH decreases or from the associated increase in PCO_2. BERGOFSKY *et al.* (6) and a previous study of our group (24), working on the effects of hypoxia on PVR, concluded that pH changes were not responsible for the changes in the pulmonary vasculature with hypoxia. HOUSLEY *et al.* (14) indicated that low pH, in the range usually found in patients with COPD was not a conditional factor of pulmonary hypertension. VISWANATHAN *et al.* (39) demonstrated that ventilation with a mixture of gases above 10 % of CO2 both in normal subjects and in patients with COPD resulted in an increased in PAP which is independent of [H^+] in blood. In contrast, other authors (7, 17, 21, 22) concluded that the increase of [H^+] does decrease the HPV response. Due to these controversial results, we decided to study the effect of blood pH with constant PCO_2 on mean PAP, mean LAP and FFR in mechanically ventilated and perfused isolated rabbit lungs.

Materials and Methods

Eighteen New Zealand rabbits with a mean body weight of 2.50 ± 0.35 kg were anaesthetized with i.p. pentobarbitone (30-40 mg/kg). A tracheostomy was performed and the lungs were ventilated mechanically (Harvard Respiration Pump Millis, Mass) at a constant tidal volume of 15 to 20 ml/kg and a mixture of 5 % CO_2, 21 % O_2 in N_2. A PEEP of 2 cm H_2O was applied before performing a sternotomy and throughout the entire protocol. After mid sternotomy, 2 ml of heparin (100 m/ml) was injected via a cannula in the right ventricle; two minutes later, the animal was exsanguinated through the same cannula and the blood volume obtained (100 ml approximately) was increased with 50 ml of 0.9 % NaCl solution plus 50 ml of 5 % Dextran solution. This mixture increases the perfusion volume and maintains an oncotic pressure of 22 ml H_2O (32). The heart and lungs were promptly removed with minimal handling and a silastic perfusion cannula was placed in the pulmonary artery through an incision in the right ventricle. A second cannula was placed in the left atrium through the left ventricle; both cannulas were fixed in their position by a ligature tied around both ventricles. This ligature was used to suspend the preparation from a force transducer (Force Displacement Transducer FTO 3, Grass Instrument Company, Quincy, Mass) which was fixed at the top of a perspex box. To minimize evaporation from the lung surface the temperature of the box was maintained at 37 °C and at constant humidity by means of water radiators in the box where water circulated constantly and at 45 °C (fig. 1). The force transducer helps differentiate pulmonary vascular volume modifications from the changes produced by the FFR variation (30, 32, 34). In this preparation, changes in the vascular volume result in rapid modifications in weight, whereas changes in FFR are reflected as a slow, constant slope in the lung's weight curve. Connections to the ventilator and perfusion circuit were placed horizontally through one side of the box so that the

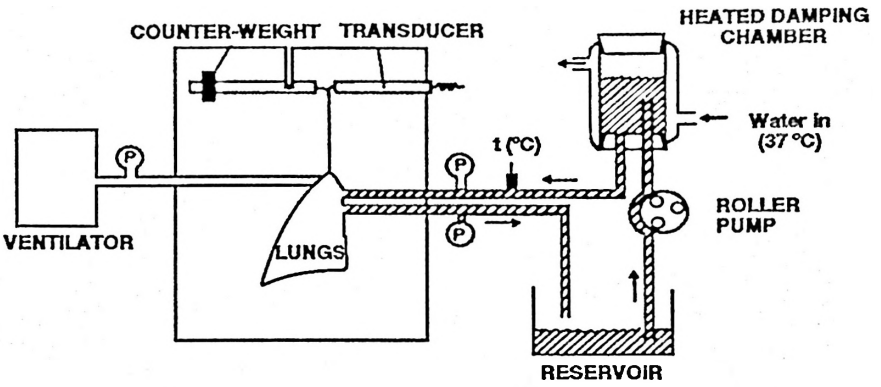

Fig. 1. Simplified diagram of the preparation.
The lungs are suspended from a force displacement transducer fixed to the top of a perspex box. Blood is pumped from the left atrial reservoir through the damping chamber and double trap to the pulmonary artery. Ventilation is achieved through the preparation trachea; p, pressure transducer; *t*, temperature.

weight transmitted to the preparation through the catheters remained constant and the changes in lung weight produced only vertical displacement of the preparation. PAP and LAP were measured through catheters placed at the end of the perfusion cannulas. Vascular and means Paw were measured with pressure transducers (Physiological Pressure Transducer, Gould, California) and displayed simultaneously with lung weight on a polygraph (Grass Instrument, Quincy, Mass). The zero reference for the vascular pressure was the left atrium and all the transducers were calibrated with a water manometer. Since the lungs were suspended vertically and the apices were approximately at atrial level (zero level), it was assumed that whole lung was homogeneously perfused (31). The perfusion flow was kept constant through all the experiment, it was performed with an occlusive peristaltic pump (Watson-Marlow Type MHRE 200 Limited, Cornwall, England) and it was always begun in the 10 min after the exsanguination. The oscillation produced by the pump was minimized, the blood flowing through a damping chamber, surrounded by water in circulation at 37 °C. The temperature was measured with a thermometer placed in the damping chamber and in the box. PO_2, PCO_2 pH and glucose concentration were adjusted during baseline period with sodium bicarbonate and 50 % dextrose in water to obtain a pH and Glucose concentration of 7.35 to 7.45 and 175 to 250 mg/dl, respectively. Glucose concentration was subsequently adjusted at 30 min intervals (40) and the pH was also repeatedly measured in extracted samples from the perfusion cannula. The lung was briefly hyperinflated (Tracheal pressure at 30 cm H_2O) to reverse atelectasy produced during the set up period. A scape valve was placed in the airway to maintain constant the Paw since this changed as respiratory rate was modified to maintain PCO_2 values constant.

All variables (pH, PO_2, PCO_2, PAP, LAP, Paw, FFR) were measured at each of 3 experimental stages,defined as Baseline: 30 min equilibration period when values of LAP, PAP, Paw, FFR, and acid base equilibration were controlled; Acidosis: 0.2 N HCl infusion was titrated to

Table I. *Hemodynamic, ventilatory and acid-base parameters.*
Values are the means ± SD. LAP: Left atrial pressure; PAP: Pulmonary artery pressure; PAW: Airway pressure, FFR: Fluid filtration rate. B: Baseline; A: Acidosis; CA: Compensated acidosis. All comparisons non-significant unless otherwise stated.

	B	A	CA
LAP (cm H_2O)	– 0.04 ± 2.96	– 0.70 ± 3.17[a]	–0.62 ± 3.23[a]
PAP (cm H_2O)	13.6 ± 3.16	18.8 ± 5.23[b]	15.9 ± 4.18[bc]
Paw (cm H_2O)	5.66 ± 1.48	5.83 ± 1.60	5.89 ± 1.54
FFR (g/min)	0.02 ± 0.04	0.01 ± 0.04	0.02 ± 0.04
pH	7.36 ± 0.05	7.18 ± 0.06[a]	7.40 ± 0.09[c]
PCO_2	35.5 ± 6.4	36.7 ± 7.6	38.5 ± 5.5
PO_2	106.0 ± 17.7	102.2 ± 18.9	104.9 ± 18.9

[a]$p < 0.05$ and [b]$p < 0.001$ versus B; [c]$p < 0.001$ versus A.

decrease the pH in the reservoir by approximately 0.2 units; and Compensated Acidosis (CA): infusion of 0.6 N $NaHCO_3$ to bring pH back to its baseline values. In acidosis and CA, respiratory rate was increased and low CO_2 mixture was used to maintain PCO_2 constant. The colloid osmotic pressure (COP) was measured at the beginning and at the end of each preparation. The oncometer design was based on the device described by AUKLAND and JOHNSEN (2), and improved by SÁNCHEZ DE LEÓN *et al.* (33).

All the experimental techniques and animal managements were previously approved by the National Institute of Scientific and Technological Research of the Central University of Caracas,Venezuela (CDCH, National Statutes, 1989).

Statistics.– The data are expressed in mean standard deviation. In this study results were checked by an analysis of variance followed by a Student's *t* analysis where appropriate, the results obtained being considered significant when p was less than 0.05 (37).

Results

The results of the different parameters measured in each one of the experimental conditions are expresed in means and SD (table I). The average pulmonary blood flow was 76.8 ± 12.9 ml/min. The COP was 22.5 ± 2.8 cm H_2O. Both values remained constant through the entire experiment. In acidosis pH decreased due to HCl infusion. There was a significant increase in PAP without changes in the rest of the measured parameters. In compensated acidosis an increase in pH resulted in a significant drop of PAP ($p < 0.01$). In this second stage there were also no significant changes in LAP, Paw, FFR, PCO_2 and PO_2.

Discussion

The isolated rabbit lung preparation allows for precise control of gasometric and hemodynamic conditions, independently of other variables such as cardiac

output and pulmonary ventilatory volume, which are maintained constant. These variables have been noted to affect PAP when they have not been controlled (6, 8, 9, 11, 15, 24, 28, 39), and the variation of the PAP reported may not necessarily reflect the changes of [H^+]. The isolated lung preparation also allows the control of the pulmonary flow,LAP, Paw and partial pressure of the inspired gases, which could modify PAP and FFR. Moreover, because the FFR is relatively small in relation to the perfusion liquid, the oncotic pressure is kept within narrow limits throughout all the experiment. However, the preparation differs from normal lungs in some important aspects (41), making comparisons with *in vivo* results therefore, difficult to extrapolate. In our isolated lung preparation the cardiac output was always kept constant using a peristaltic pump, which made possible to discard the variations of PAP produced by changes in the heart rate observed in preparations *in vivo*. By controlling Paw, the ventilatory frequency of the preparation can be changed without modifying Paw and consequently the PAP. This result, in a stable blood $PaCO_2$ with a constant Paw and constant blood flow, which logically prevents the changes in PAP secondary to modifications of Paw or $PaCO_2$. Perhaps the most important characteristic of the system used is that it allowed us to maintain PCO_2 constant, thereby isolating the pressor effect that [H^+] exerts on the pulmonary vasculature. The contradictory results found in previous works might be due to the differences in the methodologies used, which did not keep control over all the variables that are known to influence PAP. The changes observed are not due to preparation changes since the viability of our preparation has been discussed before (30-32, 34, 41).

Blood pH modifications while maintaining pCO2 constant are associated to PAP changes in isolated rabbit lungs under controlled hemodynamic conditions. The significant increase in PAP, generated by the infusion of HCl, was associated to a significant decrease in the LAP and unchanged FFR. Due to the preparation characteristics and according to previous studies (30, 34), the pulmonary vasoconstrictor response produced by the isolated pH decrease, is located at the pre and post-capillary level, as the increase in PAP not associated to an increase or decrease in FFR only can be associated with an in and out vasoconstriction, which increases the PAP but keeps the pulmonary capillary pressure and consequently the FFR constant, which is similar to what occurs with the CO_2 pulmonary vasoconstriction (34). The fast changes in the preparation weight which occurs with corresponding fast changes in LAP, PEEP or blood flow, are due to modifications in the intravascular volume and they are completely reversible when the causes that produce the change return to the pre-existent condition (20, 32, 40). Nevertheles if the change in Lap is not considered relevant biologically, the change in Pap was definitively relevant not only biologically but also clinically. In our case all the factors that could cause changes in the PAP were kept constant and the increase in PAP with unchanged FFR can only be interpreted as a pre and post capillary vasoconstriction, but with a probable predominance of the precapillary sphincter. Our findings are supported by those of RUDOLPH and YUAN (28), who observed a similar decrease in LAP and an increase in PAP due to lactic acid infusion in newborn Holstein calves. Similarly, SADA *et al.* (29) reported a decrease in the internal diameter of the arterial vessels without modifications in the venous system with the infusion of lactic acid in

the pulmonary microcirculation in cats. In humans, decompensated COPD is associated to a progressive increase in PAP, mainly secondary to decrease in PaO_2 but a concomitant increase in PCO_2 may also generate an increase in $[H^+]$, which could be an additional factor contributing to the pulmonary hypertension in this disease (10).

The infusion of $NaHCO_3$ on CA increased pH values above the pH values in B, but PAP did not revert to its original values. This might be due to the changes generated in the intracellular pH which may not totally correspond to the changes in the extracellular pH. The presence of carbonic anhydrase in the pulmonary vascular endothelial cells (27) and the results obtained by ADLER *et al.* (1) and RITTER *et al.* (26), working in platelet and diaphragmatic muscle of rats, respectively, support the existence of a difference between intracellular and extracellular pH. The intracellular pH in rabbit vascular smooth muscle in CA is probably lower than the extracellular pH, which produces a more sustained vasoconstrictor response that causes a significant difference in PAP between B and CA Furthermore PRESBERG *et al.* (25), working on in situ dog lungs, found that lactic acid i.v. infusion increased P.V.R. due to an increase in the middle segment resistance. They concluded that lactic acidosis produces microvascular obstruction with altered erythrocytes and erythrocyte fragments. In our preparation a microvascular obstruction may have been partially responsible for the non reverting of PAP to its original values. It is concluded that modifications in blood pH generates changes in PAP in isolated rabbit lungs independently of PCO_2 values. We think that the increase in PAP is due to a pre and post-capillary vasoconstrictor response to acidosis and that it does not, therefore, cause an increase in lung edema as evidenced by the unchanged FFR.

PINTO-PLATA V. M., J. C. POZO-PARILLI, A. BAUM-AGAY, C. CURIEL y R. SÁNCHEZ DE LEÓN. *Efecto del pH sanguíneo sobre la presión arterial pulmonar, la presión atrial izquierda y la tasa de filtración del fluido en pulmón aislado de conejo.* Rev. esp. Fisiol. (J. Physiol. Biochem.), 51 (3), 117-124, 1995.

Se determina el efecto producido por los cambios del pH sanguíneo sobre la presión arterial pulmonar, en 18 preparaciones de pulmón aislado de conejo, perfundidas con sangre autóloga y a un PCO_2 constante. Cada preparación se estudia en tres condiciones diferentes: Basal, a los 30 minutos del comienzo del experimento en condiciones de estabilidad; acidosis, en donde el pH sanguíneo es reducido mediante la infusión de HCl 0,2 N, con la PCO_2 sanguínea constante, mediante los cambios en el patrón ventilatorio y en la mezcla usada para ventilar; y, acidosis compensada, en donde el pH es retornado a los valores normales mediante la infusión de $NaHCO_3$ 0,7 N, pero manteniendo siempre la PCO_2 en los valores normales. La disminución del pH (acidosis) de 7,36 ± 0,05 a 7,18 ± 0,06 con la PCO_2 constante, genera un aumento significativo de la PAP (13,6 ± 3,2 a 18,8 ± 5,2 cm H_2O, $P < 0,01$); y, el aumento del pH (acidosis compensanda) desde 7,18 ± 0,06 a 7,40 ± 0,09 produce disminución de la PAP (18,8 ± 5,2 a 15,9 ± 4,2 cm H_2O). La tasa de filtración de líquidos no varía durante el experimento. Se concluye que los cambios del pH sanguíneo, aun manteniendo la PCO_2 en sangre constante, aumentan la PAP. La acidemia produce vasoconstricción pulmonar, por contribuir, quizás, a la génesis de la hipertensión pulmonar asociada con la enfermedad pulmonar crónica.

Palabras clave: Circulación pulmonar, Acidosis, Tasa de filtración de líquidos, Vasoconstricción pulmonar.

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