JCI The Journal of Clinical Investigation

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J Clin Invest. 1978;62(3):618-622. https://doi.org/10.1172/JCI109168.

Research Article

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Direct Evidence for Participation of Rat Lung Carbonic Anhydrase in CO_2 Reactions

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ABSTRACT Isolated rat lungs were ventilated with air and perfused with a blood-free Krebs-Ringer bicarbonate solution under conditions of net CO_2 elimination in the lung. Some of the effluent perfusate was drawn through a stop-flow pH electrode apparatus, arriving at the electrode within 4 s after passing through the pulmonary capillaries. pH and temperature of the fluid in the electrode chamber were continuously monitored both before and after withdrawal was suddenly stopped. Little or no change was observed in the pH of the perfusate after flow was stopped, despite the fact that CO_2 was eliminated in the lung, suggesting that the conversion of H_2CO_3 to CO_2 in the blood-free perfusion fluid was markedly accelerated and the rise in pH was complete by the time the perfusate reached the electrode. Because the effluent perfusate was shown to be free of carbonic anhydrase activity, the catalysis must have occurred during transit through the isolated lung. When acetazolamide was added to the perfusate, a rise in the pH of the perfusate after stopping flow was consistently seen. These results suggest that the carbonic anhydrase of isolated lungs accelerates the conversion of H_2CO_3 to CO_2 and enhances CO_2 elimination as perfusate passes through the pulmonary capillaries, and that the enzyme may be present on the capillary endothelial surface.

INTRODUCTION

In vitro studies of CO_2 and H^+ equilibration in erythrocyte suspensions led to predictions (1–3) that the plasma pH in arterial blood would continue to change after the blood leaves the pulmonary capillaries in vivo. Attempts to verify this prediction in animal studies have been difficult (4). Recent studies have shown that the slow pH changes that have been found in normal animals in vivo are smaller in magnitude than expected (5, 6). These findings caused us to consider the possibility that carbonic anhydrase, long thought to be present in lung tissue (7), can directly catalyze the conversion of plasma H_2CO_3 to CO_2 as blood passes through the lung capillaries, thus both enhancing pulmonary CO_2 exchange and decreasing the magnitude of the slow downstream plasma pH changes.

To study this possibility, isolated ventilated rat lungs were perfused with a blood-free Krebs-Ringer bicarbonate (KRB)[1] solution under conditions of net CO_2 elimination in the lung. The pH of the effluent perfusate, monitored in a stop-flow electrode apparatus, showed little or no change with time after leaving the lung. When acetazolamide was added to the perfusing fluid, a rise in the pH of the effluent perfusate was observed. These results indicate that (*a*) carbonic anhydrase of isolated rat lungs in these experiments accelerated the conversion of perfusate H_2CO_3 to CO_2, (*b*) lung tissue carbonic anhydrase may be present on the capillary endothelium, and (*c*) if the enzyme activity is accessible to plasma HCO_3^- in vivo, slow postcapillary pH changes may be smaller than previously predicted.

METHODS

Male Sprague-Dawley rats (210–410 g) were anesthetized with 50 mg/kg i.p. pentobarbital sodium. A tracheostomy was performed and the lungs were mechanically ventilated (Harvard model 680 ventilator, Harvard Apparatus Co., Millis, Mass.) with room air. The abdomen was opened and blood cleared from the pulmonary circulation by infusing KRB perfusate (37°C) into the portal vein after cutting the abdominal aorta. The chest was then opened and the perfusate infused at about 20 ml/min (Cole-Parmer 7014 pump, Cole-Parmer Instrument Co., Chicago, Ill.) through a pulmonary artery cannula. The lungs were removed from the thoracic cavity, a cannula was placed in the left atrium, and the lungs were hung in a water-jacketed chamber. Effluent

Dr. Crandall is the recipient of Research Career Development Award HL 00134. Dr. O'Brasky was supported by training grant HL 07027.

Received for publication 13 March 1978 and in revised form 15 May 1978.

[1] *Abbreviation used in this paper*: KRB, Krebs-Ringer bicarbonate.

perfusate was not recirculated. Pressure transducers (Statham P23, Statham Instruments, Inc., Oxnard, Calif.) were used to monitor tracheal airway and pulmonary artery pressures. A schematic diagram of the perfusion system is shown in Fig. 1.

The left atrial cannula was connected through the wall of the water jacket to a T-tube connector, one arm of which drained freely. The other arm was connected to the stop-flow pH electrode apparatus. This apparatus has been described in detail previously (4) and is shown schematically in Fig. 1. Fluid was withdrawn through the apparatus at 15 ml/min, making certain that flow always continued out through the open arm of the T tube throughout the withdrawal. After about 10–15 ml of fluid had been withdrawn, the flow through the electrode was suddenly terminated. Temperature and pH of the fluid in the electrode chamber were monitored both before and after flow was stopped, recorded on a storage oscilloscope screen (Tektronix 5103N, Tektronix, Inc., Beaverton, Oreg.), and photographed. pH changes as small as ±0.002 were measurable. The transit time of perfusate from pulmonary capillaries to the electrode was estimated at about 4 s by a method described previously (4).

The KRB perfusate consisted of 118.5 mM NaCl, 1.3 mM $CaCl_2$, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, and 23.3 mM $NaHCO_3$. The same fluid was in both reservoirs A and B (Fig. 1) except that B also contained 0.25 mg/ml acetazolamide. Both perfusates (A and B) were pre-equilibrated with gas mixtures to pH ≅7.4, P_{CO_2} ≅ 39 mm Hg, and P_{O_2} ≅ 48 mm Hg.

After hanging the lung, experimental runs were performed with perfusate A. Within several minutes of performing a run, samples of inflowing and effluent perfusate were collected anaerobically, and their pH, P_{CO_2}, and P_{O_2} measured independently on a blood-gas machine (Radiometer BMS3 Mk 2, Radiometer Co., Copenhagen, Denmark). The lung was then switched to perfusate B and the procedure repeated within 1–20 min. Subsequently, in several lungs, the perfusate was switched back once again to A and the procedure again repeated after 5–10 min.

Hemoglobin concentration in the effluent perfusate was measured spectrophotometrically at 412 nm. Carbonic anhydrase activity was determined by mixing perfusate (inflowing or effluent) with 10 mM HCl in a rapid-reaction apparatus (8) and observing the kinetics of dehydration of H_2CO_3.

RESULTS

Sequential records from experimental runs in a typical lung (rat 4) with perfusate A (acetazolamide free), B (containing 0.25 mg/ml acetazolamide), and A again are shown in Fig. 2*a*, 2*b*, and 2*c*. Corresponding pH, P_{CO_2}, and P_{O_2} of the inflowing and effluent perfusates are given in Table I. The tracings at the left side of each record represent pH (upper trace) and temperature (lower trace) during fluid withdrawal through the electrode. Small respiratory oscillations in pH can be seen (4). After flow stopped, the temperature of the fluid in the electrode chamber changed <0.1°C in all runs. pH in effluent perfusate A also did not change (Fig. 2*a* and 2*c*), whereas that in perfusate B (Fig. 2*b*) rose 0.05 with $t_{1/2}$ ≅ 3.5 s.

A summary of results is given in Table I. In each lung, there was little or no change observed in the pH of the effluent perfusate after stopping flow when perfusate A was used. With perfusate B, however, there was always a marked rise in fluid pH. The difference was significant ($P < 0.000001$). Each entry in Table I

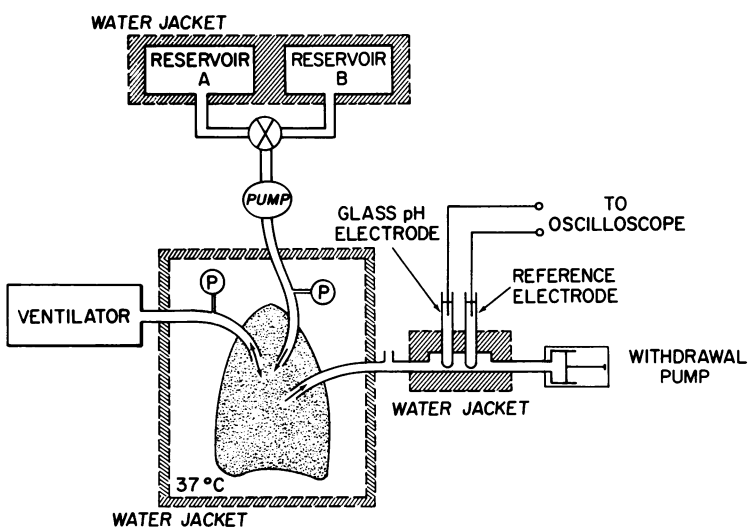

FIGURE 1 Schematic diagram of isolated lung preparation and stop-flow pH electrode apparatus. Perfusate is pumped from either reservoir A (KRB solution) or reservoir B (KRB plus acetazolamide solution) into the pulmonary artery of the isolated ventilated rat lung. The perfusate flows out through the left atrium to atmosphere and into the withdrawal pump syringe through the electrode chamber. When the flow through the electrode chamber is stopped, all the perfusate exits to the atmosphere. Tracheal and pulmonary artery pressures are monitored by transducers (denoted by Ⓟ).

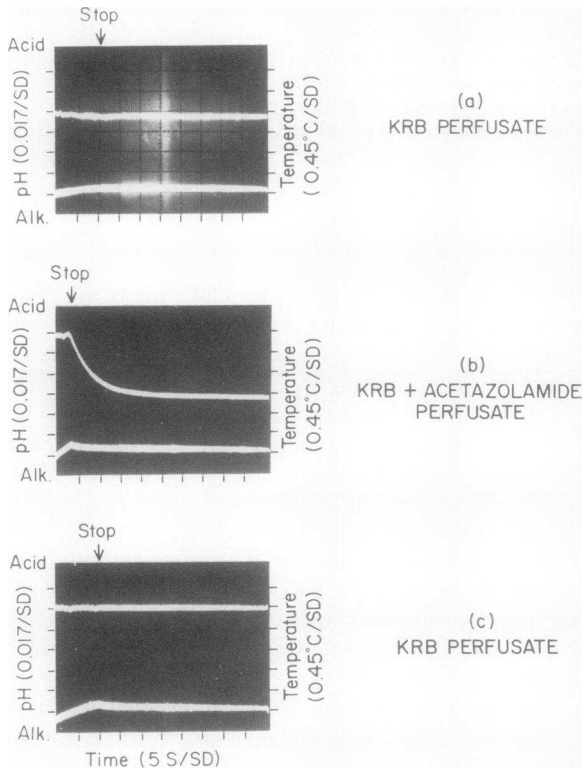

FIGURE 2 Oscilloscope records of effluent perfusate pH (upper traces) and temperature (lower traces) vs. time for lung 4. Perfusate was drawn through the electrode chamber at a constant rate of 15.3 ml/min until withdrawal was suddenly stopped (as indicated). The record (*a*) was obtained first with KRB perfusate from reservoir A. After switching to (KRB plus acetazolamide) perfusate from reservoir B the record (*b*) was obtained. The perfusate was then changed back to that from reservoir A and record (*c*) was obtained.

represents the average of at least two and as many as five repetitions of each experimental run. The average pH change observed in the stop-flow electrode with perfusate A was 0.001, whereas that for perfusate B was 0.050.

Pulmonary artery pressure and peak inspiratory airway pressure were ≅14 and 13 cm H_2O, respectively, at the beginning of the perfusions. No significant increase in these pressures was seen within the 35-min (or less) duration of the perfusions. End expiratory pressure was about 2 cm H_2O. Hemoglobin concentration in the effluent perfusate was <80 nM (essentially unmeasurable). The carbonic anhydrase assay revealed no enzyme activity in effluent perfusate A or B.

DISCUSSION

When perfusate passes through the pulmonary capillaries in our experiments, CO_2 leaves the fluid and is eliminated with expired gas (Fig. 3). This causes a disequilibrium which drives H_2CO_3 toward CO_2. For the levels of pH and P_{CO_2} in our experiments, this process, if uncatalyzed, occurs slowly (4), so the pH change associated with it should be observable in our electrode apparatus. We saw little or no change in effluent fluid pH with perfusate A (Fig. 2*a*), meaning that the reaction was accelerated before the fluid entered the electrode. Because the effluent perfusate contained no carbonic anhydrase activity, this catalysis must have occurred as the perfusate passed through the lung. We conclude that it is the lung's own carbonic anhydrase which is accessible to the perfusate and catalyzes the conversion of H_2CO_3 to CO_2, although the location of the enzyme in the lung is not exactly known.

TABLE I

Summary of Stop-Flow and pH, P_{CO_2}, and P_{O_2} Data on Isolated Rat Lung Perfusate

Rat		(KRB) Perfusate* A								(KRB plus acetazolamide) Perfusate* B							
No.	Weight	Stop-flow ΔpH	$t_{1/2}$	pH_i	$P_{CO_{2_i}}$	$P_{O_{2_i}}$	pH_o	$P_{CO_{2_o}}$	$P_{O_{2_o}}$	Stop-flow ΔpH	$t_{1/2}$	pH_i	$P_{CO_{2_i}}$	$P_{O_{2_i}}$	pH_o	$P_{CO_{2_o}}$	$P_{O_{2_o}}$
	g		*s*		*mm Hg*	*mm Hg*		*mm Hg*	*mm Hg*		*s*		*mm Hg*	*mm Hg*		*mm Hg*	*mm Hg*
1	216	0	—	7.365	39.6	40.9	7.605	22.8	111.0	0.023	2.8	7.423	36.4	61.1	7.568	25.9	115.3
2	239	0.003	3.5	7.464	33.6	59.5	7.651	22.4	129.9	0.021	2.6	7.415	38.2	49.1	7.676	21.8	114.8
3	240	0.003	3.7	7.378	39.8	43.0	7.712	19.9	140.1	—	—	—	—	—	—	—	—
4	256	0	—	7.399	40.2	36.6	7.796	17.6	134.2	0.051	3.5	7.395	42.0	43.6	7.628	24.4	134.6
5	210	0	—	7.352	43.0	33.7	7.728	19.7	132.2	0.058	4.0	7.399	40.1	40.7	7.612	26.3	133.7
6	246	0	—	7.412	36.8	50.5	7.658	20.4	130.6	0.044	3.3	—	—	—	—	—	—
7	265	0	—	7.401	40.1	44.3	7.916	12.1	142.3	0.060	3.5	7.422	35.7	54.5	7.690	19.6	143.1
8	277	0	—	7.391	40.6	44.9	7.981	11.1	145.7	0.091	4.0	7.423	38.5	43.1	7.739	20.4	141.8
9	293	0	—	7.405	38.8	51.2	7.979	11.0	137.5	0.043	3.3	7.428	39.2	51.2	7.792	17.5	131.5
10	345	0	—	7.436	35.9	50.7	7.888	12.8	128.1	0.059	3.4	7.419	38.0	44.1	7.743	18.0	131.5
11	407	0	—	7.384	38.6	53.8	7.902	10.5	136.0	0.049	3.5	7.403	39.4	53.8	7.699	18.7	139.0
Mean	272	0.001	(3.6)	7.399	38.8	46.3	7.802	16.4	133.4	0.050	3.4	7.414	38.6	49.0	7.683	21.4	131.7

* pH_i, $P_{CO_{2_i}}$, and $P_{O_{2_i}}$ are those values measured by blood-gas machine on inflowing perfusate. pH_o, $P_{CO_{2_o}}$, and $P_{O_{2_o}}$ are those values measured by blood-gas machine on effluent perfusate.

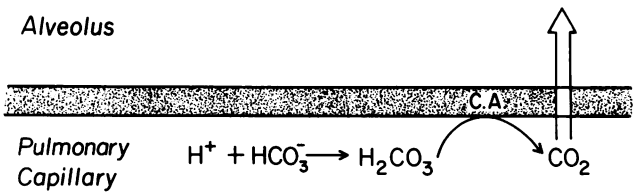

FIGURE 3 Reactions involving CO_2 in the pulmonary capillary during CO_2 elimination in the lung. The figure is drawn as though the site of catalysis is on the capillary endothelial surface although the actual site of catalysis is unknown.

With perfusate B, we observed an increasing pH with time in the stop-flow electrode (Fig. 2*b*), indicating that this sulfonamide is capable of getting to and inhibiting the lung enzyme. Furthermore, total pH change across the lung as measured in the blood-gas machine was diminished with perfusate B relative to that with A (Table I). These findings indicate that lung carbonic anhydrase accelerates the CO_2 reactions in A while the perfusate is still within the pulmonary capillaries. Acetazolamide can be washed away from its site of interaction with the lung carbonic anhydrase, as shown by the reversal of inhibition (Fig. 2*c*).

The sum of the pH change observed for perfusate B in the stop-flow electrode and the pH change that should have occurred during the transit time between lung and electrode will equal the total pH change measured in the blood-gas machine for perfusate B if acetazolamide totally inhibits the lung carbonic anhydrase. To investigate the extent of inhibition, we used a mathematical model to compute the time-course of pH change in our perfusate B as a result of a sudden fall in P_{CO_2} as might occur in the lungs. The model was similar to that developed by Gray (9), except that total CO_2 content after the initial fall in P_{CO_2} was kept constant in our calculations. The pH change computed for the lung-to-electrode transit time plus that observed in the stop-flow electrode for perfusate B was approximately the same as that measured in the blood-gas machine. We conclude that the acetazolamide in perfusate B (0.25 mg/ml) completely inhibited the lung carbonic anhydrase within 1 min after perfusion with B was begun. Based on the total pH change in perfusate A measured in the blood-gas machine, and the mean stop-flow pH change in A (Table I) similarly extrapolated back over the lung-to-electrode transit time, the conversion of H_2CO_3 to CO_2 is more than 95% complete as perfusate A passes through the lung capillaries under the conditions of our experiments.

The fact that lung carbonic anhydrase can accelerate the CO_2 reactions in perfusate A almost to completion in the brief capillary residence time suggests that the enzyme may be physically contiguous with the perfusate. The most likely possibility is that it is bound to the luminal surface of capillary endothelial cells. It could also be located within the cells or in the interstitium, requiring HCO_3^- and H^+ (or H_2CO_3) to rapidly diffuse to those sites. The latter locations are less likely, however, because the permeability of the capillary wall to HCO_3^- would have to be high, whereas that for H^+ (or H_2CO_3) would have to be extraordinarily high because of its very low concentration gradient. Other enzymes have been localized to the pulmonary capillary endothelial surface (10). Because the actual site of catalysis is unknown, Fig. 3 has been drawn assuming it to be on the endothelial cell surface.

It is unlikely that trapped erythrocytes are responsible for the observed catalysis. No hemoglobin was found in the effluent perfusate, so any trapped erythrocytes are probably located in capillaries through which perfusate is not flowing. If erythrocytes were lysed during the preparation of the isolated lung, the carbonic anhydrase so released could bind to the endothelial surface and catalyze the reactions. If this did occur, however, it probably occurs in vivo as well, and may even be the mechanism by which the enzyme gets to the endothelial surface after being manufactured elsewhere. Finally, isolated tissue preparations are likely to be somewhat edematous, although in our lungs the measured perfusion and ventilation pressures and visual inspection suggested that they were not markedly so. Interstitial carbonic anhydrase might become accessible to perfusate during pulmonary edema, perhaps as a result of the opening of intercellular spaces in the capillary endothelium.

The conclusion that isolated lung carbonic anhydrase can cause rapid equilibration of the H_2CO_3 dehydration reaction based on our direct measurements is in agreement with recent findings by indirect techniques (11, 12) in blood-free isolated rabbit lung preparations. The extent of equilibration found in the present work is closer to the 86% found in the indicator-dilution study (11) than to the 20–30% found in the steady-state CO_2 excretion study (12) although conditions were not exactly the same in the different lung preparations.

Our original in vitro and computational studies (1, 2) and those of others (3) suggested that slow postcapillary pH changes as a result of disequilibrium of $[H^+]$ between erythrocytes and plasma as blood leaves the pulmonary capillaries should be easily observable in vivo. The disequilibrium is set up within blood in the alveolar capillaries by O_2 uptake and CO_2 elimination, leading to a net fall in intracellular $[H^+]$ but little change in extracellular $[H^+]$ in the absence of carbonic anhydrase activity in plasma (4, 5). The rate of subsequent equilibration of $[H^+]$ within downstream arterial blood is limited by the speed of the CO_2 hydration-dehydration reactions in plasma. The availability to plasma of carbonic anhydrase activity, however, either because of enzyme spilled into plasma

by hemolysis or to enzyme located in the lung, would tend to diminish or abolish these slow pH changes.

Our first in vivo studies were not able to demonstrate slow plasma pH changes in the arterial blood of normal animals, although they were easily found in carbonic anhydrase-inhibited animals (4). Further recent animal work, however, has led to the observation that the slow pH changes do exist but are of smaller magnitude than originally predicted (5, 6). These data are not inconsistent with Klocke's finding (12) of 20–30% equilibration because of isolated lung carbonic anhydrase activity, but they are incompatible with the conclusion of the present work and that of Effros et al. (11) that the effluent perfuste from isolated lungs has almost reached equilibrium by the time it leaves the pulmonary capillaries. The reasons for these apparent discrepancies are not clear, but they probably include the many inherent factors that make it difficult to directly apply data obtained in isolated organ studies to in vivo situations, as well as species differences. Further work on both isolated lung and in vivo preparations will be necessary to resolve these questions and to determine the importance of the slow postcapillary plasma pH changes in normal animals. The slow pH changes occur as predicted in carbonic anhydrase-inhibited animals possibly because the inhibitors can affect lung as well as erythrocyte carbonic anhydrase.

ACKNOWLEDGMENTS

We thank Dr. A. B. Fisher for friendly and expert assistance with and discussions of the isolated lung preparation. Acetazolamide was donated by Lederle Laboratories, Pearl River, N. Y.

This work was supported in part by National Institutes of Health research grant HL19737 and by American Heart Association grant 75-992.

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