Pulmonary removal and production of endothelin in the anesthetized dog

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Dupuis, Jocelyn, Carl A. Goresky, and Duncan J. Stewart. Pulmonary removal and production of endothelin in the anesthetized dog. *J. Appl. Physiol.* 76(2): 694–700, 1994.—The single-bolus multiple-indicator-dilution technique was used to evaluate pulmonary removal of tracer ^{125}I-labeled endothelin-1 in seven anesthetized dogs. Simultaneously, pulmonary arterial and aortic blood samples were obtained and assayed to determine the levels of immunoreactive endothelin-1. When ^{125}I-endothelin-1 was compared with a plasma vascular reference (Evans blue dye), there was a single passage mean extraction of $31 \pm 8\%$. In contrast, there was no significant difference between immunoreactive endothelin-1 levels measured in blood samples from the pulmonary artery and the aorta (1.26 ± 0.58 and 1.37 ± 0.50 pg/ml, respectively; $P = 0.47$). The absence of an arteriovenous difference for bulk endothelin-1 across the lungs in the presence of tracer data indicating a substantial uptake implies that an amount of endothelin-1 quantitatively more or less equal to that removed is produced by the lung. The shapes of the dilution curves suggest that the tracer endothelin uptake by the lung is a one-way process without vascular reentry of tracer. We conclude that the dog lung is an important site for both uptake and release of endothelin-1.

pulmonary metabolism; indicator-dilution technique; vascular endothelium

ENDOTHELIN IS A NOVEL vasoconstrictor peptide produced by the vascular endothelial cells (29). The destiny of secreted endothelin remains incompletely understood, but previous data indicate that the lungs play an important role in the removal (1) and perhaps the production of this peptide (21). The pulmonary vasculature has already been established as a major removal site for the biogenic amines (norepinephrine, serotonin) and for the hydrolysis of vasoactive peptides (angiotensin, bradykinin; Refs. 6, 19, 25). These functions, together with the anatomic location of the lung in the vasculature and its vast endothelial cell surface area, have led to an appreciation of the potentially important role played by the lung in the regulation of systemic arterial vasomotor tone. From this point of view, a pulmonary role in endothelin disposition could also be important.

In different mammals (pig, rat, and human), high tissue levels of endothelin have been found in the lungs (15). Autoradiography and receptor binding studies have also shown that the lung is a quantitatively major site of endothelin binding in rats, guinea pigs, pigs, and humans (1, 15, 23, 26). High-affinity endothelin receptors with a slow dissociation rate constant have also been found homogeneously distributed in the lungs (15). The exact role of the lung in endothelin removal has, however, been controversial. No significant extraction across the pulmo-

nary circulation was found in the pig (22), whereas substantial removal of tracer endothelin-1 was found in vivo in rats and guinea pigs (1, 26) and of unlabeled endothelin was found in buffer-perfused rat and guinea pig lungs (5). In normal humans, immunoreactive endothelin-1 levels in paired samples were significantly lower in systemic arterial blood than in venous blood, suggesting that in the steady-state net endothelin clearance occurs in the lung (28). The relationship between tracer and bulk measurements has been difficult to discern.

We therefore designed experiments to examine tracer endothelin-1 uptake by the lungs in vivo in the anesthetized dog within whatever steady state for endothelin-1 was present across the lungs. We used the single-bolus multiple-indicator-dilution approach to measure pulmonary removal of trace doses of ^{125}I-labeled endothelin-1. Simultaneous pulmonary arterial and aortic plasma samples were assayed for immunoreactive endothelin-1 and were compared with the tracer dilution data to ascertain whether there was simultaneous net pulmonary production or removal of endothelin-1.

MATERIALS AND METHODS

Indicator-dilution experiments were carried out in 13 mongrel dogs. To validate the use of Evans blue dye as a vascular reference, dilution studies were carried out in six animals with Evans blue dye and ^{125}I-labeled-albumin and the outflow profiles of the two tracers were compared. Evans blue dye binds tightly to albumin (8) and has commonly been used as a vascular reference substance. The areas under the curves of the two indicators were closely matched, with virtual superposition of the two outflow profiles. Because ^{125}I-albumin is the usually utilized vascular reference for dilution studies across the lung, we have taken the coincidence to indicate that the Evans blue dye is also an appropriate vascular reference. In the succeeding seven animals, a single set of dilution curves was obtained in each animal after the injection of a mixture containing porcine ^{125}I-endothelin-1 and Evans blue dye bound to albumin.

Animal preparation. The animals were anesthetized with intravenous pentobarbital and intubated. They were allowed to breathe room air spontaneously and, to avoid atelectasis, were manually ventilated for 15 s every 5 min. Additional doses of pentobarbital were given as necessary to maintain anesthesia. The electrocardiogram was continuously monitored through four cutaneous limb leads. A right paramedial cervical incision was performed, and the right external jugular vein was dissected. A 7F multipurpose catheter was then inserted through the vein, and its tip was positioned in the right ventricular outflow tract with fluoroscopic guidance. A left cervical incision was carried out, and a collecting catheter was inserted into the left carotid artery and positioned just above the aortic valve. A right femoral artery catheter was then installed for continuous

monitoring of blood pressure. To prevent clotting of the catheters, 3,000 U of heparin were administered intravenously.

Bolus preparation and the dilution experiment. For the endothelin studies, the injection mixture contained 2 ml of Evans blue dye (5 mg/ml; New World Trading, DeBary, FL) and 5 μCi (2.26 pmol) of porcine ^{125}I-endothelin-1 (sp act 2,200 Ci/mmol; DuPont-New England Nuclear, Boston, MA). The bolus was adjusted to the dog's hematocrit by the addition of the appropriate amount of packed red blood cells. Dog albumin was added to the total volume in the amount necessary to ensure a concentration identical to that of serum (4 g/100 ml). Dilution curves were obtained by flushing 2 ml of the injection mixture with blood into the right ventricular outflow tract with subsequent collection of timed samples ($\sim$1 ml/s) from the aortic catheter in tubes containing 50 μl of heparin (1,000 U/ml). The collected samples and standards prepared from the injection mixture with blood were centrifuged at 3,000 g for 10 min. An aliquot from the supernatant was assayed in a spectrophotometer, and Evans blue dye absorbance was determined as the difference between readings at 620 and 740 nm (8). A second aliquot was assayed in a gamma-ray spectrophotometer set at the ^{125}I photopeak to determine labeled iodine activity. With this experimental design (with a nonradioactive vascular reference), the dose of ^{125}I-endothelin-1 used for the tracer study could be minimized; an essentially trace dose was used. The experimental design is therefore one of a tracer experiment within a steady state.

Measurement of plasma endothelin-1. Before the bolus injection, paired samples of 10 ml of blood were simultaneously collected from the aorta and the right ventricular outflow tract in EDTA-containing tubes and centrifuged at 1,800 g for 20 min. Plasma samples were then extracted using SepPak C$_{18}$ cartridges (Waters, Mississauga, Ontario, Canada) that had been activated with methanol, 8 M urea, and water. Endothelin was eluted using 100% methanol, yielding a recovery of 75 $\pm$ 3.3% (SD). Samples and standards (endothelin-1; Peninsula Laboratories, Belmont, CA) were reconstituted in assay buffer and incubated for 24 h with rabbit anti-endothelin-1 serum (Peninsula Laboratories) at 4°C. The addition of $\sim$4,000 counts per minute of ^{125}I-endothelin-1 (Peninsula Laboratories) was followed by a second 24-h incubation. Bound and free radioligands were separated using the second antibody method. The bound radioactivity data were evaluated after logit-log transformation.

The antibody exhibited a cross-reactivity of 10% for human "big" endothelin and 5% with endothelin-3 but no cross-reactivity with unrelated peptides [atrial natriuretic factor-(1—28), brain natriuretic peptide, vasopressin, and angiotensins I and II]. The standard curve, defined by the equation logit B/B$_0$ = 1.74 − 2.02 log pg endothelin-1, was very stable, with 50% inhibition of binding at 7.23 $\pm$ 0.58 pg/tube. The limit of detection, defined as the least amount of immunoreactive endothelin-1 distinguishable from zero at a 95% confidence level, was 0.12 pg/tube. The intra- and interassay coefficients of variation were 9 and 12%, respectively, at the midpoint of the standard curve. Heparin in concentrations exceeding those achieved in the present experiments did not interfere with the assay. High-pressure liquid chromatography of the plasma extract showed a dominant peak of immunoreactive endothelin-1 coeluting with the synthetic endothelin-1.

Other measurements. Paired arterial and venous samples were also taken for the determination of blood gas tensions immediately before the indicator-dilution experiment. Right ventricular and arterial blood pressures were recorded, and heart rate was determined from the electrocardiogram. Wet weights of lungs lacking extraparenchymal bronchi were measured after the study.

Use of tracer curves to measure blood flow, mean transit times,

and tracer endothelin-1 extraction. To provide a basis for comparison between the two indicators injected, the concentration of each was normalized in terms of the total injected; this is equivalent to defining the total amount of each material injected as 1 unit. The outflow concentration of each tracer is then defined as its outflow fractional recovery per milliliter of blood. The relative behavior of the tracers, one in relation to the other, can then be appraised from the plot of the values of fractional recovery per milliliter as a function of time. When neither leaves the circulation, both curves will superimpose. To correct for recirculation, the dilution curves were plotted on a semilogarithmic scale and the downslopes were extrapolated in a linear fashion after the classic method of Hamilton et al. (14). Blood flow (F$_b$) was then calculated with a relationship based on conservation (the indicator utilized must be completely recovered in the outflow)

$$F_b = \frac{1}{\int_0^\infty C(t)dt} \qquad (1)$$

where $C(t)$ is the outflow fractional recovery vs. time curve for Evans blue dye and the integral in the denominator is the area under the fractional recovery per milliliter vs. time curve of the Evans blue dye.

The mean transit time for each tracer was calculated by

$$\text{mean transit time} = \frac{\int_0^\infty tC(t)dt}{\int_0^\infty C(t)dt} - \bar{t}_{\text{cath}} \qquad (2)$$

where $\bar{t}_{\text{cath}}$ is the mean transit time through the catheters of the injection and collection systems.

Instantaneous tracer endothelin extraction at any given time may be calculated from

$$E(t) = 1 - C_{\text{Et}}(t)/C_{\text{Alb}}(t) \qquad (3)$$

where $E(t)$ is instantaneous tracer endothelin extraction and $C_{\text{Et}}(t)$ and $C_{\text{Alb}}(t)$ are tracer endothelin and albumin outflow fractions per milliliter, respectively, at time t.

The mean extraction of tracer ^{125}I-endothelin-1 was calculated as

$$\text{extraction} = 1 - \text{survival} \qquad (4)$$

where Survival is the cumulative survival of tracer endothelin over the entire extrapolated primary dilution curve, the definition of which is

$$\text{survival} = \frac{\int_0^\infty C_{\text{Et}}(t)dt}{\int_0^\infty C_{\text{Alb}}(t)dt} \qquad (5)$$

where the numerator and denominator are, respectively, the area under the extrapolated fractional recovery vs. time curves of the labeled endothelin-1 and Evans blue or albumin.

The application of the theoretical analyses depends on thorough cross-sectional mixing at the point of injection. Previous studies have indicated that this will be the case. Right atrial and pulmonary arterial injections give identical and reproducible values for blood flow (16). In the estimation of pulmonary extravascular water by the indicator-dilution approach, when successive injections were made into the pulmonary artery and left atrium, the variation in the measurement is <10% (31). Moreover, when dilutional estimates of extravascular lung water are related to gravimetric estimates at high flow, the two values are essentially identical (7).

Statistical analysis. Group values are given as means $\pm$ SD. A paired t test was used to assess differences between companion aortic and right ventricular endothelin-1 values.
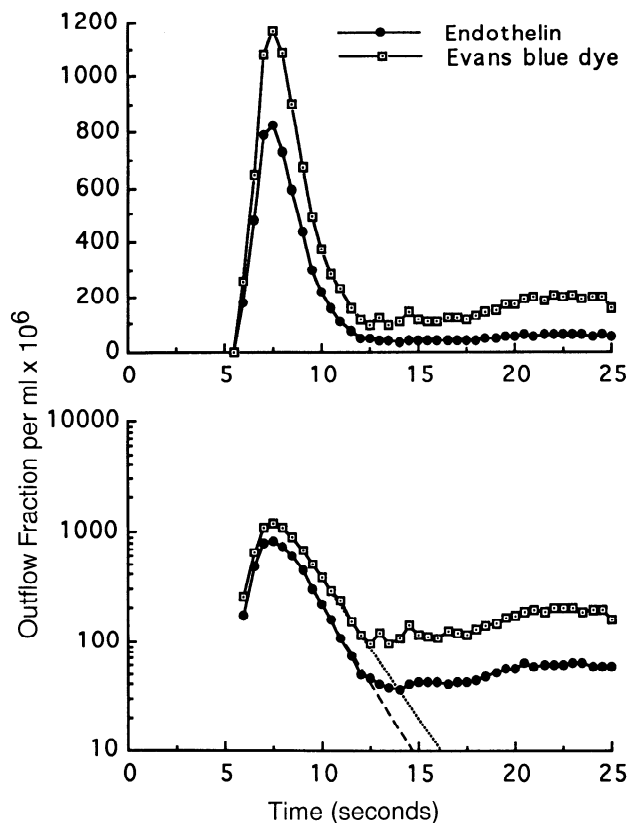
FIG. 1. Representative set of outflow indicator-dilution curves (from *experiment 7*). Fractional recovery per ml vs. time curves of vascular reference indicator Evans blue dye and ^{125}I-endothelin-1 are presented with linear (*top*) and logarithmic (*bottom*) ordinate scales.

RESULTS

Forms of the dilution data. A representative set of dilution curves (obtained during *experiment 7*) is depicted in Fig. 1 in both linear and logarithmic ordinate format. Both tracers appear simultaneously in the aorta, the initial endothelin-1 outflow fraction per milliliter being lower than that for Evans blue or albumin. The two curves then progressively separate, and the endothelin-1 values slowly become a smaller fraction of the albumin values over the primary or first passage dilution curves. This relative change is manifested as a progressive divergence on the semilogarithmic representations. Over the later parts of the data representing recirculation, there is a continuing but very minor divergence; there is no evidence of substantial late return to the circulation of the labeled endothelin-1 that was taken up by the lung during the first passage parts of the dilution curve. On the semilogarithmic representation, the downslopes of both curves become linear before recirculation; extrapolation of these downslopes was, as described, utilized to define the primary passage dilution curves for the two indicators. The ratio of the areas under the extrapolated endothelin and Evans blue curves then represents the outflow endothelin survival, and the difference between 1 and this value represents the fraction of injected tracer endothelin extracted by the lung over a single passage. The instantaneous extraction increases progressively as a function of time and levels off with the beginning of recirculation, rising only very slowly thereafter. A plot of

the natural logarithm of the ratio of the Evans blue dye fractional recovery per milliliter to that of endothelin increases linearly as a function of time over the time of the primary dilution curves (Fig. 2). The finding systematizes the relationship between the two curves and suggests that the underlying capillary transit time increases along the curve with the outflow time (11). The finding by Cua et al. (2) that outflow profiles for flow-limited tracers in the lung can be made to superimpose on their vascular reference by use of the transformation found by Goresky et al. (10) to describe flow-limited distribution of tracer in the liver is compatible both with this inference and with small variations in large-vessel transit times. In their studies of serotonin uptake in which the analysis was confined to the upslope, Dawson et al. (4) also inferred that capillary transit times increased with outflow time over this region. In the present instance, the increase is seen to extend over the whole of the primary dilution curve. Return of tracer to the circulation would have been expected to lead to a downward deviation of the log ratio vs. time curve later in time (12). This was not observed over the first passage time. The foregoing therefore suggests that the relation in the present experiments can be described by

$$C_{Et}(t) = C_{Alb}(t)e^{-k_{seq}(t-t_0)} \qquad (6)$$

where k_{seq} is an uptake or sequestration rate constant and t_0 is a common large-vessel transit time. If formulated in terms of ordinary capillary modeling, k_{seq} would correspond to PS_c/V_c, the product of the vascular permeability (P) and the surface area-to-volume ratio (S_c/V_c) for the pulmonary capillaries. Values for k_{seq} can be estimated directly from the slope of plots like that illustrated in Fig. 2. In the absence of a correction for catheter and large-vessel distortion (13), the value will be a minimum; it will be slightly smaller than the actual value. The kinetic description corresponds, of course, to the ideal case in which there is tissue uptake but no tracer return over the time of observation. With this reconstruction, it becomes appropriate to utilize the entire extrapolated curve for the computation of net tracer extraction.

Tracer endothelin-1 injection did not affect pulmonary arterial pressure values. The individual parameters derived from the dilution curves of seven animals in which tracer endothelin experiments were performed are pre-
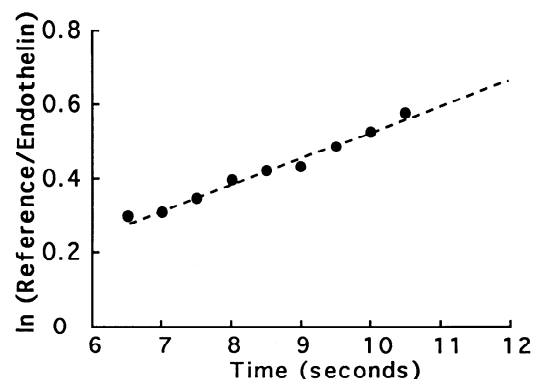

FIG. 2. Natural logarithm of ratio of Evans blue dye to tracer endothelin-1 fractional recoveries per ml vs. time. Data increase linearly until time of recirculation.

TABLE 1. *Lung weights, flows, $\bar{t}_{Et}$, $\bar{t}_{Alb}$, tracer endothelin extraction, and endothelin-like immunoassays in pulmonary arterial and aortic blood samples*

Experiment No.	Weight, kg	Lung Wet Weight, g	Blood Flow, ml/s	$\bar{t}_{Alb}$, s	$\bar{t}_{Et}$, s	Tracer Et Extraction	k_{seq}, s^{-1}	Et-LI, pg/ml	
								Pulmonary artery	Aorta
1	17	ND	22	5.08	4.95	0.20	0.064	1.05	1.02
2	24	ND	7	14.68	14.43	0.41	0.040	1.27	1.63
3	32	295	35	5.41	5.27	0.26	0.052	1.18	1.06
4	38	178	50	7.65	7.38	0.32	0.040	0.57	0.60
5	35	287	46	6.21	5.98	0.37	0.082	0.82	1.47
6	21	141	32	5.72	5.66	0.26	0.044	1.59	1.97
7	22	186	24	6.62	6.29	0.35	0.069	2.35	1.86
Mean	27	217.4	30.9	7.34	7.14	0.31	0.056	1.26	1.37
± SD	±8.0	±69.4	±14.8	±3.35	±3.31	±0.08	±0.016	±0.58	±0.50

$\bar{t}_{Et}$, mean transit time for endothelin; $\bar{t}_{Alb}$, mean transit time for albumin; Et, endothelin; k_{seq}, uptake or sequestration rate constant; Et-LI, endothelin-like immunoassays.

sented in Table 1. Mean pulmonary blood flow was 31 ± 15 ml/s (1.85 ± 0.89 l/min). One animal had a markedly low blood flow of 7 ml/s because of barbiturate overdose but maintained adequate blood pressure throughout the experiment. The average mean transit time for Evans blue dye or albumin was 7.34 ± 3.35 s, and that for tracer endothelin was, as expected, slightly shorter at 7.14 ± 3.31 s. Mean ^{125}I-endothelin-1 extraction averaged 0.31 ± 0.08 (range 0.20–0.41). Note that the animal with the lowest blood flow (longest organ transit time) achieved the highest extraction. The average value for k_{seq} was 0.056. When bulk levels were measured, there was no significant difference between the simultaneous pulmonary arterial and aortic immunoreactive endothelin-1 values (1.26 ± 0.55 and 1.37 ± 0.50 pg/ml, respectively; P = 0.47). The plasma samples from the six background experiments (no ^{125}I-endothelin injected) were also assayed for immunoreactive endothelin. These exhibited a wider variability in plasma levels, with many of the values being higher. When the two groups were combined, there was again no significant difference between the mean pulmonary arterial and aortic values (1.70 ± 0.98 and 1.77 ± 0.94 pg/ml; Fig. 3). Blood gas values and individual hemodynamic parameters are given in Table 2.
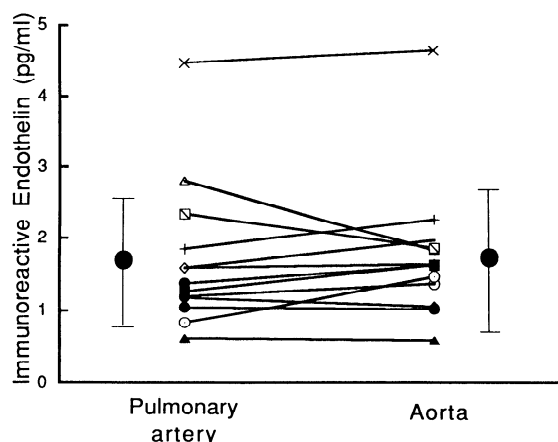

FIG. 3. Paired pulmonary artery-aorta immunoreactive endothelin-1 values from 13 anesthetized dogs. There is no significant difference between endothelin-1 mean companion values from pulmonary artery and aorta (1.70 ± 0.98 and 1.77 ± 0.94 pg/ml, respectively).

DISCUSSION

With the single-bolus indicator-dilution technique, we have found a mean of 31 ± 8% for the extraction of an ^{125}I-endothelin-1 trace dose over the time of a single pulmonary transit in the anesthetized dog. The form of the natural logarithm of the ratio of tracer albumin to tracer endothelin outflow concentrations per milliliter vs. time relationship was found to be linear over the entire course of the dilution curve, suggesting that there was no back-diffusion over this time interval. The linear increase in the extraction up to recirculation appears related to a heterogeneity of capillary transit times. A similar early profile has been observed for serotonin (4). Early in time, the outflow recovery curves are weighted by capillaries with transit times considerably shorter than the mean. The extraction for these short transit time capillaries is considerably less than that for capillaries with transit time equal to or greater than the mean transit times. A plateau is reached at the moment of recirculation after which time the extraction increases only slowly. There are several potential explanations for this finding. First, there could be backdiffusion of the extracted endothelin that appears later in time. This is, however, unlikely because previous studies have demonstrated that endothelin binds to its tissue receptor with great affinity and that it exhibits an extremely slow dissociation rate constant (15, 27). In cultured rat vascular smooth muscle cells, almost irreversible binding has previously been reported (17, 20). Second, if no backdiffusion is assumed to occur, the recirculating portion of the fractional recovery curve represents labeled endothelin-1 that has been transmitted through the systemic circulation. If substantial additional extraction had occurred in the systemic organs (kidney, liver), the instantaneous extraction should have increased further. The observed lack of further increase in extraction suggests that the systemic organs of the dog may not participate substantially in endothelin-1 extraction. Alternately, it may indicate two conformational species, one being removed poorly (3). Nevertheless, the observations indicate that lungs are a substantial site of endothelin uptake. The lung may also play a central role not only in the process being examined here, the removal

TABLE 2. *Hematocrit, hemoglobin, blood gas, and circulatory parameters*

Experiment No.	Hematocrit	Hemoglobin	pH		P_{CO_2}, Torr		P_{O_2}, Torr		Oxygen Saturation, %		Heart Rate, beats/min	Blood Pressure			
												Aorta		Pulmonary artery	
			Pulmonary artery	Aorta	Pulmonary artery	Aorta	Pulmonary artery	Aorta	Pulmonary artery	Aorta		Systolic	Diastolic	Systolic	Diastolic
1	0.47	16.7	7.28	7.31	30.6	30.1	60.5	95.6	76.7	97.3	180	175	140	34	7
2	0.45	15	7.29	7.39	32.9	19.2	34.7	62.7	55.2	91.3	180	125	95	18	3
3	0.43	14.8	7.31	7.35	35.4	33.6	50.0	87.0	78.2	98.8	185	230	150	38	10
4	0.51	18	7.30	7.35	30.5	27.7	51.3	95.3	81.7	100.0	190	245	145	51	9
5	0.46	16	7.27	7.31	35.9	33.2	49.2	84.2	76.5	98.8	170	255	150	28	10
6	0.47	17	7.27	7.36	44.0	30.0	20.0	86.0	32.2	99.3	200	205	150	35	15
7	0.41	13.5	7.22	7.29	40.7	30.5	47.0	94.0	63.0	98.0	185	210	145	32	6
Mean	0.46	15.9	7.28	7.34	35.7	29.2	44.7	86.4	66.2	97.6	184.3	206.4	139.3	33.7	8.6
±SD	±0.03	±1.5	±0.03	±0.04	±5.1	±4.8	±13.3	±11.5	±17.7	±2.9	±9.3	±44.8	±19.9	±10.0	±3.8

of endothelin-1 (1, 5, 9, 26), but also in the conversion of big endothelin to endothelin-1 (18). The fate of extracted endothelin has not yet been clarified. Subcellular fractionation of guinea pig lung homogenate after labeled endothelin-1 infusion showed that 93% of the label was associated with membranes and intracellular organelles, indicating internalization of the bound endothelin-1 (1). The findings suggest that tracer endothelin sequestration, although it may be related to receptor and second messenger mechanisms, represents primarily metabolism and local tissue clearance.

Rimar and Gillis (24) have recently carried out multiple-indicator-dilution studies in isolated rabbit lungs and hearts perfused with Krebs bicarbonate buffer containing 3% albumin. An extraction of 49% was observed in the lungs, with 5% in the heart. The findings in the lungs are more or less similar to those reported above; they emphasize the comparatively larger uptake by the lungs. Addition of 10 mM bulk endothelin-1 to the perfusion fluid reduced the pulmonary extraction to 12%. Saturation therefore appears to be a characteristic of the uptake system.

The increase in extraction with outflow time is not shared by all substrates taken up by the lungs. For benzoyl-Phe-Gly-Pro, a pharmacologically inactive angiotensin-converting enzyme substrate, conversions, which correspond to extractions, were not observed to increase along the curve with outflow time (7). This substrate undergoes hydrolysis at the capillary wall with release of product to plasma. The overall process is somewhat more complex than this, however. There is likely a simultaneous second process occurring concomitantly. A minor isomeric component more resistant to hydrolysis than its opposite, more easily cleared, configuration appears to be present, and conversion of this to its opposite configuration appears catalyzed by plasma peptidyl-prolyl *cis-trans* isomerase. There is, in addition, some plasma angiotensin-converting enzyme activity that will act on the substrate not only during passage through the capillary but also throughout the rest of its time of transit. The kinetic impact of all of the above is not completely clear. In an alternate approach, one could ask whether the angiotensin-converting enzyme activity and the endothelin uptake mechanisms are spatially coincident or whether the two have different patterns of distribution in the vasculature.

Immunoreactive endothelin-1 was simultaneously measured in the pulmonary artery and the aorta. If there were no net pulmonary production of endothelin-1, one would expect the extractions measured by immunoassay and by the tracer dilution approach to be equivalent. A lower proportional immunoassay difference across the lung will signify endothelin production by the lung. We found no significant difference between paired immunoreactive endothelin levels across the lungs, indicating that the mean pulmonary endothelin-1 rate of production more or less equals the rate at which it is removed (~30% of the rate of presentation). Even when the conclusion is tempered by the degree of variation of the immunoassay (an intra-assay coefficient of variation of 9%), the conclusion that there is significant production appears valid. The finding helps in the understanding of

previous data that showed no immunoreactive endothelin-1 extraction across the pig and human lung despite known high tissue levels (22, 30); there, too, bulk production likely masked concomitant endothelin-1 removal.

Endothelin levels are increased in different conditions associated with primary or secondary pulmonary hypertension (9, 28). In normal humans, systemic arterial and venous immunoreactive endothelin-like activity suggests that a net pulmonary extraction occurs, whereas in patients with secondary pulmonary hypertension, there were no differences in systemic arterial and venous levels. In contrast, in patients with primary pulmonary hypertension, the ratio of systemic arterial to venous immunoreactive endothelin was significantly greater than 1, suggesting a net production of the peptide by the lung (28). The present study brings into being a new way of viewing endothelin extraction studies. The tracer extraction technique, combined with immunoassay of pulmonary arterial and venous blood samples, provides a way of discriminating both organ extraction and production of endothelin. Thus, in the anesthetized dog, whereas there was a 31 ± 8% removal of ^{125}I-endothelin-1 in a single pulmonary transit time, there was a concomitant absence of a significant difference between simultaneous pulmonary arterial and venous immunoreactive endothelin-1 levels, indicating concomitant delivery of endothelin into the pulmonary circulation at a rate equivalent to its removal. We conclude that, in the dog, the lung is an important site of both uptake and release of endothelin-1.

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