

# Effects of Reduced Pulmonary Flow and Hypoxia on Metabolism of Serotonin by Rat Lungs Perfused In Situ

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To investigate the extent to which reduced pulmonary flow may affect non-ventilatory functions of the lung, pulmonary artery pressures were altered systematically in an *in vitro* perfused lung preparation. Metabolic integrity of the tissue was assessed at two levels: disposition of exogenous serotonin (5-hydroxytryptamine; 5-HT) was monitored as a specific indicator of endothelial cell metabolism; and whole-tissue rates of protein synthesis and levels of ATP were evaluated as indices of general metabolic activity and energy availability. Rat lungs were perfused with recirculating cell-free buffer (37° C) for 1 or 3 h at high (36) or low (3 ml · min<sup>-1</sup> · g<sup>-1</sup>) pulmonary flow; initial rates of 5-HT metabolism were measured over a subsequent 2-min interval of single-pass perfusion. Metabolism of 5-HT was inhibited and protein synthesis decreased 35% at low pulmonary flow. These changes did not appear to result directly from hypoxia, nor from the associated fall in tissue ATP. The effects of low flow were not reversed at high P<sub>O<sub>2</sub></sub>, nor was 5-HT metabolism inhibited by restricted oxygen availability at high flow rates. After as long as 3 h exposure to a combination of low flow, ventilation ( $\dot{V} = 0$ ), and temperature (27° C) and to the volatile anesthetic, halothane, inhibitory effects on both amine and protein metabolism were rapidly reversible. Reductions in the rate of 5-HT metabolism at reduced flow involved a decrease in the maximal velocity (V<sub>max</sub>: 8.0 to 2.2 nmol · min<sup>-1</sup> · g<sup>-1</sup>), without change in the apparent K<sub>m</sub> (2.6–3.2 μM) of the pathway for amine metabolism. These changes in endothelial metabolism thus appear to reflect a low flow-associated reduction in vascular surface area, rather than general metabolic alterations due to hypoxia *per se*. (Key words: Anesthetics, volatile: halothane. Hypoxia. Lung: blood flow. Lung, metabolism: serotonin.)

CERTAIN CLINICAL CONDITIONS associated with low pulmonary blood flow damage the pulmonary microvasculature and can result in impairment of respiratory function.<sup>1,2</sup> Injury to the pulmonary endothelium includes cytoplasmic, endoplasmic reticular and mitochondrial swelling,<sup>3</sup> and increased permeability of the endothelial barrier to water and solutes.<sup>2,4</sup> In addition, alterations in the metabolic activity of the endothelium have been observed. In anesthetized dogs, temporary removal of the lung from the circulation leads subse-

quently to increased plasma levels of prostaglandins,<sup>5</sup> possibly as a result of decreased extraction by the lung.<sup>6,7</sup> Other studies have shown that extraction of serotonin (5-HT) and norepinephrine is either increased, decreased, or unchanged after cardiopulmonary bypass in man.<sup>8,9</sup> Changes in the structural and metabolic integrity of the pulmonary endothelium have been implicated in the development of pulmonary complications following bypass,<sup>3</sup> and have been proposed as early indicators of ensuing pulmonary insufficiency.<sup>10</sup>

Conditions which occur in the lung under conditions such as those cited above include restricted pulmonary flow, hypoxia, decreased temperature, and exposure of the tissue to anesthetic agents and to blood-borne substances released during trauma. These conditions, individually or in combination, may be involved in initiating the observed metabolic changes. *In vitro*, hypoxia decreases the rate of protein synthesis, and anesthetic agents inhibit both protein synthesis and metabolism of 5-HT.<sup>11,12</sup> The present studies extended those observations by examining the effects of reduced pulmonary flow on nonventilatory lung functions. Metabolic effects were noted at two levels. First, 5-HT metabolism was taken to be a specific index of endothelial cell integrity; second, synthesis of lung proteins and tissue levels of high-energy phosphates were taken as general indicators of overall cellular metabolism and energy availability, respectively. This allowed the consequences of changes at the vascular surface to be resolved from the general inhibitory effects of hypoxia. The results suggest that, in the perfused lung, inhibition of 5-HT metabolism at low pulmonary flow reflects a lowered vascular surface area, and does not result from hypoxia *per se*.

## Materials and Methods

### LUNG PERFUSION

Male Sprague-Dawley rats (175–200 g) obtained from Charles River Laboratories were allowed free access to food (Agway RMH 3000; Agway, Inc., Syracuse, NY) and water. Animals were anesthetized by intraperitoneal injection of sodium pentobarbital (65 mg/kg; Fort Dodge Laboratories, Inc., Fort Dodge, IA). Lungs were perfused as described previously<sup>11</sup> with modifications to permit rapid introduction of 2-[<sup>14</sup>C]5-HT into the pulmonary vasculature.<sup>12</sup> To ensure that blood was

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cleared from the perfusion circuit, the animals were heparinized (4 mg/kg body weight *iv*) prior to cannulation of the pulmonary vasculature; in addition, the first 30 ml of perfusate to pass through the lungs were discarded.<sup>11</sup> After a preliminary period of equilibration of 10–180 min during which perfusate free of 5-HT was recirculated, 5-HT uptake and metabolism were measured during a 2-min interval of single-pass perfusion using buffer from a second perfusate reservoir containing radiolabelled 5-HT. Amine uptake was linear over 2 min and remained so independent of pulmonary flow (data not shown). When indicated, the rate of protein synthesis was estimated during the equilibration period; radiolabelled phenylalanine was added to the perfusate at the beginning of recirculation, as detailed below.

The perfusion buffer was Krebs-Henseleit bicarbonate buffer containing 5.6 mM glucose, 19 amino acids at concentrations measured in rat plasma,<sup>11</sup> 0.69 mM phenylalanine, and 4.5% (w/v) bovine serum albumin (fraction V; Pentex; Miles Laboratories, Naperville, IL). Where indicated, 5-hydroxy-2-[<sup>14</sup>C]tryptamine ([<sup>14</sup>C]5-HT; Amersham) was added to the buffer used for single-pass perfusion at 1–20  $\mu$ M (specific radioactivity, 1–15  $\times 10^3$  dpm/nmol). Ring-2,6-[<sup>3</sup>H]-phenylalanine (Amersham, Arlington Heights, IL) was added to the recirculating perfusate at a final specific radioactivity of 820 dpm/nmol. All perfusate buffers were equilibrated with warmed, humidified O<sub>2</sub>/N<sub>2</sub>/CO<sub>2</sub> (20:75:5), O<sub>2</sub>/CO<sub>2</sub> (95:5), or N<sub>2</sub>/CO<sub>2</sub> (95:5), as indicated. In each case, the same gas mixture was supplied both to the airways (unless no ventilation was provided) and to the perfusate reservoir.<sup>11</sup> Ventilation was at a peak airway pressure of 15 cm H<sub>2</sub>O (72 breaths/min) and a positive end-expiratory pressure (PEEP) of 2 cm H<sub>2</sub>O, using a small animal respirator (either Harvard Apparatus, Millis, MA or Analytical Specialties, Inc., St. Louis, MO), to provide a tidal volume of 10 ml/kg body weight. Each 5 min, the lungs were sighed to prevent atelectasis. In experiments indicated, minute ventilation was reduced to zero and the lungs were allowed to collapse against a PEEP of 2 cm H<sub>2</sub>O. When halothane (2-bromo-2-chloro-1,1,1-trifluoroethane; Fluothane, Ayerst Laboratories, Inc., New York, NY) was present (2% v/v), it was added to the gas phase supplying both the perfusate reservoir and the respirators using a Fluotec 3 vaporizer (Frazer-Sweatman, Inc., Lancaster, NY), as documented previously.<sup>12</sup>

Perfusate flow ( $Q$ ; ml  $\cdot$  min<sup>-1</sup>  $\cdot$  g<sup>-1</sup>) was held constant during the equilibration and uptake intervals, at the rates indicated. In the perfused preparation employed, a pulmonary artery pressure of 20 cm H<sub>2</sub>O results in pulmonary flow of about 36 ml  $\cdot$  min<sup>-1</sup>  $\cdot$  g<sup>-1</sup>. This represents the "high flow" condition. Lower flows, ranging from 2 ml  $\cdot$  min<sup>-1</sup>  $\cdot$  g<sup>-1</sup> upward, were established at re-

duced perfusion pressure by decreasing the speed of the peristaltic pump which supplied perfusate to the pulmonary artery. Pulmonary flow was measured by timed collection of the venous effluent in a graduated cylinder. Outflow pressure was 0, with respect to the left atrium. Previous studies established the stability of rat lungs perfused *in situ*, as well as the absence of hemodynamic responses to levels of 5-HT as high as 100  $\mu$ M.<sup>11,12</sup>

The temperature of the preparation was either 27° C or 37° C. The perfusion apparatus was held at 37° C by an air convection system which included thermostat-controlled incandescent light bulbs. When the lamps were removed, the temperature of the apparatus stabilized at 27° C.

#### MEASUREMENT OF 5-HT METABOLISM

The content of 5-HT and its metabolite, 5-hydroxyindolacetic acid (5-HIAA), in tissue and perfusate samples was estimated in perchloric acid extracts as validated previously<sup>12</sup> using ion-exchange chromatography.<sup>13</sup> Briefly, weighed samples of right lung or perfusate (2 ml) were deproteinized by homogenization (Polytron homogenizer; Brinkman Instruments, Westbury, NY) in perchloric acid (PCA) at a final concentration of 0.5 M. Acid-soluble material was neutralized with KOH, then centrifuged (10,000  $\times$  g, 10 min). A portion of the PCA supernatant was applied to a column (0.9  $\times$  2 cm) of Bio-Rex 70 (Bio-Rad Laboratories, Richmond, CA); 5-HIAA was eluted with water, followed by 5-HT with 0.25 M HCl. Radioactivity in the eluates was determined by liquid scintillation spectrometry using Formula 947 scintillation cocktail (New England Nuclear, Boston, MA). Tissue 5-HT and 5-HIAA content were calculated on the basis of the specific radioactivity of [<sup>14</sup>C]5-HT added to the perfusate, and were corrected for loss in the extraction procedures, as determined in each sample of tissue or perfusate.<sup>12</sup>

Unless indicated otherwise, production of 5-HIAA by the lung was employed to estimate 5-HT metabolism. The entire tissue content of 5-HT could be accounted for by distribution of the unmetabolized amine in extracellular water; furthermore, 5-HIAA was not released into the perfusate in detectable amounts during a 2-min interval at any flow rate employed. Changes in lung 5-HIAA content, therefore, reflected the rate of uptake and deamination of 5-HT. Because 5-HT did not accumulate in the lung under any of the conditions examined, uptake appeared to remain rate-limiting to the metabolic pathway.<sup>14</sup> Initial rates of 5-HT metabolism by the present preparation remained stable during at least 180 min of perfusion.<sup>12</sup>

In some experiments, 5-HT metabolism ( $V$ ) was esti-

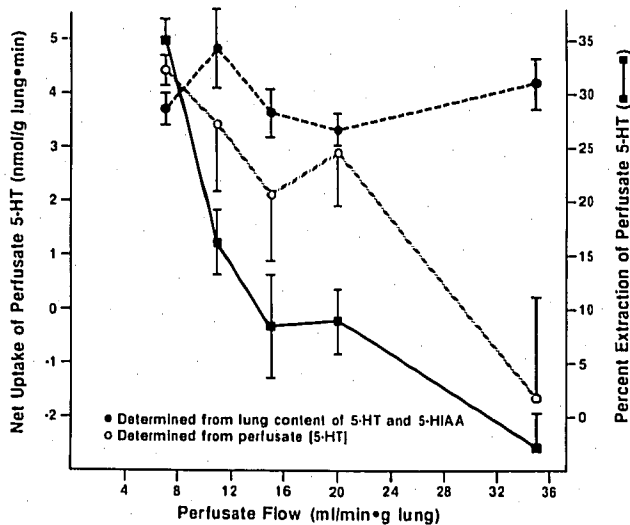


FIG. 1. Effect of perfusate flow on measurement of 5-HT uptake by perfused lungs. Lungs were perfused as described in "Methods" at the pulmonary flows indicated. Net uptake of 5-HT (2  $\mu$ M) was determined by analyzing tissue content of 5-HT + 5-HIAA (closed circles) or by estimating disappearance of 5-HT in the perfusate (open circles), as described in the text. The period of preliminary perfusion was 10 min; 5-HT metabolism was measured over a subsequent 2-min interval. Values represent the mean  $\pm$  SE of six observations. The small negative values shown at high flow rates do not differ significantly from zero.

mated based on removal of the amine from the perfusate:

$$V = Q \times (C_{in} - C_{out}),$$

where  $V$  represents amine uptake ( $\text{nmol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ ),  $Q$  is pulmonary flow ( $\text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ ),  $C_{in}$  is the concentration of 5-HT in the perfusate reservoir ( $\text{nmol} \cdot \text{ml}^{-1}$ ), and  $C_{out}$  is the concentration of the amine in the venous effluent. Percent extraction of perfusate 5-HT ( $E$ ) was calculated as:

$$E = 100 \times (C_{in} - C_{out}) / C_{in}.$$

#### MEASUREMENT OF PROTEIN SYNTHESIS

Conditions required for accurate estimates of the rate of protein synthesis in perfused lungs were detailed earlier.<sup>15,16</sup> Acid-insoluble material from tissue PCA extracts (above) was washed three times with 0.5 M PCA containing an excess (1 g/l) of non-radiolabelled phenylalanine, once with 0.5 M PCA, and was then dissolved in 0.3 M sodium hydroxide. Aliquots were taken for assay of protein content<sup>17</sup> and radioactivity, to determine incorporation ( $\text{dpm} \cdot \text{h}^{-1} \cdot \text{mg protein}^{-1}$ ) of [<sup>3</sup>H]phenylalanine into lung protein. Rates of protein synthesis ( $\text{nmol of phenylalanine incorporated} \cdot \text{h}^{-1} \cdot \text{mg protein}^{-1}$ ) were calculated based on the specific radioactivity ( $\text{dpm} \cdot \text{nmol}^{-1}$ ) of phenylalanine in the perfusate. Previous experiments<sup>16</sup> showed that high extracellular

concentrations of phenylalanine, as used in the present study, minimized dilution of the specific radioactivity of the amino acid with non-radioactive phenylalanine released by proteolysis. Under these conditions, the specific radioactivities of phenylalanine in the extracellular, intracellular, and aminoacyl-tRNA pools were equal, and remained in a steady state throughout at least 3 h of perfusion. In the experiments reported here, amino acids in PCA extracts of both lungs and perfusate were measured by HPLC;<sup>18</sup> intracellular concentrations and specific radioactivities were calculated as described earlier.<sup>15,19</sup>

#### OTHER ANALYTICAL PROCEDURES

At the end of perfusion, the left lung was frozen rapidly at the temperature of liquid nitrogen; tissue ATP content was estimated enzymatically.<sup>11</sup> [<sup>14</sup>C]Sorbitol and [<sup>3</sup>H]phenylalanine spaces were determined in PCA extracts of tissue prepared as detailed previously.<sup>15</sup> Based on the observations outlined above, uptake and metabolism of 5-HT by perfused lungs were treated as a single process for purpose of calculating apparent kinetic parameters.<sup>12</sup> Apparent  $K_m$  and  $V_{max}$  were estimated using the direct linear plot suggested by Eisenthal and Cornish-Bowden.<sup>20</sup>

Significance of differences among means was determined by one-way analysis of variance; significance between means was determined using Dunnett's test. Values of  $P < 0.05$  were considered to be significant.

#### Results

##### MEASUREMENT OF 5-HT UPTAKE BY THE LUNG

In a number of published studies, the metabolic integrity of the pulmonary endothelium has been assessed by determining removal of biogenic amines from the plasma or perfusion buffer across the pulmonary circulation.<sup>6,21,22</sup> The accuracy and sensitivity of this approach are diminished as either amine concentration or pulmonary flow is increased. As the present kinetic studies demand measurements of 5-HT metabolism over a wide range of amine concentrations and pulmonary flows, measurements of arterio-venous substrate differences were not feasible under all conditions. This is illustrated in figure 1, where uptake of perfusate 5-HT is shown at low amine concentration as a function of pulmonary flow. Whether the data are expressed as net uptake (open circles) or as percent 5-HT extracted by the lung (closed squares), increasing flow from 7  $\text{ml} \cdot \text{min}^{-1} \cdot \text{g lung}^{-1}$  into the physiological range caused estimates of amine metabolism to decrease and to become substantially more variable. Although, at low flow, 35% of perfusate 5-HT was extracted by the tissue, no arterio-venous concentration difference could

TABLE 1. Effect of Decreased Pulmonary Flow and Ventilation on Uptake and Metabolism of 5-HT

Experimental Group	N	Equilibration Period (60 min)		Uptake Period (2 min)		5-HIAA Production nmol/g lung · min	5-HT Uptake Determined From:	
		Q ml/min · g lung	V ml/min · 100 g BW	Q ml/min · g lung	V ml/min · 100 g BW		Lung Content of 5-HT + 5-HIAA nmol/g lung · min	Change in Perfusate [5-HT] nmol/g lung · min
I	6	24 ± 1.8	72	26 ± 2.0	72	5.40 ± 0.55	6.37 ± 0.55	N.D.
II	6	3 ± 0.2	0	4 ± 0.4	72	2.84 ± 0.25*	3.85 ± 0.28*	2.71 ± 0.38
III	6	3 ± 0.5	0	26 ± 2.4	72	4.13 ± 0.35	5.47 ± 0.56	N.D.
IV	6	28 ± 2.2	72	6 ± 0.4	72	4.12 ± 0.36	4.71 ± 0.41	5.76 ± 0.79
V	6	25 ± 1.6	0	5 ± 0.7	0	4.00 ± 0.22	5.02 ± 0.33	5.62 ± 0.85
VI	6	35 ± 1.3	0	35 ± 1.3	0	4.30 ± 0.20	5.89 ± 0.28	N.D.

Lungs were perfused as described in "Methods" at the pulmonary flow (Q) and minute ventilation (V) indicated. The equilibration period of 60 min was followed immediately by a 2-min interval, during which uptake and metabolism of 5-HT were estimated as described in

the text; the perfusate concentration of 5-HT was 2  $\mu$ M. Values represent mean  $\pm$  SE of the number of observations. N.D. = not determined.

\*  $P < 0.01$  vs. group I.

be detected at high flow rates. As 5-HT concentration was increased toward levels required to saturate the pathway of its metabolism, inadequate sensitivity of these measurements was encountered at progressively lower flows (data not shown).

When total amine uptake was estimated directly from the sum of tissue content of 5-HT and its metabolite 5-HIAA (fig. 1, closed circles), rates were in good agreement with the determinations above at low flow (net uptake,  $4.4 \pm 0.3$ ; tissue content,  $3.7 \pm 0.3$  nmol · min<sup>-1</sup> · g<sup>-1</sup>). Measurements of metabolism based on tissue analysis were, however, flow independent in the range above 7 ml · min<sup>-1</sup> · g<sup>-1</sup>, and averaged 4 nmol · min<sup>-1</sup> · g<sup>-1</sup> over the entire range of flows studied. Based on these observations, and on the facts that tissue 5-HT is confined to the extracellular space,<sup>12</sup> and that carrier-mediated uptake of the amine into the cell, rather than its metabolism, is the rate-limiting step in its removal from the vascular space,<sup>12,14</sup> the rate of accumulation of 5-HIAA in the lung was taken subsequently as a direct measure of 5-HT transport and metabolism and, therefore, of the metabolic integrity of the pulmonary endothelium.

#### EFFECTS OF PULMONARY FLOW AND VENTILATION ON 5-HT UPTAKE AND METABOLISM

Table 1 shows the effects of an interval of altered pulmonary flow and ventilation on metabolism of 5-HT by the lung. As compared to controls provided high flow and ventilation (group I), low pulmonary flow, and cessation of ventilation for 60 min led to a 40% decrease in uptake and metabolism of 5-HT (2  $\mu$ M), as measured at low flow (group II). Return of pulmonary flow to control values only during the interval while uptake was measured immediately reversed the depression of 5-HT metabolism (group III). Resumption of ventilation was

not required for reversal (data not shown). In contrast, acute exposure of the lungs to low pulmonary flow with or without ventilation (following 60 min of high pulmonary flow) did not affect metabolism of the amine (groups IV and V). Thus, the inhibitory effects of reduced flow were not immediate in onset, but were rapidly reversible. Cessation of ventilation alone was also without effect on 5-HT disposition (group VI). Data from these experiments, in which 5-HT was present at low concentration (2  $\mu$ M), show good agreement between rates obtained from analysis of tissue or perfusate samples at low to moderate rates of pulmonary flow.

The effects of ventilation and perfusate flow on the synthesis of lung proteins were examined under similar conditions. First, protein synthesis was estimated during 60 min of perfusion at high pulmonary flow (fig. 2); a wide range of minute ventilations was achieved by varying both ventilatory rate and tidal volume. These alterations did not result in a significant change in the synthesis of lung proteins, which averaged about 2.0 nmol phenylalanine incorporated per h/mg lung protein. Indeed, cessation of ventilation during either 60 min (fig. 2) or 180 min (data not shown) of perfusion did not depress the rate of protein synthesis.

Synthesis of lung proteins was dependent, however, on pulmonary flow. At flow rates of 8 ml · min<sup>-1</sup> · g<sup>-1</sup> or higher, control synthetic rates were maintained for at least 60 min. When flow was reduced to 6 ml · min<sup>-1</sup> · g<sup>-1</sup> or lower during this interval, synthesis was reduced by 30–40% (fig. 3).

#### EFFECTS OF PROLONGED REDUCTIONS IN PULMONARY FLOW ON METABOLIC ACTIVITY OF THE LUNG

The effects of more prolonged periods (hours) of restricted pulmonary flow were investigated in perfused

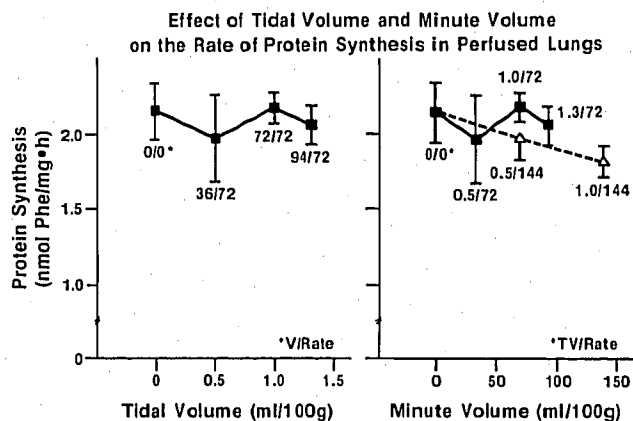


FIG. 2. Effects of minute ventilation on protein synthesis in perfused lungs. Lungs were perfused and protein synthesis measured as described in "Methods"; ventilatory parameters were altered as indicated. Ventilatory frequencies (rate) are expressed as breaths/min; tidal volumes as ml/100 g body weight. *Left panel:* tidal volume (TV) was increased at a constant frequency of 72. *Right panel:* frequency (closed symbols, 72; open symbols, 144) and tidal volume were increased simultaneously. Values represent the mean  $\pm$  SE of six to ten observations.

lungs by extending the equilibration interval to 3 h, followed by subsequent measurement of 5-HT metabolism under the same perfusion conditions (table 2). Protein synthesis was monitored during the equilibration period, and tissue levels of ATP were determined at the end of perfusion.

When flow was restricted for 3 h, 5-HIAA production decreased further to only 20–30% of the control rate, while protein synthesis remained inhibited about 60% (compare groups I and II, table 2). Inhibition of 5-HT metabolism was observed at amine concentrations of both 1 and 20  $\mu$ M, and was accompanied by a substantial reduction in tissue ATP. With similar flow restriction, lowering the temperature to 27° C prevented the fall in ATP, but did not ameliorate the ef-

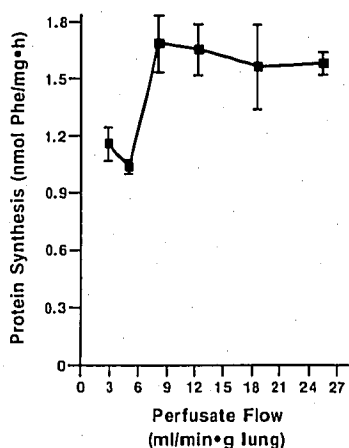


FIG. 3. Effect of pulmonary flow on synthesis of lung proteins. Lungs were perfused for 60 min at the pulmonary flow indicated. Protein synthesis was estimated as in "Methods." Values are the mean  $\pm$  SE of four to 12 observations.

fects of flow restriction on 5-HIAA production. Protein synthesis was depressed an additional 50% at 27° C, consistent with the expected effect of a 10° C drop in temperature (table 2, group III). Independent of temperature, low flow decreased the maximal velocity of 5-HT metabolism approximately fourfold. No change in the apparent  $K_m$  of the process was evident.

Reduction in pulmonary flow to less than 10% of control values may have resulted in tissue hypoxia and associated hemodynamic changes. In order to distinguish between effects of hypoxia and of flow restriction *per se*, lungs were exposed to either 95% O<sub>2</sub>:5% CO<sub>2</sub> ("high P<sub>O<sub>2</sub></sub>") or 95% N<sub>2</sub>:5% CO<sub>2</sub> ("hypoxia"). In previous studies, 95% N<sub>2</sub>:5% CO<sub>2</sub> effected a reduction in effluent perfusate P<sub>O<sub>2</sub></sub> to 18 mmHg and lowered tissue ATP content to less than half that present at 20% O<sub>2</sub>.<sup>11</sup> In the present experiments, 95% N<sub>2</sub>:5% CO<sub>2</sub> resulted in a similar reduction in tissue ATP and in a 60% inhibition of the rate of protein synthesis (table 2, group VI). However, at high pulmonary flow, hypoxic tissues maintained 5-HIAA production at control rates, even though indices of overall metabolism remained depressed (table 2, group VI). Thus, hypoxia did not have a direct effect on the uptake and metabolism of 5-HT by the pulmonary endothelium. In these experiments, only small changes in pulmonary resistance due to hypoxia were observed.

With high flow, equilibration of the perfusate with 95% O<sub>2</sub>:5% CO<sub>2</sub> increased the rate of 5-HT metabolism significantly above the control (20% O<sub>2</sub>) value. Elevation of P<sub>O<sub>2</sub></sub> at low flow (table 2, group V) prevented loss of ATP (compare group II), but did not result in maintenance of control rates of protein synthesis or 5-HT metabolism (table 2, groups II, IV, and V). This experiment supports dissociation of the effects of low flow and tissue hypoxia on amine metabolism. Again, the decreased rate of production of 5-HIAA appeared to result from a decrease in the V<sub>max</sub> of 5-HT metabolism.

Table 3 shows the effect of simultaneous exposure of the lungs to a combination of low pulmonary flow, low temperature, lack of ventilation, and a volatile anesthetic agent (group I). This combination of variables was intended to simulate some of the additional conditions to which the lung might be exposed under clinical conditions of restricted blood flow. During 180 min, protein synthesis decreased to 30% of control and subsequent production of 5-HIAA was inhibited by 76% (compare table 2, groups I and II). Thirty minutes after these parameters were restored to control values, the effect on 5-HT metabolism was completely reversed (table 3, group II). The rate of protein synthesis, measured during the 15–30-min interval of the recovery period, reached 67% of the control. The latter value

TABLE 2. Effect of Prolonged Periods of Flow Restriction on Pulmonary Metabolism

Experimental Group	N	Q ml/min · g lung	V ml/min · 100 g	T °C	O <sub>2</sub> %	Protein Synthesis nmol Phe/mg · h	[5-HT] μM	5-HIAA Production			ATP nmol/g dry
								nmol/g lung · min	Km μM	Vmax nmol/g lung · min	
I (control)	4	32.4 ± 1.6	72	36.4 ± 0.6	20	1.90 ± 0.16	1	2.04 ± 0.12	2.6	8.0	10.52 ± 0.46
	3	32.7 ± 1.2	72	36.0 ± 1.5	20		20	7.06 ± 0.22			
II (low Q)	3	2.5 ± 0.5	0	36.3 ± 0.8	20	1.06 ± 0.11*	1	0.46 ± 0.11*	3.2	2.2	4.85 ± 0.69*
	4	2.1 ± 0.3	0	36.1 ± 0.4	20		20	1.92 ± 0.62*			
III (low Q, T)	3	2.8 ± 0.3	0	27.2 ± 0.4	20	0.56 ± 0.04*	1	0.65 ± 0.03*	2.4	2.2	9.91 ± 0.86
	4	2.6 ± 0.2	0	27.5 ± 0.3	20		20	2.01 ± 0.06*			
IV (high P <sub>O<sub>2</sub></sub> )	4	30.0 ± 1.6	72	36.8 ± 0.5	95	2.02 ± 0.14	1	3.60 ± 0.28*	2.0	9.1	9.51 ± 0.60
	3	36.0 ± 1.0	72	36.3 ± 0.9	95		20	9.39 ± 1.20			
V (high P <sub>O<sub>2</sub></sub> , low Q)	3	2.9 ± 0.1	0	37.0 ± 0.4	95	1.33 ± 0.15†	1	0.76 ± 0.21†	2.0	3.2	9.33 ± 0.71
	3	3.3 ± 0.2	0	37.0 ± 0.4	95		20	2.29 ± 0.32†			
VI (hypoxic)	3	29.3 ± 3.3	72	36.3 ± 0.7	0	0.72 ± 0.05*	1	2.17 ± 0.56	N.D.	N.D.	4.92 ± 1.08*

Lungs were perfused as described in table 1. The period of equilibration was 180 min; 5-HT metabolism was estimated during a subsequent 2-min uptake interval under the same perfusion conditions. Protein synthesis was measured during the equilibration period. The gas phase was O<sub>2</sub>:N<sub>2</sub>:CO<sub>2</sub> (20:75:5; 95:0:5; 0:95:5) as indicated. Metabolic rates in group VI (hypoxic) were corrected for an increase in lung

weight due to edema.<sup>11</sup> Neither concentration of 5-HT caused changes in pulmonary hemodynamics or lung water content. Values represent the mean ± SE of N observations. N.D. = not determined.

\* P < 0.01 vs. corresponding control in group I.

† P < 0.01 vs. corresponding control in group IV.

reflects a combination of the time required for reversal to become complete and the relatively long interval required for synthesis measurements. Significant changes in the tissue content of high-energy phosphate were not observed under the conditions of this experiment.

Altered substrate delivery may play a role in accounting for reduced rates of amine and protein metabolism under low flow conditions. At low pulmonary flow, sorbitol space was reduced by 30% (table 4). As sorbitol is a marker of extracellular water, the observed reduction in distribution may at least partially represent decreased vascular volume. In contrast, the equilibration of phenylalanine, the distribution of which includes the intracellular compartment, was not altered by restricted pulmonary flow (table 4). In addition, the specific radioactivity of intracellular phenylalanine reached a level

equal to that measured in the perfusate. Table 5 shows that the intracellular concentrations of three essential amino acids, threonine, lysine, and histidine, decreased at low pulmonary flow. Intracellular levels of two compounds increased when flow was restricted. In an earlier study, protein synthesis was not inhibited at intracellular concentrations of these amino acids as low as or lower than those observed here.<sup>19</sup> Together, these results suggest that the flow-dependent inhibition of synthesis of lung proteins probably does not reflect a limitation of amino acid availability or an artifact of incomplete equilibration of the radioactive precursor.

### Discussion

Most published studies of pulmonary uptake of 5-HT have been based on estimates of removal of the amine

TABLE 3. Effect of Anesthesia and Reduced Flow, Ventilation, and Temperature on Pulmonary Metabolism

Experimental Group	Equilibration Period (3 h)				Uptake Period (2 min)				5-HIAA Production nmol/g lung · min	Protein Synthesis nmol Phe/mg · h	ATP nmol/g dry
	Q	V	T	Halothane %	Q	V	T	Halothane %			
I	3.0 ± 0.3	0	27.9 ± 0.4	2	3.0 ± 0.3	0	27.9 ± 0.4	2	0.48 ± 0.09	0.57 ± 0.07	7.09 ± 0.81
II	2.8 ± 0.4	0	26.3 ± 0.3	2	30.9 ± 1.9	72	36.9 ± 1.1	0	2.53 ± 0.52*	1.27 ± 0.12*	9.66 ± 1.05

Lungs were perfused as described in table 2 at the pulmonary flow (Q; ml/g lung · min), minute ventilation (V; ml/min · 100 g body weight) and temperature (T; °C) indicated. The gas phase was O<sub>2</sub>:N<sub>2</sub>:CO<sub>2</sub> (20:75:5). In experiment II, Q, V, and T were returned to control values following the equilibration period. Exposure to halothane was also stopped at this time. Thirty minutes were allowed for recovery before 5-HIAA production was determined; 15 min of recovery were allowed prior to estimation of the rate of protein synthesis. The

concentration of 5-HT in these experiments was 1 μM. In an additional experiment, flow was increased to the control rate after 180 min under low flow conditions. During the subsequent 2-min interval, 5-HIAA production was 2.17 ± 0.15 nmol/g lung · min, a rate equal to that observed in control tissues (compare table 2, group I; 1 μM 5-HT). Values represent the mean ± SE of four observations.

\* P < 0.01 vs. group I.

TABLE 4. Effect of Pulmonary Flow on Phenylalanine Equilibration

Parameter	Low Q	High Q
Pulmonary flow ml/min · g lung	2.7 ± 0.2*	31.9 ± 1.5
Sorbitol space ml/g lung	0.31 ± 0.01*	0.44 ± 0.02
Phenylalanine space ml/g lung	0.68 ± 0.01	0.67 ± 0.04
Intracellular phenylalanine specific radioac- tivity (SA) % of extracellular SA	99 ± 2	106 ± 6
Protein synthesis nmol phenyl- alanine/mg protein · h	0.34 ± 0.03*	1.89 ± 0.17

Lungs were perfused for 180 min as described in "Methods" at the pulmonary flow (Q) indicated. The specific radioactivity of extracellular [<sup>3</sup>H]phenylalanine was 820 dpm/nmol. Values represent the mean ± SE of six observations.

\*  $P < 0.001$  vs. high Q.

from the fluid perfusing the vasculature. While this is an effective approach to determine the amount of 5-HT that escapes inactivation by the lung, a useful parameter for investigation of non-ventilatory lung function *in vivo*, it provides only limited information regarding amine uptake and metabolism by the tissue itself. Differences in amine concentration between arterial and

TABLE 5. Effect of Pulmonary Flow on Intracellular Concentrations of Essential Amino Acids

Amino Acid	Low Q	High Q
Arginine	55 ± 5 (160 ± 11)	65 ± 5 (186 ± 11)
Threonine	801 ± 73* (346 ± 12)	1514 ± 115 (389 ± 20)
Methionine	78 ± 2 (71 ± 8)	114 ± 17 (83 ± 7)
Valine	428 ± 56 (17 ± 6)	558 ± 82 (195 ± 10)
Tryptophan	15 ± 3 (113 ± 5)	B.D. (111 ± 11)
Leucine	372 ± 66 (191 ± 8)	282 ± 56 (201 ± 9)
Isoleucine	181 ± 34 (160 ± 13)	95 ± 29 (189 ± 25)
Tyrosine	306 ± 38 (75 ± 7)	321 ± 36 (80 ± 6)
Histidine	18 ± 2* (36 ± 5)	64 ± 5 (85 ± 10)
Lysine	341 ± 28* (300 ± 15)	178 ± 12 (371 ± 27)

Lungs were perfused for 180 min with plasma levels of amino acids as described in "Methods" at the pulmonary flows indicated in table 4. Values are means ± SE of data from six lungs. B.D. = below detection. Concentrations (μM) of both intracellular and (parenthesis) extracellular essential amino acids are presented.

\*  $P < 0.001$  vs. high Q.

effluent perfusate are influenced both by 5-HT concentration and by the rate of pulmonary flow. The present studies show that estimates of amine metabolism based on the loss of circulating 5-HT are accurate only at sub-physiological flow rates and low amine concentrations. As pulmonary flow is increased toward the physiological range, amine extraction decreases to a value which cannot be reliably quantitated. This phenomenon is exaggerated at high perfusate 5-HT concentrations which are required for estimates of kinetic parameters. On the other hand, the actual amount of 5-HT taken up by the lungs is independent of flow at rates above 6 ml · min<sup>-1</sup> · g<sup>-1</sup>. Thus, at near physiological flows, as well as at substrate concentrations which permit evaluation of the capacity of the endothelial uptake system (V<sub>max</sub>), analysis of tissue metabolite levels is required.

The present findings compliment other studies relating pulmonary flow and uptake of 5-HT. Using an indicator-dilution technique in blood-perfused lungs of dogs undergoing right heart bypass, Pitt *et al.*<sup>5</sup> showed that, while extraction of tracer quantities of 5-HT was flow-independent at near normal flow rates, it rose significantly when flow was decreased to 10–20% of control. Similarly, Rickaby *et al.*<sup>22</sup> observed a decrease in the percentage of 5-HT taken up by isolated dog lungs when blood flow was increased from 4.9 to 9.7 ml · min<sup>-1</sup> · g lung<sup>-1</sup>. Wiersma and Roth<sup>21</sup> also reported flow-dependence of 5-HT extraction by isolated perfused rat lungs. In the latter study, however, calculated amine uptake by lung tissue appeared essentially flow-independent, in general agreement with the present data.

The low flow-induced depression in rates of 5-HT metabolism and protein synthesis observed in the present study could have resulted directly from tissue hypoxia, from general limitations to substrate availability, or from a change in the surface area of the endothelium exposed to perfusate. Under high flow conditions, perfusate equilibrated with a gas mixture containing 20% O<sub>2</sub> supplies about four times the oxygen requirements of the tissue, without counting the contribution of ventilatory gas.<sup>11</sup> Reduction of flow to 3 ml/min · g (without ventilation; table 2) was expected to reduce the oxygen supply to less than half that required to support metabolism.<sup>23,24</sup> This restriction in oxygen availability is of similar magnitude to that observed in lung preparations exposed to 95% N<sub>2</sub>:5% CO<sub>2</sub>, where perfusate P<sub>O<sub>2</sub></sub> is 18 mmHg,<sup>11</sup> in order to produce tissue hypoxia. Furthermore, both reduced ATP content and protein synthesis provide evidence that the lungs were hypoxic at low pulmonary flow.

When the imbalance between oxygen supply and demand was re-established at low flow, either by reducing

the temperature of the preparation or by increasing perfusate  $P_{O_2}$  (95%  $O_2$ ), ATP was restored, but 5-HT metabolism remained depressed. These results suggest that the lung is hypoxic during periods of restricted flow, but they do not support an exclusive role for hypoxia or for an associated reduction in high energy phosphate stores in the depression of 5-HT metabolism. In fact, the similar magnitude (75–80%) of the low flow-associated reduction in 5-HT metabolism at both normal and high oxygen (table 2) suggests a limited hypoxia-associated component in the inhibition. This conclusion is in agreement with some, but not all, published reports of the extent of inhibition of 5-HT uptake by hypoxia which, in lungs of rodents and lagomorphs, ranges from 9–68%.<sup>14,25,26</sup> The basis of these discrepancies is unknown, but may lie in species differences or variations in perfusion protocols.

The observed reduction in the maximal rate of 5-HT metabolism, without a change in apparent  $K_m$ , suggests that, in the absence of reduced substrate supply, endothelial surface area was decreased under low flow conditions. A reduction in surface area may be due to changes in recruitment and/or distension of vessels in the pulmonary capillary bed. Fanburg and Glazier<sup>27</sup> reported changes in the extent of conversion of angiotensin I to angiotensin II when dog lungs were perfused under Zone 2 and 3 conditions. They attributed this phenomenon to changes in endothelial surface area due to recruitment and derecruitment of capillaries. In the present preparation, these possibilities remain to be resolved.

The modest but significant inhibition of protein synthesis under conditions of low flow, but adequate energy availability (table 2, group V), was unexpected. Although previous studies showed that a reduced supply of amino acids can inhibit protein synthesis in perfused lungs,<sup>19</sup> this was the case only under conditions far more extreme than those presently encountered. In the context of the earlier studies, the flow-dependent decrease in intracellular levels of essential amino acids shown in table 5 appears too small to provide an inadequate supply of substrate for the protein synthetic pathway. Furthermore, the data on phenylalanine equilibration and specific activity (table 4) provide no evidence to suggest a flow-related artifact of incomplete equilibration of the radiolabel across the tissue. This result is in accord with earlier data, which indicate that the amino acid is not compartmented within the lung under the conditions presently employed.<sup>15</sup> More extensive and specific studies are thus required for interpretation of this result, especially in the context of microcompartmentation of the amino acid within specific cells, which may contribute disproportionately to the overall rate of protein synthesis at the tissue level.

Exposure of the lungs to reduced temperature and to halothane in addition to restricted flow for 3 h did not reduce ATP levels significantly, as compared to high flow controls. Metabolism of 5-HT and protein synthesis were depressed markedly, but reversibly. Similarly, in lungs perfused 1 (table 1) or 3 h (table 3) at low flow, restoration of high flow resulted in an immediate and complete return of 5-HIAA production to control values. These results are consistent with the interpretation that, in the perfused lung, inhibition of amine metabolism at low pulmonary flow reflects changes in capillary surface area, not permanent hypoxic damage to the tissue.

In summary, the present study provides preliminary information regarding only some of the factors involved in the very complex changes to which the lung is exposed during intervals of restricted blood flow *in vivo*. Extrapolation of these findings to the mechanism of low flow-induced pulmonary injury to humans is not possible at present. Nonetheless, in the model employed, several conditions commonly associated with reduced flow led to reversible depression of pulmonary metabolism. The isolated perfused lung, while it provides the advantage of a well-controlled preparation with relatively normal architecture and cell-cell relations, does not allow reversible endothelial injury and changes in vascular surface area to be clearly distinguished. The present observations suggest, however, that a change in capillary surface is the likely mediator of the effects of reduced flow on amine metabolism.

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