

# Factors influencing pulmonary and cutaneous arterial blood flow in the toad, *Bufo marinus*

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WEST, NIGEL H., AND WARREN W. BURGGREN. *Factors influencing pulmonary and cutaneous arterial blood flow in the toad, Bufo marinus*. Am. J. Physiol. 247 (Regulatory Integrative Comp. Physiol. 16): R884-R894, 1984.—In the conscious, undisturbed toad, *Bufo marinus*, pulmonary arterial blood flow increased during periods of lung ventilation and decreased in intervening periods of pulmonary apnea. In unidirectionally ventilated, anesthetized toads, lung inflation produced by increasing the outflow resistance to pulmonary gas flow to 3 cmH<sub>2</sub>O caused a significant increase in pulmonary arterial blood flow and a significant decrease in cutaneous arterial blood flow. Changes in flow were associated with reciprocal changes in calculated vascular resistance. Mean pulmocutaneous pressure and cardiac frequency did not change significantly. Thus lung inflation (in the absence of changes in the composition of intrapulmonary gases) increased the proportion of total pulmocutaneous flow routed to the lungs and decreased the proportion directed to the skin. Unidirectional ventilation with air + 5% CO<sub>2</sub> at constant lung volume produced a significant decrease in pulmonary arterial blood flow, an increase in calculated pulmonary arterial flow resistance, and a small increase in the flow to the cutaneous artery. Concomitant mild hypoxia potentiated the effects of pulmonary hypercapnia, although hypoxia alone was less effective than hypercapnia alone in decreasing pulmonary flow. Pulmonary arterial blood flow was decreased by infusion of acetylcholine into the pulmocutaneous artery, but epinephrine had no effect on either the pulmonary or cutaneous artery at doses below those that produced systemic effects. Atropine blocked all changes in pulmonary arterial blood flow. This and other evidence suggest that calculated arterial resistance changes are due to reflex changes in the tone of vascular smooth muscle. Intrapulmonary CO<sub>2</sub>-sensitive mechanoreceptors possess appropriate response characteristics to mediate the afferent limb of the reflex.

pulmocutaneous artery; ventilation-perfusion matching

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AN EFFECTIVE TRANSFER of respiratory gases depends both on diffusion of CO<sub>2</sub> and O<sub>2</sub> across a gas exchange membrane and on their convective transport in blood perfusing the gas exchanger. Mismatches between these processes can have deleterious consequences for the partial pressure gradients of respiratory gases, their flow rates to tissues, and the magnitude of gas stores (4). It is not surprising therefore that the relationship between pulmonary ventilation and perfusion in air-breathing vertebrates is regulated to optimize gas transport.

In endotherms, lung ventilation and perfusion occur

continuously, and the ratio between them varies relatively little within the context of this steady state. The absence of large intrapulmonary shunts in the normal lung and placement of the left and right ventricles in series, rather than in parallel as in some lower vertebrates, mean that changes in pulmonary vascular resistance will have little overall effect on pulmonary flow. However, localized changes in pulmonary vascular resistance regulate regional ventilation-perfusion ratios within the limits of the regional differences in the ventilation-perfusion relationship imposed by gravitational forces (22).

In contrast, reflex changes in pulmonary perfusion may play an important role in optimizing gas exchange in the intermittently ventilating, ectothermic vertebrates. Optimization of the ventilation-perfusion relationship during intermittent ventilation implies that pulmonary perfusion is controlled not to maintain a steady state but rather to match increases in lung blood flow to episodes of lung ventilation. Since the pulmonary and systemic circulation are located in parallel, the potential for large changes in pulmonary flow exists. The circulation of the anuran amphibian represents a particularly intriguing system in which to examine the control of pulmonary perfusion. Not only can the distribution of blood between the pulmocutaneous and systemic circuits be adjusted by changes in the extent of intraventricular shunting, but the potential for shunting also exists more peripherally at the cutaneous-pulmonary arterial junction (19). Although lung inflation per se and lung inflation with N<sub>2</sub> or O<sub>2</sub> have been reported to influence the distribution of pulmocutaneous and systemic arterial blood flow in anurans (8, 18), a description of the influence of lung inflation on the distribution of flow between the pulmonary and cutaneous arteries has not been attempted.

Therefore in this investigation we set out to investigate changes in pulmonary and cutaneous arterial blood flow in response to mechanical and chemical stimuli from the lungs in the tropical toad, *Bufo marinus*. The lungs were unidirectionally ventilated to disassociate the effects of lung inflation from attendant changes in intrapulmonary gas tensions. Gas compositions were then changed at constant lung volume. We chose to concentrate on the effects of pulmonary hypercapnia rather than hypoxia, because there is already some evidence that hypercapnia

causes pulmonary vasoconstriction in *Bufo* (21). Finally, simultaneous measurement of pulmocutaneous arterial pressure enabled us to calculate changes in vascular resistance in the pulmonary and cutaneous circuits in response to the above stimuli.

## MATERIALS AND METHODS

### *Animals*

Fifteen tropical toads (*B. marinus*) were used in this study. Three toads ( $384 \pm 12$  g) were used in preliminary experiments as chronic, conscious preparations. Another 12 toads ( $494 \pm 20$  g) were used as unidirectionally ventilated, anesthetized preparations. The toads were obtained from a commercial supplier in the US and maintained in large aquaria, with access to water. The animals were held at room temperature (22–25°C).

### *Surgical Preparation*

The animals were anesthetized by immersion in tricaine methane sulfonate (MS 222, Sandoz; 1:1,000) adjusted to pH 7.0. For preparation of unidirectionally ventilated toads, small incisions were made in the skin and body wall of the flanks. The caudal apices of both unicameral lungs were then cannulated with PE-205 tubing, which was sutured into the lungs. The flank skin and body wall were closed separately, and the cannulas were secured to them. The animals were positioned dorsal side down on a Plexiglas operating table and covered with moist paper toweling. MS 222 solution was applied to the toweling as necessary to maintain anesthesia. Unidirectional ventilation of the lungs was initiated by delivering humidified air via a flowmeter at a rate of  $47 \text{ ml} \cdot \text{min}^{-1}$  into one lung cannula. Outflow resistance was adjusted by positioning the contralateral cannula under a water column.

Once satisfactory unidirectional ventilation was established, the pectoral girdle was split in the midventral line to expose the heart and central arteries and veins. The pericardium was left intact. The left extrinsic pulmonary artery was exposed and gently dissected away from the associated connective tissue, while great care was taken to avoid damage to the pulmonary vagus, whose fibers run superficially over the extrinsic pulmonary artery at this point (6). A 1.0- or 1.5-mm-lumen-diameter Zepeda electromagnetic flow probe was positioned on the extrinsic pulmonary artery between its junction with the pulmocutaneous artery and its division into the major intrinsic pulmonary branches, two of which were clearly visible through the lung tissue in most preparations. The position of the flow probe was adjusted so as not to impede flow in the adjacent cutaneous artery. A suitable length of the cutaneous artery was then cleared, the laryngeal nerve was carefully dissected away from the artery if necessary, and a 1.0-mm-lumen-diameter flow probe was placed on the cutaneous artery. Only in the larger toads was ipsilateral placement of the two flow probes possible. In seven preparations, flow probes were placed on the left extrinsic pulmonary artery and the left cutaneous artery; in four preparations they were placed

on the left extrinsic pulmonary artery and the right cutaneous artery; in one preparation they were placed on the left pulmocutaneous artery and the right cutaneous artery. Results from ipsilateral and bilateral placement of the probes were not statistically different; consequently the results from both types of preparation have been expressed together. Zero flow was determined periodically by vessel occlusion. Flow probes were calibrated by excising and cannulating the pulmocutaneous arch. The arch was perfused with heparinized toad blood delivered via a Sage Instrument infusion pump at five different nominal flow rates. Absolute flow rates were then calculated by weighing the contents of collection vials; calibration curves over the appropriate range of flows were linear. To measure pulmocutaneous arterial pressure, a 22-gauge Huber point needle was inserted into the pulmocutaneous division of the truncus arteriosus and held in place with cyanoacrylate adhesive. The placement of the needle was confirmed by dissection postmortem.

The preparation of toads for chronic experiments was similar. Only a pulmonary arterial flow probe was fitted in these toads. The leads were led out ventrally and secured to the pectoral girdle. The pectoral girdle, body wall, and skin were closed separately. The apex of the ipsilateral lung was cannulated with PE-90 tubing, the end of which was sealed to allow normal lung inflation during recovery. These animals were allowed to recover for 2 days before pulmonary flow recordings were made.

### *Instrumentation*

In unidirectionally ventilated toads, extrinsic pulmonary and cutaneous arterial flows were recorded with two Zepeda SWF-4 electromagnetic flowmeters, whereas pulmocutaneous arterial pressure was sensed by a Narco P1000B pressure transducer, with zero level situated at the level of the animal's heart. The pulsatile pressure signal, or the extrinsic pulmonary flow signal, was fed to a Narco 7302 biotachometer to give an instantaneous measurement of cardiac frequency. Extrinsic pulmonary arterial flow, cutaneous arterial flow, pulmocutaneous pressure, and cardiac frequency were recorded on a Narco mark IV four-channel recorder writing on rectilinear coordinates. Physiological variables were recorded from conscious, undisturbed toads 2 days postoperatively. The toads were held at 22°C in moist boxes constructed of expanded polystyrene, which diffused light and insulated against sound and vibration. Intrapulmonary pressure was sensed by a Grass PT5 transducer. Both flow and pressure signals were fed to Beckman 9853a couplers and 461D preamplifiers. Records were displayed on a Beckman R511A pen recorder writing on rectilinear coordinates.

### *Experimental Protocol*

All experiments using unidirectionally ventilated toads were performed at 24–25°C. Two main experimental protocols were utilized to differentiate between the changes in flow distribution associated with lung infla-

tion per se and those occurring in response to changes in the composition of lung gases. Outflow resistance to pulmonary gas flow in the control condition was 2 cmH<sub>2</sub>O in both protocols.

*Effects of lung inflation.* The cardiovascular responses to through flow of humidified air at outflow resistances of 0–4 cmH<sub>2</sub>O were investigated. An increase in outflow resistance to unidirectional ventilation produced an increase in lung inflation, with no change in the composition of intrapulmonary gas. Typically the lungs were ventilated with an outflow resistance of 0 cmH<sub>2</sub>O for 5 min, inflated by increasing the outflow resistance for 5 min, and then returned to an outflow resistance of 0 cmH<sub>2</sub>O for a further 5 min before being returned to control conditions.

*Effects of intrapulmonary hypercapnia and hypoxia.* To determine the effects of hypercapnic intrapulmonary gas tensions on the distribution of pulmocutaneous flow, unidirectionally ventilated toads were switched from a flow of humidified room air at control to air containing 1–5% CO<sub>2</sub>. Gas flow rate was held constant by flowmeter adjustments. The degree of lung inflation was also constant, with the outflow resistance held at 2 cmH<sub>2</sub>O. The effects of hypoxia were investigated by unidirectional ventilation with N<sub>2</sub>, 50% N<sub>2</sub>-50% air, and 25% N<sub>2</sub>-75% air. In some experiments the additive effects of hypoxia and hypercapnia were investigated. The gases were mixed with a Wösthoff pump, humidified, and delivered to the experimental animal via a two-way valve. Five-minute experimental periods were used, and the animals were not subjected to further experimental gas flow for at least 15 min or until the recorded cardiovascular variables had returned to control levels for 5 min. The time lag for gas delivery to the animal from switching the valve was 23 s, determined by a Beckman OM-14 O<sub>2</sub> analyzer. A blood gas analyzer (Instrumentation Laboratories, Micro 13) was used to measure the O<sub>2</sub> and CO<sub>2</sub> partial pressures (PO<sub>2</sub> and PCO<sub>2</sub>, respectively) and pH of pulmocutaneous blood in six anesthetized toads subject to unidirectional ventilation with air and various hypoxic and hypercapnic gas mixtures. In one animal, systemic arterial PO<sub>2</sub>, PCO<sub>2</sub>, and pH were also measured.

*Pharmacologic agents.* The flow responses to bolus injections of 50–200 μl of 10<sup>-4</sup> M epinephrine and 20–100 μl of 10<sup>-4</sup> M acetylcholine into the pulmocutaneous artery via the pressure cannula were investigated in 10 toads. In five toads the muscarinic receptor antagonist atropine (1 mg·kg<sup>-1</sup>) was injected via the same route at the end of the experiment.

#### Data Analysis

Flow and pressure were recorded as instantaneous flow and pulsatile pressure. Mean pulmocutaneous arterial pressure was determined from the pulsatile trace using a square-counting technique. Mean blood flow was calculated from the blood velocity traces by the area integration method outlined in Geddes and Baker (9). Pulmonary or cutaneous stroke volume was determined by integration of the area under a representative flow curve with a weighing technique. Flow resistance (mmHg·

ml<sup>-1</sup>·min) was calculated from the values of mean pulmocutaneous arterial pressure and flow in the pulmonary and cutaneous arteries. Total unilateral pulmocutaneous arterial flow was calculated as the arithmetic sum of pulmonary and cutaneous arterial flows. Pulmocutaneous resistance (Rpca) was calculated as the sum of parallel cutaneous (Rca) and pulmonary arterial resistances (Rpa)

$$Rpca = 1/[(1/R_{ca}) + (1/R_{pa})]$$

In an additional toad subjected to the experimental protocols, the left and right atrial pressures were recorded and the diastolic pressures were used to approximate pulmonary and systemic venous pressures, respectively. Under control conditions, left atrial diastolic pressure (LADP) varied between 0.7 and 1.0 mmHg and right atrial diastolic pressure (RADP) between 1.0 and 1.1 mmHg and represented 2.6–3.4% of mean pulmocutaneous arterial pressure. Both LADP and RADP rose slightly with both protocols but never exceeded 5% of mean pulmocutaneous arterial pressure. Pulmonary and systemic venous pressures were therefore considered to be invariable and negligible for the purpose of the calculation of resistance.

The data were analyzed to provide means ± SE where appropriate. Discrimination between treatment means was performed by analysis of variance (ANOVA). The fiducial limit of significance was considered to be *P* < 0.05.

## RESULTS

### *Relationship Between Lung Ventilation and Pulmonary Arterial Blood Flow in Conscious, Unrestrained Toads*

Figure 1A illustrates the relationship between left pulmonary arterial blood flow and the changes in intrapulmonary pressure brought about by periodic lung ventilation in a conscious, unrestrained toad. Pulmonary blood flow fell during the short periods of pulmonary apnea when buccal ventilation still occurred (23) and increased during periods in which the lungs were inflated, primarily by an increase in pulmonary stroke volume with no increases in cardiac frequency on lung inflation. Figure 1B illustrates the effects of the spontaneous start of pulmonary ventilation on pulmonary blood flow and cardiac frequency after a period of 7 min 20 s without lung ventilation. In this case the increase in pulmonary blood flow as lung ventilation resumed was brought about by an increase in both pulmonary stroke volume and cardiac frequency.

In Fig. 2, mean pulmonary blood flow at the end of a period of pulmonary apnea is plotted against the length of the preceding period of apnea in the same conscious, unrestrained toad. Periods between spontaneous periodic lung ventilation ranged from 1 to 7 min. There was a good relationship (*P* < 0.001) between the preceding length of pulmonary apnea and mean flow. An investigation of the mechanisms involved in the control of these marked changes in pulmonary blood flow in relation to ventilation is the further subject of this paper.

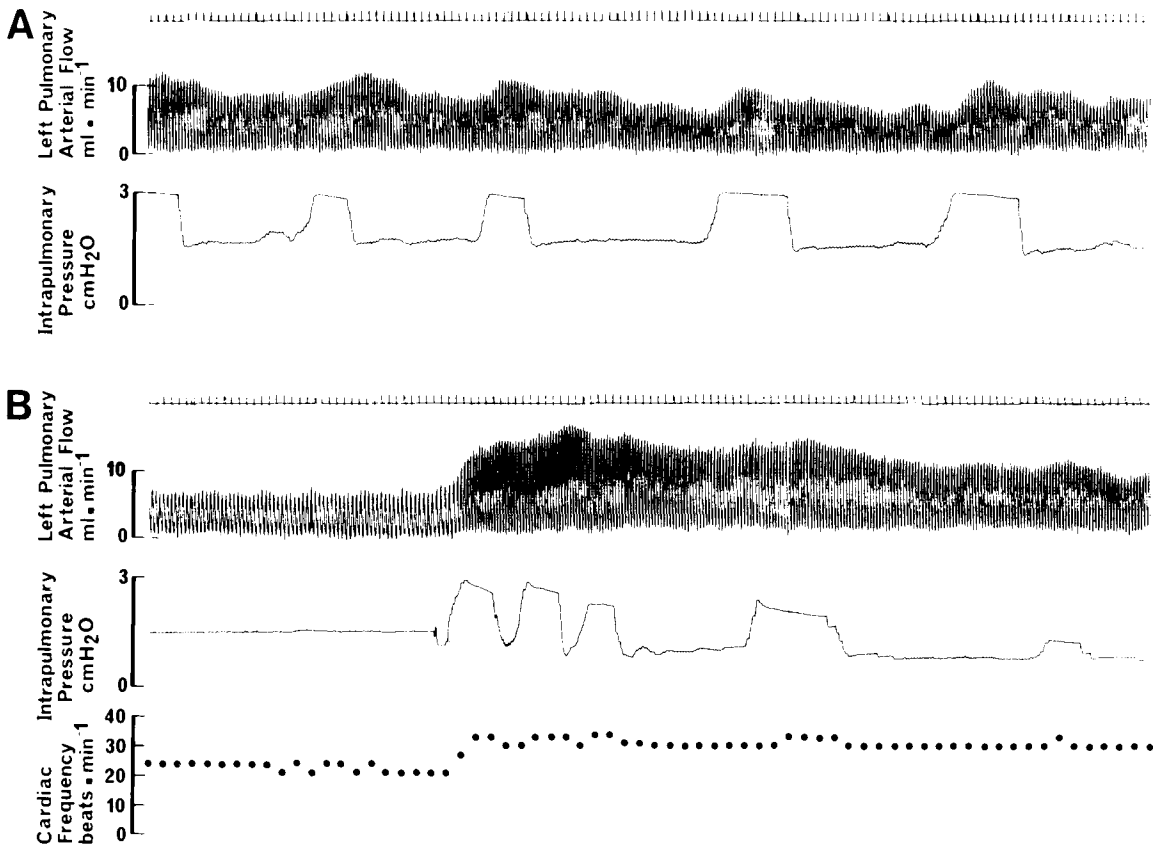


FIG. 1. A and B: changes in left pulmonary arterial blood flow in response to intermittent lung ventilation in conscious unrestrained 362-g *Bufo marinus* at 22°C. B: increase in pulmonary arterial flow and tachycardia produced by resumption of lung ventilation after 7 min 20

s of voluntary pulmonary apnea. Traces: 5-s time marker; instantaneous left pulmonary arterial blood flow; intrapulmonary pressure recorded from left lung; and, in B, cardiac frequency.

*Responses to Lung Inflation During Unidirectional Ventilation*

The cardiovascular responses to a change in the out-flow resistance to pulmonary airflow from 0 to 3 cmH<sub>2</sub>O in a unidirectionally ventilated, anesthetized toad are shown in Fig. 3, whereas the mean values for five trials in five animals are illustrated in Fig. 4. An outflow resistance of 3 cmH<sub>2</sub>O was chosen to simulate a typical intrapulmonary pressure produced by lung inflation in the conscious toad (see Fig. 1). Artificial lung inflation produced no significant changes in mean pulmocutaneous arterial pressure (Figs. 3 and 4). Cardiac frequency increased on lung inflation in three animals (Fig. 3), although this effect was not statistically significant overall (Fig. 4).

With the resistance to pulmonary gas flow at 0 cmH<sub>2</sub>O and the lungs deflated, unilateral pulmocutaneous arterial blood flow was 16.8 ± 2.3 ml · min<sup>-1</sup> 20 s before lung inflation (Fig. 4). Of this flow the pulmonary artery received 86% whereas the cutaneous artery received 14%. Reciprocal changes in blood flow through the pulmonary and cutaneous arteries occurred when the lungs were inflated. Mean pulmonary blood flow increased significantly from a control value of 14.4 ± 1.7 to 20.4 ± 2.1 ml · min<sup>-1</sup> by 60 s after lung inflation, whereas cutaneous arterial flow fell significantly from 2.4 ± 0.6 to 1.7 ± 0.5 ml · min<sup>-1</sup> by 160 s. By 300 s total pulmocutaneous flow

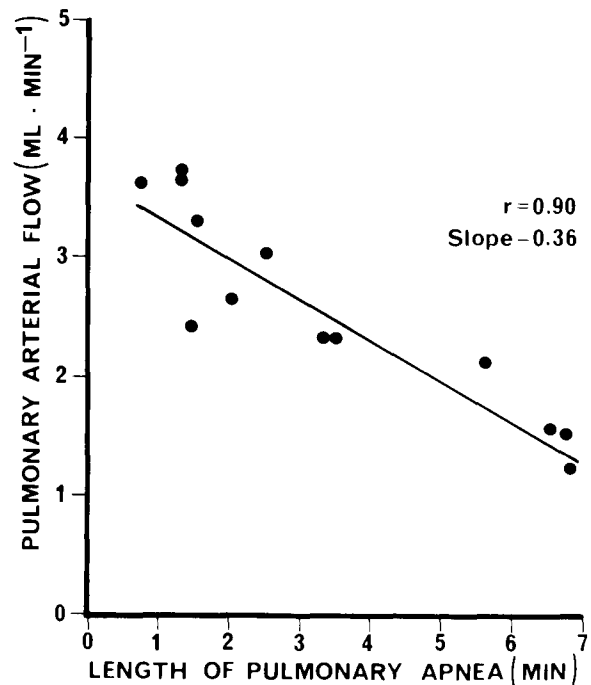


FIG. 2. Relationship between mean left pulmocutaneous arterial blood flow, at end of period of pulmonary apnea, and length of preceding period of buccal ventilation, recorded over 1 h at 22°C in same animal shown in Fig. 1.

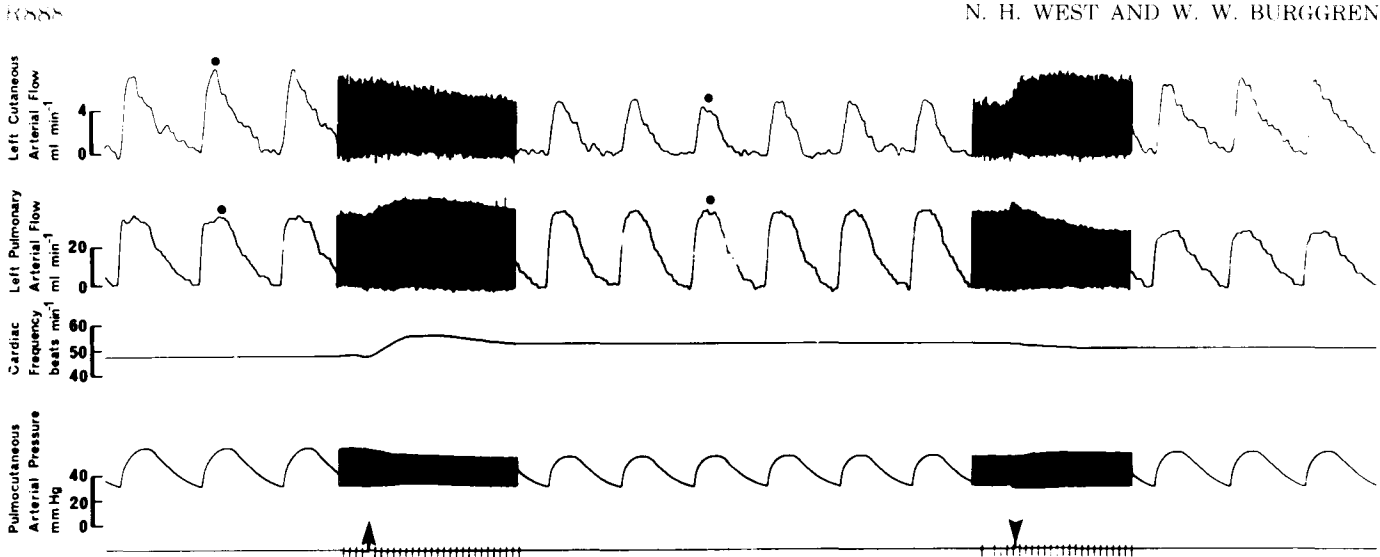


FIG. 3. Typical responses to lung inflation in unidirectionally ventilated anesthetized toad (511 g). Traces: instantaneous left cutaneous arterial blood flow, instantaneous left pulmonary arterial blood flow, cardiac frequency, pulmocutaneous arterial pressure, 10-s timer, and event marker. Outflow resistance to unidirectional ventilation with

humidified air is changed from 0 to 3 cmH<sub>2</sub>O at 1st arrow and returned to 0 cmH<sub>2</sub>O after 5 min inflation at 2nd arrow. Airflow was 47 ml·min<sup>-1</sup>. Note reciprocal changes in flow and increase in cardiac frequency on lung inflation. Filled circles, flow curves from which stroke volume calculated (see RESULTS).

was  $21.8 \pm 1.4$  ml·min<sup>-1</sup> (Fig. 4). The pulmonary artery now received 92% of total pulmocutaneous flow, whereas the cutaneous artery received 8% of flow. Pulmonary arterial stroke volume rose from 0.340 ml at control to 0.428 ml during inflation in the trial illustrated in Fig. 4, whereas cutaneous arterial stroke volume fell from 0.060 to 0.028 ml.

The increase in pulmocutaneous arterial flow and the reciprocal changes in pulmonary and cutaneous arterial flow were associated with a decrease in calculated pulmonary arterial flow resistance and a reciprocal increase in cutaneous arterial resistance (Fig. 4). Unilateral pulmocutaneous arterial flow resistance was  $1.6 \pm 0.2$  mmHg·ml<sup>-1</sup>·min 20 s before lung inflation and fell to  $1.1 \pm 0.2$  mmHg·ml<sup>-1</sup>·min at 300 s (Fig. 4). Pulmonary arterial flow resistance was significantly reduced from  $1.9 \pm 0.3$  to  $1.3 \pm 0.2$  mmHg·ml<sup>-1</sup>·min by 80 s, whereas cutaneous arterial flow resistance was significantly increased from  $12.1 \pm 0.6$  to  $16.5 \pm 1.8$  mmHg·ml<sup>-1</sup>·min by 160 s after lung inflation.

In the control state, with unidirectional ventilation of humidified air at 47 ml·min<sup>-1</sup>, PO<sub>2</sub> was  $36.4 \pm 4.5$  Torr; PCO<sub>2</sub> was  $12.1 \pm 1.0$  Torr, and pH was  $7.651 \pm 0.030$  ( $n = 11$ ) for pulmocutaneous arterial blood. One animal was removed from ventilation for 30 min to simulate anesthetized conditions typical of previous published studies on the amphibian circulation. Arterial blood gases recorded from the aorta were 13.0 Torr PO<sub>2</sub>, 16.2 Torr PCO<sub>2</sub>, and 7.415 pH; whereas the values recorded from the pulmocutaneous artery were 8.0 Torr PO<sub>2</sub>, 15.3 Torr PCO<sub>2</sub>, and 7.484 pH. This suggests that unidirectional ventilation is necessary during anesthesia in the toad to maintain blood gas levels similar to those recorded from unanesthetized animals.

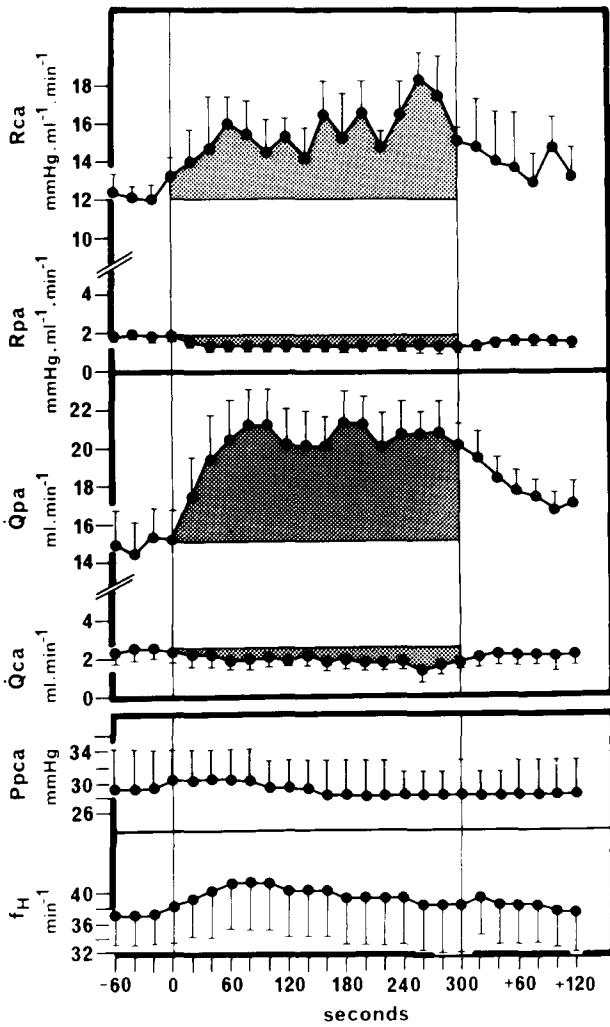
#### Responses to Unidirectional Ventilation with Air + 5% CO<sub>2</sub>

Figure 5 illustrates the cardiovascular responses to

unidirectional ventilation with humidified air + 5% CO<sub>2</sub> in an anesthetized toad, whereas Fig. 6 illustrates the results from five trials in five animals. In all trials the outflow resistance to unidirectional ventilation was 2 cmH<sub>2</sub>O, and the flow rate was 47 ml·min<sup>-1</sup>. Mean pulmocutaneous arterial pressure rose from  $27 \pm 3$  mmHg at control to  $32 \pm 3$  mmHg at 300 s (Fig. 6). Cardiac frequency responses were not consistent; three animals showed a prompt bradycardia, whereas three animals showed a mild tachycardia.

At control, 20 s before switching to the 5% CO<sub>2</sub> gas mixture, and with an outflow resistance of 2 cmH<sub>2</sub>O, unilateral pulmocutaneous arterial blood flow was  $18.5 \pm 1.7$  ml·min<sup>-1</sup>. The pulmonary artery received 87% of the total flow, whereas the cutaneous artery received 13%. Mean pulmonary arterial blood flow fell significantly from  $16.1 \pm 1.2$  ml·min<sup>-1</sup> at control to  $14.1 \pm 1.2$  ml·min<sup>-1</sup> by 60 s after switching from air to air + 5% CO<sub>2</sub>. Lag time in gas delivery to the animal was 23 s. There was an apparent increase in cutaneous arterial blood flow, from  $2.4 \pm 0.5$  ml·min<sup>-1</sup> at control to  $2.9 \pm 0.5$  ml·min<sup>-1</sup> at 300 s, although this was statistically insignificant (Fig. 5). At the end of the experimental period, total unilateral pulmocutaneous arterial flow had fallen to  $14.0 \pm 2.0$  ml·min<sup>-1</sup>. The pulmonary artery now received 79% of total pulmocutaneous arterial flow, whereas the cutaneous artery received 21%. Pulmonary arterial stroke volume fell from 0.401 ml at control to 0.280 ml in response to air + 5% CO<sub>2</sub> flow in the trial illustrated in Fig. 5, whereas cutaneous arterial stroke volume rose from 0.018 to 0.024 ml.

The decrease in pulmocutaneous arterial flow in response to 5% CO<sub>2</sub> was associated with a marked increase in calculated pulmonary arterial flow resistance with no significant change in cutaneous arterial resistance. Unilateral pulmocutaneous flow resistance was  $1.5 \pm 0.2$  mmHg·ml<sup>-1</sup>·min 20 s before switching to 5% CO<sub>2</sub> flow and rose to  $2.5 \pm 0.4$  mmHg·ml<sup>-1</sup>·min by 300 s of 5% CO<sub>2</sub> flow. Pulmonary arterial flow resistance was signifi-



icantly increased from  $1.7 \pm 0.2 \text{ mmHg} \cdot \text{ml}^{-1} \cdot \text{min}$  at control to  $2.5 \pm 0.2 \text{ mmHg} \cdot \text{ml}^{-1} \cdot \text{min}$  at 100 s. Cutaneous arterial flow resistance was  $12.1 \pm 1.5 \text{ mmHg} \cdot \text{ml}^{-1} \cdot \text{min}$  at control and  $11.8 \pm 1.5 \text{ mmHg} \cdot \text{ml}^{-1} \cdot \text{min}$  at 300 s.

The threshold for a detectable fall in pulmonary arterial blood flow was 1–2%  $\text{CO}_2$  for all the animals reported in Fig. 6, with consistent effects being produced at 5% (21).

*Responses to Pulmonary Anoxia, Hypoxia, and Interactions with Hypercapnia*

The effects of unidirectional ventilation with humidified  $\text{N}_2$  and hypoxic gas mixtures were determined in five toads. The outflow resistance to pulmonary gas flow was constant at  $2 \text{ cmH}_2\text{O}$ . Four animals showed an increase in cardiac frequency in response to  $\text{N}_2$  flow (Fig. 7A). Maximum frequency was achieved at 120–180 s after switching to  $\text{N}_2$  flow, with heart rate rising from  $40 \pm 5$  to a maximum of  $47 \pm 4 \text{ beats} \cdot \text{min}^{-1}$  ( $n = 4$ ). One animal became bradycardic. Mean pulmocutaneous arterial pressure rose significantly by 80–140 s after switching to  $\text{N}_2$  flow, rising from  $25.3 \pm 0.6 \text{ mmHg}$  at control to a maximum of  $31.8 \pm 2.8 \text{ mmHg}$  ( $n = 4$ ) (Fig. 7A). Overall a small increase in pulmonary arterial flow occurred in these animals by 300 s of  $\text{N}_2$  flow (Fig. 7A), whereas pulmonary vascular resistance showed an insignificant increase from  $1.3 \pm 0.3 \text{ mmHg} \cdot \text{ml}^{-1} \cdot \text{min}$  at control to a

FIG. 4. Responses to lung inflation from 0 to 3  $\text{cmH}_2\text{O}$  in unidirectionally ventilated, anesthetized toads. *Panels:* cutaneous arterial resistance (Rca), pulmonary arterial resistance (Rpa), pulmonary arterial minute flow (Qpa), cutaneous arterial minute flow (Qca), mean pulmocutaneous arterial pressure (Ppca), cardiac frequency ( $f_H$ ). *Vertical lines,* inflation and deflation, respectively. *Vertical bars,* SE;  $n = 5$ ; mean mass,  $468 \pm 29 \text{ g}$ .

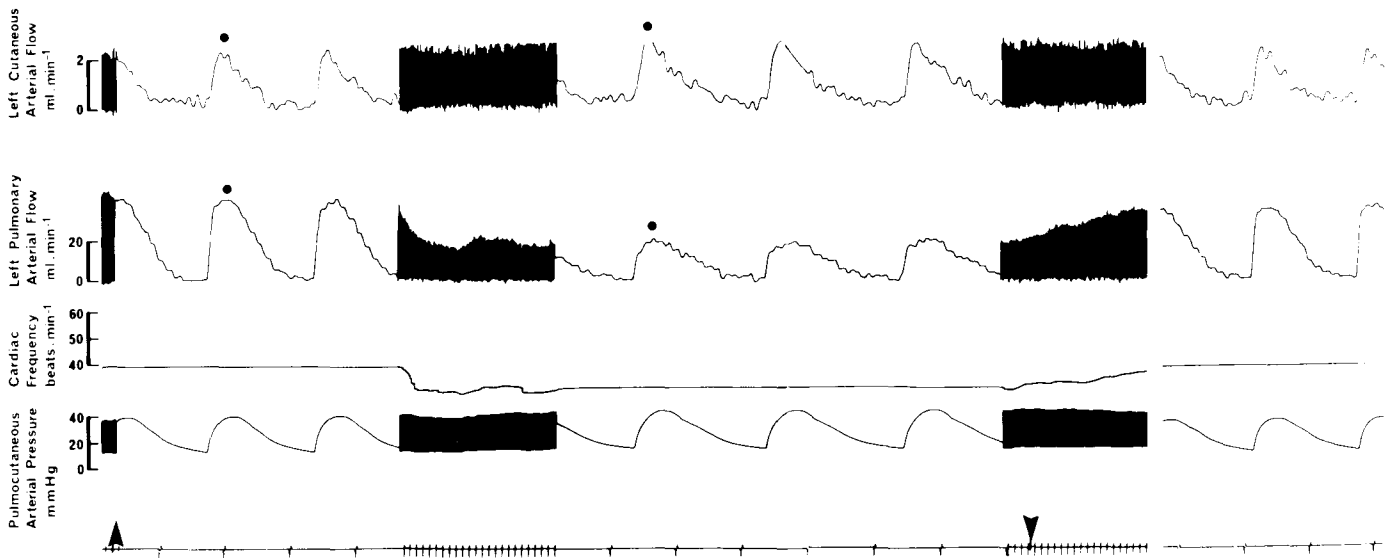


FIG. 5. Representative responses to unidirectional ventilation with air + 5%  $\text{CO}_2$  in anesthetized toad (381 g) at  $2\text{-cmH}_2\text{O}$  constant inflation pressure and  $47\text{-ml} \cdot \text{min}^{-1}$  gas flow rate. *Traces:* instantaneous left cutaneous arterial blood flow, instantaneous left pulmonary arterial blood flow, cardiac frequency, pulmocutaneous arterial pressure, and 10- and 1-s timers and event marker. Ventilation is switched from air to air + 5%  $\text{CO}_2$  at 1st arrow (far left) and returned to air after 5 min

gas flow at 2nd arrow. Note pronounced fall in pulmonary arterial blood flow and, in this animal, pronounced bradycardia in response to hypercapnia. *Right-hand panel* is not continuous but shows variables 10 min after return to air flow. *Filled circles,* flow curves from which stroke volume was calculated (see RESULTS). Test gas reached lungs 23 s after event mark.

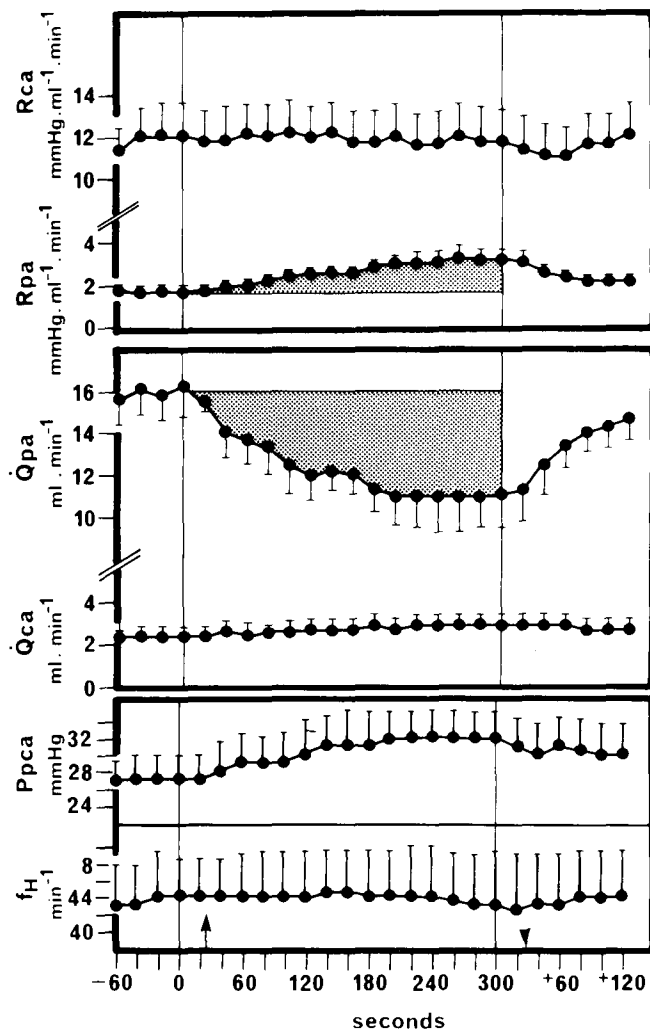


FIG. 6. Responses to air + 5%  $\text{CO}_2$  in unidirectionally ventilated, anesthetized toads at 2-cmH $_2$ O intrapulmonary pressure. Panels as in Fig. 4. Vertical lines, time of switching to and from test gas; vertical arrows, estimated time of arrival of test and control gases at lungs; vertical bars SE;  $n = 5$ ; mean mass, 504  $\pm$  36 g.

maximum of  $2.8 \pm 0.8$  mmHg·ml $^{-1}$ ·min.

Unidirectional ventilation with hypoxic gas mixtures (5–6%  $\text{O}_2$ ) produced small decreases in pulmonary flow (Fig. 7B) but was not as effective as pulmonary hypercapnia (20%  $\text{O}_2$ -5%  $\text{CO}_2$ ) in reducing pulmonary arterial blood flow (Fig. 7C). In three animals it was possible to demonstrate a synergistic effect on pulmonary arterial blood flow between the stimuli of pulmonary hypercapnia and mild hypoxia (Fig. 7, B–D). The addition of hypoxic and hypercapnic stimuli produced a spectacular fall in pulmonary arterial flow, which was greater than the sum of the two independent effects (Fig. 7D). In the example shown, unidirectional ventilation with 20%  $\text{O}_2$ -5%  $\text{CO}_2$  was calculated to increase pulmonary vascular resistance from 3.1 mmHg·ml $^{-1}$ ·min at control to a maximum of 6.8 mmHg·ml $^{-1}$ ·min. The addition of a hypoxic stimulus to the hypercapnic stimulus (5%  $\text{CO}_2$ -5%  $\text{O}_2$ ) increased maximum pulmonary vascular resistance to 20.4 mmHg·ml $^{-1}$ ·min.

### Responses to Pharmacologic Agents

Epinephrine, acetylcholine, and finally atropine were routinely introduced into the pulmocutaneous artery via the pressure cannula toward the end of an experiment. Epinephrine ( $10^{-4}$  M, 50–200  $\mu\text{l}$ ) increased both cutaneous and pulmonary flow. This was accomplished by increases in mean pulmocutaneous arterial pressure (Fig. 8). We could find no evidence for cutaneous arterial constriction on epinephrine infusion below doses that raised arterial pressure. Pulmonary arterial flow, on the other hand, proved to be sensitive in a dose-dependent manner to  $10^{-4}$  M acetylcholine infused into the pulmocutaneous artery. In the example shown, flow was essentially arrested by 100  $\mu\text{l}$  acetylcholine (Fig. 8). At high doses with a greatly reduced pulmonary flow, diastolic flow did not reach zero in the cutaneous artery (Fig. 8).

Atropine (1 mg·kg $^{-1}$ ) was introduced into the pulmocutaneous artery under circumstances of high pulmonary arterial blood flow (lungs inflated) or low flow (ventilated with hypercapnic gas) in five toads. In all cases atropinization produced an initial rapid fall in pulmonary arterial blood flow, followed by an increase to a maintained high, constant level. After atropinization, toads were unresponsive to protocols that had previously modulated pulmonary arterial flow.

### DISCUSSION

In the conscious toad, pulmonary arterial blood flow increases during lung ventilation and falls progressively during intervening periods of buccal ventilation. The end of a period of pulmonary apnea is characterized by a relatively low lung volume and intrapulmonary gases that are hypercapnic and hypoxic in their composition compared with those during lung ventilation. We have demonstrated in anesthetized, unidirectionally ventilated *B. marinus* that the distribution of pulmonary and cutaneous arterial blood flow is influenced independently both by lung volume and by the chemical composition of the intrapulmonary gas. Earlier, related studies on the distribution of pulmocutaneous and systemic blood flow in anuran amphibians did not dissociate the factors of lung volume and the resulting changes in the chemical composition of the gas within the lungs (6, 8, 18).

Lung inflation produced by changing the outflow resistance to unidirectional lung ventilation with air from 0 to 3 cmH $_2$ O resulted in a significant increase in pulmonary arterial blood flow and a decrease in cutaneous arterial flow. Total pulmocutaneous flow rose in the absence of significant changes in pulmocutaneous arterial pressure, whereas the lung received an increased proportion of pulmocutaneous flow. In *Xenopus* the increase in pulmocutaneous arterial flow on spontaneous lung inflation occurred without a corresponding fall in flow in the systemic arteries, suggesting that cardiac output fluctuates with lung ventilation cycles (18). The increase in cardiac frequency on voluntary or experimental lung inflation in some of our experiments suggests that this may also be true for *Bufo* under these circumstances.

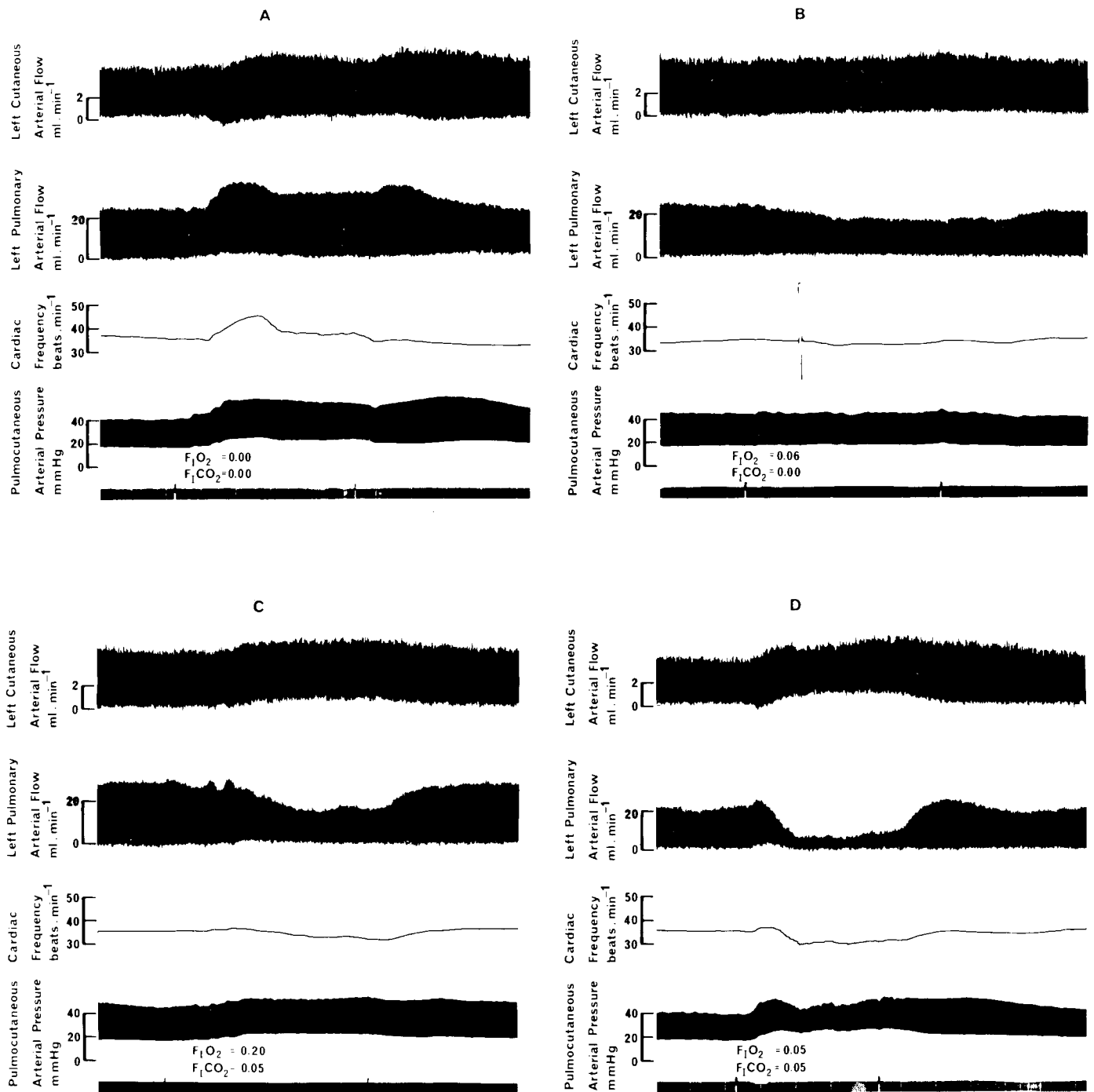


FIG. 7. Effect of intrapulmonary gas composition on cutaneous pulmonary arterial blood flow in unidirectionally ventilated anesthetized toad (489 g) at 2-cmH<sub>2</sub>O intrapulmonary pressure and 47-ml·min<sup>-1</sup> gas flow. A: responses to pulmonary anoxia produced by N<sub>2</sub> flow. B: responses to hypoxia, produced by 70% air-30% N<sub>2</sub>. C: responses to hypercapnia, air + 5% CO<sub>2</sub>. D: responses to hypercapnic hypoxia, 60%

air-5% CO<sub>2</sub>-25% N<sub>2</sub>. Traces in A-D: instantaneous left cutaneous arterial flow, instantaneous left pulmonary arterial flow, cardiac frequency, pulmocutaneous arterial pressure, and 1-s timer and event marker. Time lag for gas delivery was 23 s. Note synergistic effects of hypoxia and hypercapnia on pulmonary flow. F<sub>I</sub>O<sub>2</sub> and F<sub>I</sub>CO<sub>2</sub>, fractional inspired O<sub>2</sub> and CO<sub>2</sub>, respectively.

Unidirectional ventilation at constant lung volume with air + 5% CO<sub>2</sub> produced a reduction in pulmocutaneous arterial blood flow, which was largely due to a fall in pulmonary arterial flow. A statistically insignificant increase in flow occurred in the cutaneous artery, resulting in a fall in total pulmocutaneous arterial flow and redistribution of pulmocutaneous blood flow away from

the lung, with pulmonary flow being 87% of total pulmocutaneous flow before hypercapnia and 79% during hypercapnic ventilation. To our knowledge the only previous report of the potent effect of intrapulmonary CO<sub>2</sub> concentration on pulmonary blood flow in anuran amphibians is that of Smith (21). He found, using a qualitative plethysmographic technique in *B. marinus*, that



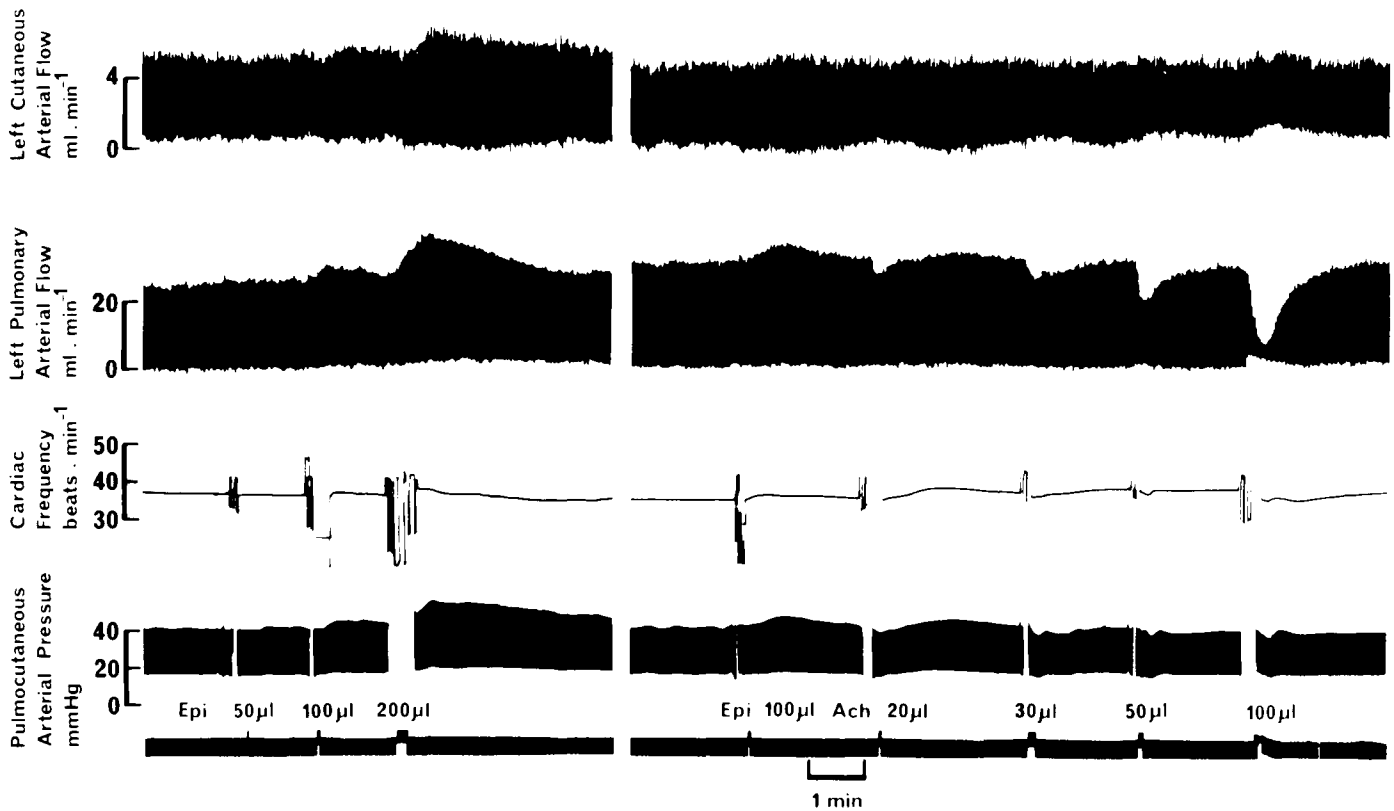


FIG. 8. Responses to infusion of  $10^{-4}$  M epinephrine (Epi) and  $10^{-4}$  M acetylcholine (ACh) into pulmocutaneous artery of unidirectionally ventilated toad (392 g). Outflow resistance to unidirectional ventilation was 2 cmH<sub>2</sub>O. *Left-hand panel:* epinephrine infusion ultimately produced increases in cutaneous and pulmonary arterial blood flow by increasing pulmocutaneous arterial pressure. No evidence was found

for cutaneous arterial vasoconstriction in response to exogenous epinephrine. *Right-hand panel:* acetylcholine infusion produced dose-related decreases in pulmonary arterial blood flow. Cutaneous diastolic flow did not reach zero during periods in which pulmonary vasculature was constricted. Cardiac frequency trace has been retouched.

unidirectional flow of 5% CO<sub>2</sub> through the lung produced a marked reduction in pulmonary vascular engorgement. As in the present study a few animals responded to 1% CO<sub>2</sub>, but 5% CO<sub>2</sub> gave consistent responses.

The effect of intrapulmonary anoxia or hypoxia on pulmonary flow in our animals was more equivocal than that of hypercapnia (cf. Ref. 8). Although unidirectional ventilation with N<sub>2</sub> produced an increase in pulmocutaneous arterial flow in some animals, this was apparently due to an elevation in pulmocutaneous arterial pressure. Increases in cardiac frequency, pulmocutaneous arterial pressure, and probably cardiac output may have been mediated by chemoreceptors within the carotid labyrinth under these circumstances (10). Similarly, unidirectional ventilation with hypoxic gas mixtures, although causing smaller systemic effects, produced no statistically significant effects on pulmonary or cutaneous arterial flow, although in all individuals there was a slight fall in pulmonary arterial flow. However, the addition of the stimulus of mild intrapulmonary hypoxia to a hypercapnic stimulus greatly potentiated the responses to hypercapnia alone.

Although changes in pulmonary and cutaneous arterial flow were determined experimentally, it was necessary to calculate values of vascular resistance from these and pulmocutaneous arterial pressure measurements. A limitation of all such calculations is that a change in vascular

resistance may have active components, due to changes in the tone of vascular smooth muscle, and passive components, due to changes in the transmural pressure gradient of distensible vessels. Only if resistance and transmural pressure covary is it certain there has been an active change in vessel radius (15). In our experiments, calculated pulmonary vascular resistance fell when the outflow resistance to lung ventilation was increased from 0 to 3 cmH<sub>2</sub>O, whereas cutaneous vascular resistance was calculated to rise. There was no significant increase in pulmocutaneous arterial pressure, suggesting that the fall in pulmonary resistance was not due to passive distension of the pulmonary resistance vessels by an increase in positive transmural pressure. Indeed, for unprotected vessels of the intrinsic pulmonary vasculature there is presumably a decrease in transmural pressure during both experimental and volitional positive-pressure lung inflation in the toad. It appears therefore that the observed changes in blood flow and calculated changes in resistance were caused by reciprocal active changes in vascular radius in the pulmonary and cutaneous circuits. On unidirectional ventilation with 5% CO<sub>2</sub> at a constant outflow resistance, there was no significant change in calculated cutaneous resistance, whereas there was a significant increase in pulmonary resistance. Pulmocutaneous arterial pressure rose, although this rise was insignificant by 5 min. It is reason-

able to assume, particularly in view of the covariation in calculated pulmonary resistance and pulmocutaneous pressure, that active vasoconstriction occurred in the pulmonary artery under these circumstances.

Several lines of evidence suggest that the changes we observed in pulmonary blood flow in *B. marinus* are both reflex and dependent on active changes in vascular resistance mediated by the cholinergic, vagal pulmonary innervation.

1) Not all toads were responsive to lung inflation and/or pulmonary hypercapnia at the start of experimentation, whereas deep anesthesia could abolish responsiveness in previously responsive animals. If purely mechanical factors attendant on lung inflation were important in modulating pulmonary flow, all animals should have responded to lung inflation (see also Ref. 8).

2) Muscarinic receptor blockade by atropine abolished the changes in pulmonary blood flow in response to both lung inflation and intrapulmonary hypercapnia, whereas acetylcholine produced constriction of the pulmonary vasculature. As in the case of pulmocutaneous flow in *Xenopus* (8), pulmonary arterial flow was maintained at high constant levels after atropinization, even in the face of lung deflation and/or pulmonary hypercapnia. Modulation of pulmonary vascular resistance by neural, cholinergic (pulmonary vagal) mechanisms is therefore strongly implicated in the observed responses.

3) In *Rana*, section of the pulmonary vagus causes the ipsilateral extrinsic pulmonary artery to become permanently dilated, whereas stimulation of the distal end of the cut vagal trunk, with the cardiac vagus cut, results in constriction of a segment of the pulmonary artery with no apparent changes in cardiac frequency or output (6, 14). In *Bufo*, bilateral pulmonary vagotomy abolishes pulmonary vasoconstriction in response to hypercapnic pulmonary gas (21).

4) Unidirectional ventilation of one lung with air + 5% CO<sub>2</sub> in *B. marinus* reduced the vascular engorgement of both the ventilated and nonventilated lung (21). This implies either the presence of receptors sensitive to the composition of pulmonary blood in the nonventilated lung (see below) or central integration of sensory information from the ventilated lung, with a resulting motor outflow to this, and the contralateral, unventilated lung. The possibility that central irradiation between respiratory neurons and neurons of the cardiovascular center is a primary mechanism of coupling between pulmonary ventilation and blood flow seems slight, at least in anesthetized animals. In anesthetized toads, ventilation efforts that were not effective in inflating the lungs caused no increase in pulmonary flow, although this was marked if lung inflation was successful. This was also the case in *Xenopus* for pulmocutaneous flow (18).

The anatomic basis for the observed changes in pulmonary blood flow in *B. marinus* has yet to be determined. However, in common with other intermittently ventilating vertebrates (1, 2, 5, 17), at least some anuran amphibians possess a highly vasoactive extrinsic pulmonary arterial segment, which constricts in response to vagal stimulation or the application of acetylcholine (6). In *Rana temporaria* a muscular sphincter is present in

the pulmonary artery at its junction with the cutaneous artery. A small branch of the pulmonary vagus innervates the sphincter. This is probably also the situation in *B. marinus*, for a muscular sphincter is present in the cogeneric anuran *B. bufo* (M. L. De Saint-Aubain, personal communication).

The functional significance of the above changes in the distribution of blood flow between the pulmonary and cutaneous arteries may be that they serve, together with changes in the proportionate distribution of cardiac output between the pulmocutaneous and systemic circuits (8, 18, 19), to match pulmonary perfusion to intermittent lung ventilation. Lung inflation, low intrapulmonary PCO<sub>2</sub>, and high intrapulmonary PO<sub>2</sub> will increase pulmonary but not cutaneous arterial flow. On the other hand, low lung volumes, high intrapulmonary PCO<sub>2</sub>, and low PO<sub>2</sub> will cause a reduction in pulmonary arterial flow, whereas flow to the skin will be maintained. In our experiments, exogenous acetylcholine caused a profound diminution in pulmonary arterial flow, without significantly affecting cutaneous arterial flow, whereas muscarinic receptor blockade by atropine produced a large, constant flow in the pulmonary artery. Below doses at which systemic effects were observed, exogenous epinephrine had no detectable effect on pulmonary or cutaneous arterial flow or resistance. Luckhardt and Carlson (14) claim that epinephrine has a biphasic action on the pulmonary artery of *Rana*, being a vasodilator in small, and a vasoconstrictor in larger, doses. The results certainly suggest that the pulmonary artery is the main vasoactive element under the conditions of our experiments, although it has been shown that the cutaneous artery is innervated by excitatory adrenergic nerve fibers from the vagosympathetic trunk, with probably little cholinergic control (20). Adrenergic fibers supplying the pulmonary artery, on the other hand, are vasodilatory (3).

We hypothesize that pulmonary mechanoreceptors may serve as transducers for the sensory limb of the reflex changes in pulmonary blood flow. Anuran amphibians possess both rapidly and slowly adapting pulmonary mechanoreceptors, which increase their discharge frequency in response to lung inflation (13, 16). Neural information from receptors could therefore mediate the observed vascular responses to lung inflation by causing a reduction in vasomotor tone in the pulmonary vasculature, decreasing pulmonary flow resistance. Both rapidly and slowly adapting pulmonary mechanoreceptors decrease their discharge frequency as intrapulmonary CO<sub>2</sub> increases. An increase in intrapulmonary CO<sub>2</sub> would therefore tend to diminish the effects of a given degree of lung inflation, resulting in a relatively greater vasomotor tone in the pulmonary vasculature and increasing pulmonary flow resistance. Such reflex responses depend on effective stimuli. Do such stimuli exist in the intermittently ventilating toad? In common with other anurans, intrapulmonary pressure increases during episodes of lung ventilation, accompanied by substantial increases in lung volume (23). Intrapulmonary pressure rose from 1.5 to 3 cmH<sub>2</sub>O on voluntary lung inflation in conscious *Bufo*, which would have been adequate to

stimulate pulmonary mechanoreceptors with the same characteristics as those in *Rana* (13, 16). As pointed out by Smith (21), apnea produces hypercapnia in several species of anurans (7, 11, 12), and according to our data this would serve to increase pulmonary arterial flow resistance, routing blood away from the lungs to the skin under these conditions.

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