Halothane inhibits hypoxic pulmonary vasoconstriction in the presence of cyclo-oxygenase blockade

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Using an isolated lung the effects of halothane on hypoxic pulmonary vasoconstriction (HPV) were studied in the presence of cyclooxygenase blockade. The pulmonary vasculature can be divided into arterial, middle and venous segment resistances. Analysis of the vascular pressure-flow relationship further separates resistance into a flow dependant resistance (1/slope) and a zero-flow pressure intercept (PCRIT). We ventilated six lobes with control (35 per cent O_2) and hypoxic (three per cent O_2) gas mixtures with the addition of either 0, 0.5, 1.0, or 2.0 per cent halothane. We found that after addition of indomethacin (5 $mg \cdot kg^{-1}$), ventilation with three per cent O_2 increased total resistance by 87 per cent over baseline with the increase primarily in the middle vascular segment. During normoxic ventilation PCRIT was 7.9 cm H_2O and this increased significantly with hypoxia to 11.5 cm H_2O). Only 2.0 per cent halothane blocked the increases in middle segment resistance and in PCRIT. We conclude that following cyclooxygenase blockade, halothane inhibits HPV by acting on middle segment vessels.

Nous avons étudié, sur poumon isolé, les effets de l'halothane sur la vasoconstriction pulmonaire hypoxique (HPV) en présence d'inhibiteur de la cyclooxygénase. Les secteurs artériel, médian et veineux contribuent successivement à la résistance vasculaire pulmonaire. En analysant les courbes pression-débit, on peut calculer la résistance (1/pente) et la pression d'ouverture (PCRIT). Nous ventilions six lobes pulmonaires avec de l'oxygène à 35 pour cent (contrôle) ou à trois pour cent (hypoxie) en y ajoutant 0, 0,5, 1,0 ou 2,0 pour cent d'halothane. Après l'injection de 5 $mg \cdot kg^{-1}$ d'indométhacine et avec trois pour cent d'O_2, la résistance totale augmentait de 87 pour cent par rapport au contrôle, surtout en secteur médian. La PCRIT était significativement moins élevée en condition normoxique, passant de 7,9 à 11,5 cm H_2O en condition hypoxique. Il fallait deux pour cent d'halothane pour bloquer cette augmentation de résistance et de pression d'ouverture. Donc, malgré une inhibition de la cyclooxygénase, l'halothane renverse l'HPV du secteur vasculaire médian.

Key words
ANAESTHETICS VOLATILE: halothane;
LUNG: blood flow, HPV;
HYPOXIA.

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Decreased regional partial pressure of oxygen causes regional pulmonary vasoconstriction thus diverting blood from hypoxic regions and minimizing venous admixture. Halothane has been shown to inhibit this hypoxic pulmonary vasoconstriction in isolated lobes[1] and intact animals.[2] Recently Marshall *et al.*[3] demonstrated in isolated rat lung that cyclooxygenase blockade with ibuprofen partially blocked the inhibition of hypoxic pulmonary vasoconstriction caused by halothane. They speculated that halothane might interact directly with enzymes bound to the cell membrane thus altering cellular arachidonic acid metabolism. We wondered if this effect was due to a specific interaction of halothane with the mediator(s) of hypoxic pulmonary vasoconstriction. Alternatively halothane might act as a non-specific vasodilator and cause general vasodilatation of the pulmonary vasculature. Since cyclooxygenase blockade may decrease the release of vasodilating prostaglandins such as PGI_2 induced by hypoxia,[4] we elected to test the effects of halothane on hypoxic pulmonary vasoconstriction following cyclooxygenase inhibition. We postulated that the effects of halothane on hypoxic pulmonary vasoconstriction would be more readily discernible if hypoxic pulmonary vasoconstriction were first augmented by cyclooxygenase blockade with indomethacin.

Using a vascular stop-flow technique, vascular resistance can be partitioned into arterial, venous and middle vascular segments.[5] Hypoxia has been shown primarily to increase middle segment resistance in both pigs',[6] and dogs', lungs.[7] Hakim *et al.* found that other agents such as histamine or alpha agonists affected either arterial or venous segments and did not alter middle segment resistance.[7] We have previously examined the effects of halothane on hypoxic pulmonary vasoconstriction in isolated canine lobes. We partitioned pulmonary vascular resistance into arterial, middle and venous segment resistances and we also examined the pressure-flow relationship of the lobar vasculature.[8] We found that halothane decreased vascular tone in the middle vascular segment and this decrease in tone could account for halothane's effects on pulmonary vascular resistance. We wondered whether halothane would affect hypoxic pulmonary vasoconstriction in a qualitatively similar manner to our previous findings even in the presence of cyclooxygenase blockade. This would imply that halothane affected hypoxic pulmonary vasoconstriction through a mechanism which did not require the modulation of prostaglandin production. Further it would imply that halothane inhibited hypoxic pulmonary vasoconstriction rather than acting as a non-specific vasodilator. We therefore decided to evaluate the interaction of hypoxia, halothane and cyclooxygenase inhibition on hypoxic pulmonary vasoconstriction using the vascular stop-flow technique in conjunction with pressure-flow analysis. As well, since Marshall *et al.*[3] found only a partial inhibition of hypoxic pulmonary vasoconstriction with 0.57 per cent halothane, we elected to evaluate several concentrations of halothane ranging from 0.5 to 2 per cent.

Methods

Animal preparation

Six mongrel dogs, 18–24 kg, were anaesthetized with thiopentone 500 mg IV, and their tracheas intubated with a 10 mm cuffed tracheal tube. These experiments were performed in accordance to the guidelines of the University of Saskatchewan Animal Care Committee. The lungs were mechanically ventilated (Harvard ventilator) at a tidal volume (V_T) of 20 ml · kg^{-1} on room air. Throughout the remainder of the surgical procedure anaesthesia was maintained with intermittent doses of sodium pentobarbitone, 50–100 mg IV, as necessary. A large bore catheter inserted into the abdominal aorta via the femoral artery was used for phlebotomy and drug administration.

Following muscle paralysis with succinylcholine 40 mg IV, the left upper lobe was exposed through the left fifth intercostal space. Positive end-expiratory pressure (PEEP) of 3 cm H_2O was added during the surgical procedures. The left upper lobe was surgically removed to facilitate exposure of the left lower lobe. Heparin, 300 U · kg^{-1}, was administered and then a stainless steel cannula was inserted into the left lower lobar vein via the left atrial appendage. The bronchus was intubated through a bronchotomy with a 6 mm cuffed tracheal tube and the cuff was inflated. After ensuring that the entire lobe could be inflated, we sutured the bronchial tube in place. The left lower lobar artery was then dissected and a plastic cannula was inserted. We collected 400 ml of blood from the femoral artery catheter and diluted it with saline and heparin, 5,000 units, to a total volume of 600 ml and haematocrit of 20–25 per cent. The blood was passed through a filter to remove any aggregates or particles before being added to the extracorporeal circuit (Figure 1). After the cannulae were inserted, the animals were sacrificed by an injection of 10 ml supersaturated KCl solution and 40 mg succinylcholine. The cannulated lobe was left within the thoracic cavity and was connected to the extracorporeal circuit. The lobe was perfused by gravity from an arterial reservoir. Pulmonary venous blood passively drained into a venous reservoir, was pumped by a roller pump through a heat exchanger and filter and was then returned to the arterial reservoir. Lobar blood flow, $\dot{Q}_L$, was measured near the venous cannula by a previously calibrated electromagnetic flow probe (Carolina Instruments). Care was taken to prevent air embolization during the surgical procedures and subsequent lobar perfusion.

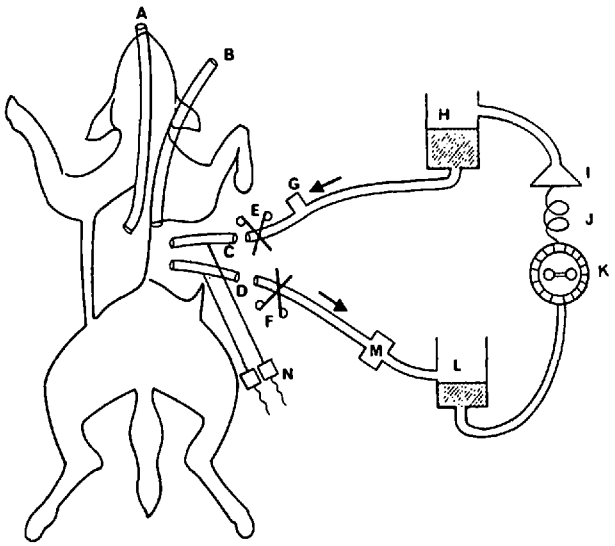

FIGURE 1 The experimental model. A = tracheal tube, B = bronchial cannula, C = inflow cannula, D = outflow cannula, E and F = points of occlusion of flow, G = bubble trap, H = inflow reservoir, I = filter, J = heat exchanger, K = roller pump, L = outflow reservoir, M = electromagnetic flow probe, N = vascular pressure transducers.

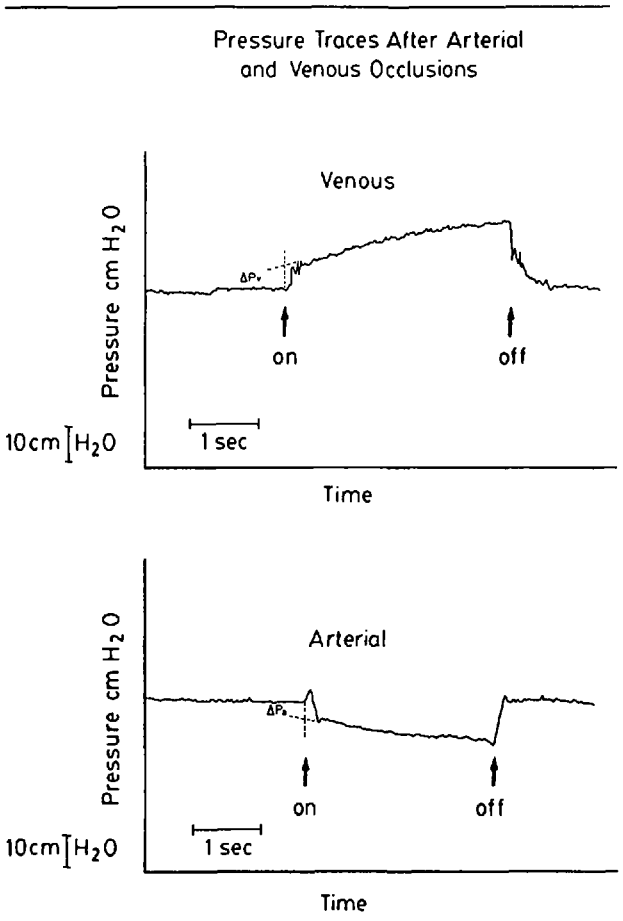

FIGURE 2 A representative venous occlusion (upper curve) and an arterial occlusion (lower curve). The arrows represent the beginning and end of each occlusion. Each occlusion lasted 2–3 sec. The change in pressure was calculated from the stable baseline preceding the occlusion. Each slow pressure change was extrapolated back to the instant of occlusion. The initial pressure transient increases following an occlusion were not included in the straight line extrapolation. The changes in pressure from the stable baseline to the extrapolated pressure were the rapid pressure changes (ΔPa and ΔPv).

The lobar bronchus was ventilated independently of the remainder of the lung by a second Harvard ventilator (PEEP = 0; VT = 150–200 ml). The ventilator rate was adjusted to maintain lobar PCO_2 near 40 mmHg. Lobar arterial pressure (Pa) and venous pressure (Pv) were set by adjusting the height of the respective reservoirs. The Pa and Pv were measured at pressure ports near the arterial and venous cannulae respectively. The ports were connected to pressure transducers by low compliance tubing and all pressures zero referenced to the top of the lobe at end expiration. Before commencing the experimental protocol, at least 30 min was allowed for the preparation to stabilize as assessed by constant $\dot{Q}_L$ over a five-minute period. Heparin, 1000 units, and 50 per cent dextrose (1 ml) were added to the extracorporeal circuit every 30 min. The dextrose maintained a glucose level between 7–9 $mmol \cdot L^{-1}$.

Calculations

Using a modification of methodology described by Hakim *et al.*[5] and Rock *et al.*,[6] we divided total pulmonary vascular resistance into arterial segment resistance (Ra), middle segment resistance (Rm) and venous segment resistance (Rv). We calculated the distribution of resistances using the pressure changes induced by occlusion of the venous or arterial blood flow (Figure 2). After an arterial occlusion there is first a fast (ΔPa) followed by a slow decrease in pressure. ΔPa is considered to be the pressure decrease across the arterial vascular segment.[5] The lower panel of Figure 2 illustrates the effects of arterial occlusion on the arterial pressure signal. The value of ΔPa was calculated by manually fitting a straight line through the first one second of the slow decrease in pressure and extrapolating this back to the time of occlusion. The initial transient overshoot in pressure following an occlusion was not included in fitting the straight line through the slow decrease in arterial pressure. The change from the stable baseline value of Pa to the pressure extrapolated to the time of occlusion was assumed to be ΔPa. The upper panel of Figure 2 shows the effect of a venous occlusion on the venous pressure signal. After releasing the arterial clamp and following return of flow, the venous cannula was rapidly occluded and a rapid rise in pressure (ΔPv) followed by a slow rise in pressure was recorded at the outflow port. ΔPv is considered to be the pressure decrease across the venous segment.[5] ΔPv was calculated in a similar manner to ΔPa. Total resistance (RTOT) was calculated as Pa-Pv/$\dot{Q}_L$. Ra and Rv were calculated as ΔPa/$\dot{Q}_L$ and ΔPv/$\dot{Q}_L$ respectively. Rm was calculated as RTOT − (Ra + Rv).

We measured the lobar pressure-flow (P-$\dot{Q}$) relationship with the lobe under Zone 2 conditions for flow (Pv < −10 cm H_2O and alveolar pressure = 0). In this setting the driving pressure for flow was equal to Pa. Values of lobar flows with their respective driving pressures from each individual experiment were fitted to a straight line by linear regression and the straight line was then extrapolated to zero flow. The zero-flow pressure intercept was assumed to be the mean critical closing pressure (PCRIT) of the pulmonary vasculature (Figure 3). The slopes of the P-$\dot{Q}$ relationship were also obtained by linear regression. The slope of the pressure-flow line is a measure of conductance to flow. Therefore resistance to flow is equal to the inverse of the slope (1/slope) of the P-$\dot{Q}$ relationship. Mean PCRIT and mean 1/slope for each period was obtained by meaning the individual PCRIT and 1/slope values from each animal.

Experimental protocol

During stabilization of flow, indomethacin, 5 $mg \cdot kg^{-1}$, was added to the venous reservoir. We also administered a continuous infusion of indomethacin, 0.5 $mg \cdot kg^{-1} \cdot hr^{-1}$, into the extracorporeal circuit for the duration of the experiment. Following the stabilization period, baseline flow was set by adjusting Pa to 22 cm H_2O and Pv to 3.5 cm H_2O. We manually adjusted the heights of the venous and arterial reservoirs as necessary to maintain constant values of Pa and Pv and thereby maintained Zone 3 conditions for flow except during determinations of the lobar P-$\dot{Q}$ relationships during which we set Zone 2 conditions for flow. Each lobe received five paired periods of ventilation with a control gas mixture (35 per cent O_2, seven per cent CO_2, and 58 per cent N_2) followed by a hypoxic gas mixture (three per cent O_2, seven per cent CO_2, and 90 per cent N_2) to which halothane (0, 0.5, 1.0 or 2.0 per cent) was added. We used a precalibrated Ohio halothane vaporizer to generate the different concentrations of halothane to be added to the control and hypoxic gas mixtures. Each lobe was initially ventilated with the control gas mixture followed by the hypoxic gas mixture (periods Ci and Hi respectively). Following this the lobes were ventilated with paired periods of control gas followed by the hypoxic gas to which one of the three concentrations of halothane was added. We randomized the order of ventilation with halothane to the sequence 0.5, 1.0 and then 2.0 per cent halothane added or to the sequence 2.0, 1.0 and 0.5 per cent halothane added, i.e., each lobe received ventilation with either increasing or with decreasing concentrations of halothane. The lobe was finally ventilated with the control gas (period Co) and then hypoxic gas (Ho) without any added halothane. We refer to the ten ventilatory periods as initial control (Ci), initial hypoxia (Hi), control + halothane 0.5 per cent ($C_{0.5}$), hypoxia + halothane 0.5 per cent ($H_{0.5}$), control + halothane 1.0 per cent ($C_{1.0}$), hypoxia + halothane 1.0 per cent ($H_{1.0}$), control + halothane 2.0 per cent ($C_{2.0}$), hypoxia + halothane 2.0 per cent ($H_{2.0}$), final control (Co), final hypoxia (Ho).

Venous and arterial occlusions were performed in triplicate at the end of each ventilation period. The occlusions were obtained after stopping the ventilator at end expiration. Immediately preceding the occlusions, blood was withdrawn from the venous cannula for blood gas and haematocrit determinations. Blood was analyzed for PO_2, PCO_2, and pH at 37° C (Corning 162-2) using appropriately calibrated electrodes and then corrected for blood reservoir temperature[9] after calculating haemoglobin saturation from a standard nomogram.[10] Inspired gas was also withdrawn prior to occlusions and was analyzed for N_2, O_2, CO_2 and halothane concentrations using a SARA mass spectrometer.

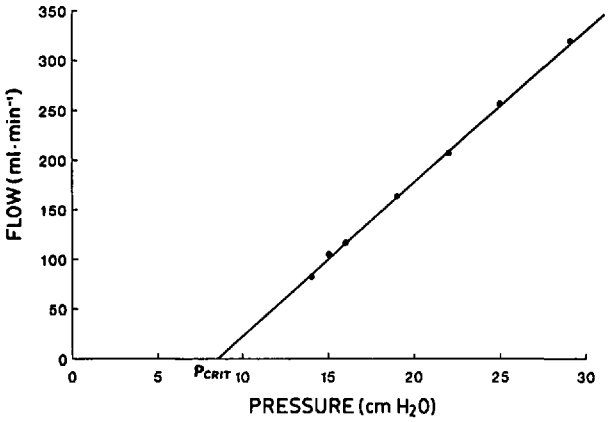

FIGURE 3 A representative pressure-flow plot. Lobar flow ($\dot{Q}_L$) is shown on the Y-axis and arterial pressure is shown on the X-axis. The interrupted line represents the best fit line through the points determined by linear regression. The critical closing pressure (PCRIT) is shown as the extrapolated zero-flow pressure intercept. In this example PCRIT = 8.6 cm H_2O. The slope of the line represents conductance to flow. Note that for a fixed arterial pressure flow could increase with either a decrease in PCRIT or an increase in conductance.

We obtained a P-$\dot{Q}$ plot for each lobe after vascular occlusions. Briefly, following vascular occlusion determinations, the venous reservoir was set below the lung base (Pv < −10 cm H_2O). Varying flows were obtained by decreasing the height of the arterial reservoir in steps of 2–3 cm H_2O. To allow for flow stability, $\dot{Q}_L$ was measured at each arterial pressure at end expiration after stopping the ventilator for 20 sec. We obtained a minimum of six pressure-flow measurements and following these measurements, Pv was reset to baseline values (3.5 cm H_2O) and the next ventilatory period was started. In this manner for each ventilatory period we obtained measurements of driving pressures and their respective flows in Zone 2 conditions and we obtained vascular occlusion measurements under Zone 3 conditions.

Statistics

The values of blood gases, inspired gases and haemodynamic measurements were compared by analysis of variance (ANOVA). Where the F statistic showed significance, the mean values were compared by t test. Sidak's multiplicative inequality was used to correct the t statistic for the number of comparisons made between groups.[11,12] Total resistance and its subdivisions (Ra, Rm and Rv), mean PCRIT and mean 1/slope values were also compared between the paired control and hypoxic ventilatory periods by t test. A P value less than 0.05 was considered to show a significant difference. All values are represented as mean ± standard deviations.

TABLE I Values of haemodynamic variables, blood gases and inspired gas concentrations for each ventilatory period.

	C_I	H_I	$C_{0.5}$	$H_{0.5}$	$C_{1.0}$	$H_{1.0}$	$C_{2.0}$	$H_{2.0}$	C_O	H_O
Pa	22	23	21	24	22	23	21	22	22	23
(cmH_2O)	±1.8	±1.0	±2.2	±1.2	±2.1	±1.5	±3.2	±2.9	±2.4	±1.6
Pv	3.5	3.2	3.8	3.3	3.8	3.3	3.5	3.5	3.7	3.3
(cmH_2O)	±0.8	±1.0	±0.7	±0.5	±0.8	±0.8	±1.0	±1.0	±0.8	±0.5
$\dot{Q}_L$*	133	78	134	78	144	96	168	170	133	71
(cmH_2O)	±23	±15	±8	±13	±15	±21	±21	±36	±10	±9
Temp	36.6	36.6	36.9	36.8	36.8	36.9	36.8	37	37	37
°C	±0.2	±0.1	±0.3	±0.2	±0.4	±0.2	±0.2	±0.1	±0.2	±0.2
Hct	23	23	23	23	23	23	23	23	24	24
(%)	±1	±1	±1	±1	±1	±1	±1	±1	±2	±2
pH	7.24	7.26	7.21	7.24	7.22	7.26	7.23	7.27	7.21	7.23
	±0.03	±0.03	±0.02	±0.03	±0.03	±0.02	±0.04	±0.04	±0.03	±0.03
$PvCO_2$	40	39	39	37	37	38	38	37	39	39
(mmHg)	±4	±3	±3	±1	±2	±2	±2	±4	±1	±1
PvO_2*	193	41	192	42	188	43	197	44	198	40
(mmHg)	±7	±4	±11	±4	±10	±3	±5	±5	±19	±4
FIO_2*	33	3.4	33	3.8	33	3.9	33	4	33	3.8
(%)	±1	±0.3	±0.9	±0.4	±1	±0.6	±1	±0.4	±1	±0.2
$FICO_2$	6.1	6.1	6.0	6.1	5.9	6.2	6.1	6.2	6.1	6.1
(%)	±0.5	±0.4	±0.1	±0.4	±0.2	±0.4	±0.3	±0.4	±0.3	±0.3
FIHal	0	0	0.43	0.43	1.10	1.12	1.97	1.97	0	0
			±0.06	±0.02	±0.12	±0.14	±0.05	±0.04		

Where Pa = arterial pressure, Pv = venous pressure, $\dot{Q}_L$ = lobar blood flow, temp = temperature, hct = haematocrit, $PvCO_2$ = venous PCO_2, PvO_2 = venous PO_2, FIO_2 = inspired concentration O_2, $FICO_2$ = inspired concentration of CO_2 and FIHal = inspired concentration of halothane. *denotes significant difference by ANOVA. The paired control and hypoxic ventilatory periods are denoted by C and H respectively. The subscripts I, 0.5, 1.0, 2.0 and O refer to initial, †halothane 0.5%, halothane 1.0%, halothane 2.0% and final periods respectively. See text for further explanation of various ventilatory periods. Note that $\dot{Q}_L$ is similar during periods $H_{2.0}$ and $C_{2.0}$.

Results

Haemodynamic and ventilatory variables

Table I illustrates the mean values of haemodynamic variables, inspired gas concentrations and blood gases obtained during the five paired ventilatory periods under Zone 3 conditions for flow. There were no differences in the mean values for arterial and venous pressures between the paired hypoxic and control ventilatory periods. Similarly, there were no differences in temperature, haematocrit, or pH between the paired hypoxic and control periods. Inspired CO_2 concentration ($FICO_2$) and venous PCO_2 ($PvCO_2$) were similar between hypoxic and control ventilatory periods. During the hypoxic ventilatory periods (H_I, $H_{0.5}$, $H_{1.0}$, $H_{2.0}$), the mean inspired O_2 concentration ($FICO_2$) ranged from 3.4 to 4.0 per cent. The PvO_2 was similarly low with mean values from 40 to 44 mmHg. Mean values of both PvO_2 and FIO_2 were significantly lower during hypoxic periods compared with their respective control ventilatory periods (C_I, $C_{0.5}$, $C_{1.0}$ and $C_{2.0}$ respectively). Inspired halothane concentrations (FIHAL) were similar between paired ventilatory periods and were near the preselected values of 0.5, 1.0 and 2.0 per cent. ($C_{0.5}$ and $H_{0.5}$, $C_{1.0}$ and $H_{1.0}$, and $C_{2.0}$ and $H_{2.0}$ respectively). $\dot{Q}_L$ was significantly lower during each hypoxic period compared with its respective control period except during ventilation with 2.0 per cent halothane. $\dot{Q}_L$ was similar between periods $C_{2.0}$ and $H_{2.0}$ with values of 168 ± 21 and 170 ± 36 $ml \cdot min^{-1}$ respectively.

Vascular occlusions

The values for pulmonary vascular resistance and its subdivisions during each ventilatory period are shown in Table II. Values of RTOT, Ra, Rm and Rv were compared between the paired control and hypoxic ventilatory periods at similar values of FIHAL. During hypoxic ventilation RTOT was significantly larger than during its paired control period except during ventilation with 2.0 per cent halothane. During periods $C_{2.0}$ and $H_{2.0}$ RTOT was similar with values of 0.11 ± 0.03 and 0.11 ± 0.04 $cm\ H_2O \cdot ml^{-1} \cdot min^{-1}$ respectively. Mean values for Rm showed the same pattern with Rm significantly larger

TABLE II Values for pulmonary vascular resistance and pressure-flow relationship during each ventilatory period.

	Cl	*Hl*	$C_{0.5}$	$H_{0.5}$	$C_{1.0}$	$H_{1.0}$	$C_{2.0}$	$H_{2.0}$	*Co*	*Ho*
RTOT	0.140 ±0.029	0.262* ±0.065	0.125 ±0.009	0.265* ±0.058	0.124 ±0.021	0.208* ±0.051	0.108 ±0.031	0.112 ±0.035	0.135 ±0.026	0.273* ±0.051
Ra	0.056 ±0.013	0.014* ±0.011	0.049 ±0.008	0.014* ±0.013	0.048 ±0.010	0.0126* ±0.011	0.046 ±0.009	0.045 ±0.009	0.052 ±0.011	0.008* ±0.007
Rm	0.039 ±0.011	0.184* ±0.046	0.030 ±0.010	0.180* ±0.033	0.036 ±0.009	0.141* ±0.035	0.028 ±0.016	0.033 ±0.017	0.032 ±0.009	0.185* ±0.023
Rv	0.045 ±0.019	0.063 ±0.044	0.046 ±0.007	0.071 ±0.043	0.040 ±0.011	0.054 ±0.024	0.034 ±0.014	0.035 ±0.017	0.051 ±0.016	0.080 ±0.044
PCRIT (cm H_2O)	7.9 ±1.7	11.5* ±2.3	7.1 ±1.2	12.5* ±2.0	8.1 ±1.4	11.4* ±1.4	7.5 ±2.6	6.0 ±2.5	7.2 ±2.0	10.9* ±3.3
1/slope	0.082 ±0.017	0.12* ±0.03	0.081 ±0.016	0.13* ±0.02	0.080 ±0.018	0.12* ±0.03	0.077 ±0.022	0.083 ±0.025	0.084 ±0.019	0.13* ±0.01

Table II illustrates mean values of total resistance (RTOT) and its subdivisions; arterial segment resistance (Ra), middle segment resistance (Rm) and venous segment resistance (Rv). Mean values of critical opening pressures (PCRIT) and resistances (1/slope) derived from the vascular pressure-flow relationships are also illustrated. Units for resistances including 1/slope are in cm $H_2O \cdot ml^{-1} \cdot min^{-1}$. See table I for ventilatory period abbreviations. *denotes significant difference ($p < 0.05$) between paired control and hypoxic periods.

during hypoxic periods than during control periods except during ventilation with 2.0 per cent halothane. Mean values of Ra were significantly lower during hypoxic periods than during their paired control periods except during ventilation with 2.0 per cent halothane. During ventilation with 2.0 per cent halothane, Ra was similar between hypoxic and control periods. Rv did not change significantly between hypoxic and control periods.

P-$\dot{Q}$ relationship

Table II also shows the values for mean PCRIT and mean 1/slope from the individual P-$\dot{Q}$ lines. Mean PCRIT values increased significantly during hypoxic periods when compared with their paired control periods except during ventilation with 2.0 per cent halothane. Excluding periods $C_{2.0}$ and $H_{2.0}$, mean PCRIT values increased by between 3.6 and 4.4 cm H_2O when paired control and hypoxic periods were compared. During periods $C_{2.0}$ and $H_{2.0}$, mean values of PCRIT were similar. Mean 1/slope followed a similar pattern with values significantly higher during hypoxic periods compared with their respective normoxic periods except during periods $C_{2.0}$ and $H_{2.0}$.

Discussion

Model of vascular occlusion

We used methodology similar to that described by Hakim *et al.*[5] to partition pulmonary vascular resistance into arterial, middle, and venous segment resistances. The lobe vasculature can be compared to an electrical circuit composed of three resistors (Ra, Rm and Rv) in series separated by two capacitors (Ca and Cv) in parallel. Opening the circuit upstream from resistor Ra (equivalent to occluding arterial flow in the lobe) results in an immediate voltage drop to the level set by capacitor Ca. A similar analogy holds for the measurement of venous resistance. The capacitor then continues to either discharge through downstream segments (arterial occlusion) or to charge from upstream flow (venous occlusion). In the lobe, acutely occluding flow allows pressure to equilibrate rapidly with the pressure set by downstream (arterial) or upstream (venous) capacitance vessels. This rapid change in pressure following occlusion is related to the resistive pressure decrease across the vascular segment which is being tested.

The differences between our model and the models used by others have been extensively discussed previously.[8] Briefly, Hakim *et al.*[5,7] set flow constant and allowed arterial pressure to vary with changes in resistance. We set constant arterial and venous pressures and allowed flow to vary with changes in resistance. Secondly, Hakim *et al.*[7] and Rock *et al.*[6] used a three-way piston valve or solenoid to occlude arterial flow. We obtained arterial occlusions using a manual clamp. Finally, Hakim *et al.*[7] calculated ΔPa by fitting the slow decrease in the arterial pressure to an exponential curve and then extrapolating this curve back to the time of occlusion. We fitted the slow decrease in the arterial pressure to a straight line and then extrapolated the straight line back to the time of occlusion in order to calculate ΔPa. Despite these methodological differences,[8] our results should be comparable with those of other investigator.[5-7]

Experimental conditions

We attempted to control variables which might independently effect vascular resistance and thereby confound

interpretation of our results. We prospectively maintained the lobe vasculature in Zone 3 conditions for flow during vascular occlusions and we maintained near constant arterial and venous pressures throughout all ventilatory periods. By this we hoped to minimize vascular recruitment or derecruitment and to maintain similar transmural vascular distending pressures between all ventilatory periods. We allowed lobar venous pH to remain in an acidotic range in order to augment potentially the strength of the hypoxic response.[14] pH was maintained at similar values between paired periods of ventilation. Since haematocrit can independently affect middle segment resistance[15] we prospectively set haematocrits to similar values between control and hypoxic periods. The concentration of inspired halothane was also similar between the paired hypoxic and control periods. Finally $PvCO_2$ was similar between the paired periods and should not have been a cause of any of the differences which we noted. The primary difference between each pair of control and hypoxic periods was the FIO_2 and the resultant PvO_2. The FIO_2 was high enough during control periods such that it should not have caused any hypoxic pulmonary vasoconstriction. Mean values of PvO_2 during hypoxic periods were all near 40 mmHg and should have affected resistance to a similar degree.

Cyclooxygenase blockade

In these experiments we administered indomethacin after the animals experienced the surgical stress of lobar vascular isolation. Since indomethacin does not affect preformed prostaglandins, it is possible that there were already high levels of vasoconstricting prostaglandins in the lobar perfusate. This is suggested by comparison with our previous experiments where we found a lower total resistance during control ventilation when indomethacin was administered prior to the vascular isolation of the lobe.[16] The high initial resistance was maintained for the duration of the experiments and therefore does not likely influence our results. Although we did not measure prostaglandin levels to ensure their subsequent blockade, we used doses of indomethacin which are similar to those which other investigators have found effective at blocking prostaglandin production in dogs' lungs.[17–19] We had previously found that in the absence of a hypoxic stimulus indomethacin administration alone does not affect middle segment resistance.[16] We therefore designed our experiments to compare total resistance and its subdivisions during paired control and hypoxic ventilatory periods in the presence of cyclooxygenase inhibition at varying halothane concentrations.

Comparing groups H_I and C_I demonstrates that prior to addition of halothane (in the presence of cyclooxygenase blockade) hypoxic ventilation resulted in near doubling of total resistance compared with control gas ventilation. We had previously found that hypoxia in the absence of cyclooxygenase blockade resulted in total resistance increasing by 30 per cent.[8,16] This was similar to the 50 per cent increase in resistance with hypoxia noted by Hakim *et al.*[7] The larger increase in total resistance following cyclooxygenase blockade is consistent with the hypothesis that hypoxia may induce production of a vasodilating prostaglandin.[20]

Vascular segmental changes

We found that the middle vascular segment was the only segment to demonstrate increases in resistance with hypoxia. This is similar to the findings of previous studies by Hakim *et al.*[7] in dogs and Rock *et al.*[6] in pigs. We also found that at lower concentrations of inspired halothane (0.5 or 1.0 per cent) both total resistance and middle segment resistance still increased significantly during hypoxic ventilation. At a halothane concentration of 2.0 per cent, however, neither total resistance nor middle segment resistance demonstrated significant increases with hypoxia. There was a trend for middle segment resistance to decrease as halothane concentration increased consistent with a dose–response curve for halothane.

Arterial segment resistance decreased significantly during hypoxic ventilation when compared with control ventilation with either 0, 0.5 or 1.0 per cent halothane. During ventilation with a 2.0 per cent inspired halothane the arterial segment resistance did not differ between control and hypoxic periods. There are three possible causes for this apparent vasodilatation of the arterial segment with hypoxia. It is possible that hypoxia specifically altered the arterial capacitance vessels and did not affect the venous capacitance vessels. Then the locus of the arterial capacitance vessels may have shifted upstream toward the origin of the arterial segment. This would result in the arterial segment consisting of a physically shorter segment and therefore resistance through this shortened segment would decrease. Alternatively the arterial capacitance vessels may have preferentially increased their resting pressure and therefore the pressure drop across the arterial segment would decrease. However, changes in capacitance vessels do not entirely explain the return of arterial segment resistance toward baseline levels during ventilation with 2.0 per cent halothane. The third possible explanation is that with the intense constriction of the middle segment vessels there is a greater pressure present at the distal end of the arterial segment. This could result in increased transmural pressure in the arterial segment with a resultant increase in vascular distension and therefore a lower arterial segment resistance. With vasodilatation of the middle segment, the arterial segment distension would decrease and the

resistance would increase as noted during hypoxic ventilation with 2.0 per cent halothane.

Pressure-flow relationship

The effects of halothane on arterial segment resistance are small (0.04 cm $H_2O \cdot ml^{-1} \cdot min^{-1}$) compared with the much larger changes in middle segment resistance (0.15 cm $H_2O \cdot ml^{-1} \cdot min^{-1}$). The net effect of halothane administration is a marked decrease in pulmonary vascular resistance. Halothane acts as a vasodilator at the level of the middle segment vessels and at higher concentrations is able to ablate completely the vasoconstriction induced by hypoxia. In order to define further the effects of halothane on hypoxic pulmonary vasoconstriction we evaluated its effects by analyzing the vascular pressure-flow relationship.[6] This relationship can be used to divide changes in resistance those due to changes in the slope of the P-$\dot{Q}$ line and into those due to changes in critical closing pressure. The slope of the P-$\dot{Q}$ line corresponds to the conductance (the inverse of resistance) to flow through a rigid pipe and can be described by the Poiseuille equation.[21] The critical closing pressure, as assessed by the extrapolated zero-flow pressure intercept, corresponds to the resistance to flow due to a Starling resistor effect.[22] The pressure drop through a rigid pipe increases with increasing flow and therefore the pressure drop is dependent upon the level of flow. However, once flow is established across a Starling resistor, the pressure drop across the Starling resistor is constant and therefore the pressure drop can be considered to be independent of flow. Similarly, once the critical closing pressure of the vascular bed is exceeded it no longer limits flow and the pressure drop across it can be considered flow-independent. Total pulmonary vascular resistance may be increased by an increased critical closing pressure, a decreased conductance or a combination of both decreased conductance and increased critical closing pressure.

Interactions of halothane, indomethacin and resistance

Vessels in the middle vascular segment respond to hypoxia with increased tone and this increased tone is measured as an increase in critical closing pressure.[6] During the paired periods CI, HI and CO, HO, we observed that critical closing pressure was significantly increased during hypoxic ventilation compared with control ventilation. Comparing periods $C_{0.5}$ with $H_{0.5}$ and $C_{1.0}$ with $H_{1.0}$, hypoxia still resulted in an increased critical closing pressure. During periods $C_{2.0}$ and $H_{2.0}$, the addition of 2.0 per cent halothane blocked the increase in critical closing pressure seen with hypoxia. Combining the results of the vascular occlusions with the results of the P-$\dot{Q}$ relationships would suggest the hypoxia-induced increases in resistance occur at the level of the middle vascular segment. Hypoxia increased the tone of the middle segment vessels and this increased tone is expressed as an increase in critical closing pressure. In the presence of cyclooxygenase inhibition, halothane in concentrations of 2.0 per cent will decrease vascular tone and block the hypoxia-induced increases in vascular resistance. We also observed a significant decrease in conductance to flow as measured by a decreased slope of the P-$\dot{Q}$ lines. The decreased conductance is the equivalent of an increase in resistance (1/slope). This component of resistance was also increased by hypoxia and was also ablated only at the highest concentration of halothane. Since we found that neither arterial nor venous resistance increased with hypoxia, we believe that the middle vascular segment may also contain vessels which can narrow their calibre and thereby result in decreased conductance to flow. This would be similar to the results of Rock *et al.* in pigs' lungs except they found the locus of the decreased conductance to be at the arterial segment.[6] Thus halothane may alter middle segment calibre as well as middle segment critical closing pressure. At higher concentrations of halothane hypoxic pulmonary vasoconstriction is completely ablated even in the presence of indomethacin-induced blockade of prostaglandin production.

Summary

In this experiment we found that in the presence of cyclooxygenase blockade, halothane inhibited hypoxic pulmonary vasoconstriction only at a concentration of 2.0 per cent. We believe these experiments demonstrate that the site of action of halothane is at the level of the middle segment of the pulmonary vasculature. This is similar to the site of action of hypoxia alone[6,7] and would suggest that both halothane and hypoxia exert their effects in the same vascular segments. These effects, in turn, are modified with cyclooxygenase blockade. Since halothane could still ablate hypoxic pulmonary vasoconstriction even with cyclooxygenase inhibition, these experiments suggest that interactions with prostaglandins are not necessary for halothane to alter hypoxic pulmonary vasoconstriction. Our experiments do not, however, rule out an interaction of halothane with leukotrienes such as that suggested by Marshall *et al.*[3]

Our results should not be extrapolated directly to the clinical setting. The isolated lobe preparation allows vascular pressures to be controlled to a degree not possible in the intact animal. The isolated lobe is also unique in that secondary neuronal and humoral influences[23] can be eliminated and the direct effects of a drug on the pulmonary vasculature can be tested. The difficulty in extrapolating these results to the clinical setting is that these same secondary neural or hormonal reflexes may

modify the end effects of the drug. Therefore although our results demonstrate that halothane inhibits hypoxic pulmonary vasoconstriction, this effect may be masked in the clinical setting by secondary humoral or neurogenic reflexes. At this time, it seems prudent to consider halothane as a potential cause of systemic hypoxaemia when hypoxic pulmonary vasoconstriction is required to maintain adequate arterial oxygenation. In our previous studies, using identical methodology in the absence of cyclooxygenase inhibition, we found that halothane in a concentration of 0.5 per cent blocked hypoxic pulmonary vasoconstriction.[8] In the present study 2.0 per cent halothane was required to obtain similar results. This suggests that inhibition of prostaglandin production may antagonize the effects of halothane as a pulmonary vascular vasodilator. We therefore speculate that cyclooxygenase blockade or infusion of vasoconstricting prostaglandins might be clinically useful in settings where maximal hypoxic pulmonary vasoconstriction is useful (i.e., one lung anaesthesia).

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