

Endothelium-derived Relaxing Factor Is Not Responsible for Inhibition of Hypoxic Pulmonary Vasoconstriction by Inhalational Anesthetics

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Inhalational anesthetics inhibit hypoxic pulmonary vasoconstriction (HPV). One mechanism suggested for this action is stimulation of release of endothelium-derived relaxing factor. The present study has tested this hypothesis. These studies were performed in 66 ventilated and perfused isolated rat lungs. There were three study protocols. Study I examined the effect on HPV of the inhibition of soluble guanylate cyclase by methylene blue (MB). In the presence or absence of MB, the lungs constricted to hypoxia with pulmonary artery pressure increases of 8.6 ± 0.2 cmH₂O and 11.5 ± 0.4 cmH₂O, respectively, and halothane, enflurane, and isoflurane caused a reversible 50% decrease in the pulmonary pressor response, but acetylcholine (ACh) was vasodilatory in the saline group and vasoconstrictor in the MB group. In Study II a dose-response curve was established for the potent stimulator (Sin 1) of the enzyme guanylate cyclase. In the presence of MB the dose-response curve for Sin 1 was shifted to the right with an increase in the ED₅₀ for Sin 1 from 44 μ M for the control to 85 μ M for the MB group. In Study III, baseline pulmonary artery pressure was increased with U46619, and the hypoxic pressor response was increased (28.9 ± 2.5 cmH₂O), but halothane again caused a 50% decrease (11.0 ± 1.8 cmH₂O) in the response to hypoxia. In summary, when soluble guanylate cyclase activity is inhibited by MB, the inhibition of hypoxic pulmonary vasoconstriction by halothane, isoflurane, or enflurane was unaltered, and release of endothelium-derived relaxing factor (EDRF) is therefore not an essential mechanism underlying this action. (Key words: Anesthetics, volatile: halothane, isoflurane, enflurane. Lung, isolated, blood flow: hypoxic pulmonary vasoconstriction. Methylene blue. Parasympathetic nervous system: acetylcholine.)

ALVEOLAR HYPOXIA and/or hypoxemia cause constriction of pulmonary arteries (hypoxic pulmonary vasoconstriction [HPV]) and inhalational anesthetics inhibit this constriction in a dose-dependent manner.¹ The mechanisms by which hypoxia causes constriction and inhalational anesthetics inhibit this response are unknown.

One hypothesis is that alveolar hypoxia and/or hypoxemia reduce the production of the endothelium-derived relaxing factor (EDRF)² and that inhalational anesthetics interfere with this sequence. Blaise *et al.*³ presented data suggesting that the vasodilatory action of isoflurane on systemic vessels was through its action on endothelial cells;

and Muldoon *et al.*⁴ reported that inhalational anesthetics reduced the ability of some systemic vessels to dilate in response to a vasodilator and this too was endothelium-related.

Endothelial cells contribute to vasodilation through the release of endothelium-derived relaxing factor (EDRF).⁵ Endothelium-derived relaxing factor is synthesized and released from endothelial cells on stimulation by a number of agents such as acetylcholine, adenosine diphosphate (ADP), and the calcium ionophore, A23187.⁶ Endothelium-derived relaxing factor diffuses to the vascular smooth muscle, where it activates the soluble form of guanylate cyclase. This enzyme converts guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP). The concentration of cGMP has been found to have a positive correlation with vascular smooth muscle relaxation.^{7,8} Methylene blue (MB) prevents activation of this guanylate cyclase in the vascular smooth muscle and thus prevents relaxation through the cGMP pathway.⁹ The present study has used this property of methylene blue to investigate four questions; Does MB inhibit guanylate-cyclase-dependent responses in the whole lung? Does MB alter the HPV response? What effect does MB and therefore the inhibition of the action of EDRF have on the reduction of hypoxic pulmonary vasoconstriction by inhalational anesthetics? Does increased tone in the pulmonary vasculature change the response to pulmonary hypoxia or change the inhibitory action of an inhalational anesthetic on HPV?

Methods

Following institutional approval, 66 adult female rats were anesthetized by an intraperitoneal injection of sodium pentobarbital (30 mg/kg). A jugular vein was exposed and cannulated and either methylene blue (30 rats) or saline (36 rats) was slowly infused; the milligram dose of MB infused was based on 0.04 times the rat's body weight in grams, and this gave a concentration in the whole animal of approximately 1×10^{-4} M.

The MB was allowed to circulate in the anesthetized animal for 45 minutes. At the end of this time, a tracheostomy was performed, the lungs were exposed by means of a sternotomy, and 100 IU of heparin was injected into the heart. Both the pulmonary artery and left ventricle were cannulated. The pulmonary artery cannula was "t" shaped and one arm of the "t" was attached to a Statham®

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transducer for measurements of pulmonary artery pressure. A perfusate solution was pumped from a heated reservoir by a Harvard® peristaltic pump through a pediatric Fenwal® filter into the pulmonary artery. The rate of flow was maintained constant at $0.06 \text{ ml} \cdot \text{g body weight}^{-1} \cdot \text{min}^{-1}$ and the perfusate was returned to the reservoir by the left ventricular cannula. The perfusate consisted of half blood and half physiologic salt solution¹⁰ containing 3% bovine albumin and sodium meclofenamate (5 mg/l). The blood was obtained from anesthetized donor rats by means of a cardiac puncture.

The lungs were removed from the chest cavity and suspended in a heated chamber and were ventilated at 60 breaths per minute with a Harvard® Rodent ventilator with a normoxic gas mixture (21% oxygen, 5% carbon dioxide, balance nitrogen) and a minute volume of 180 ml/min. A positive end-expiratory pressure of 2 cmH₂O was applied. An additional amount of MB was added to the reservoir at this time for studies numbered I and II, to obtain a final MB concentration of $0.5 \times 10^{-5} \text{ M}$, and the same volume of saline was added to the reservoir for the saline groups.

The lungs were allowed to stabilize for 30 min, during which time they were ventilated with the normoxic gas mixture. At the end of this period the isolated lungs were challenged for 6 min with an hypoxic gas mixture (3% oxygen, 5% carbon dioxide, balance nitrogen) followed by a 6-min normoxic ventilation. This pattern of ventilation was repeated at least three times until the pulmonary artery pressure response to hypoxia stabilized. This procedure was performed in all animals before the commencement of the studies.

To answer each of the specific questions, the animals then were assigned to three different study protocols.

STUDY I

Study I concerned the effect of methylene blue on inhibition of hypoxic response by inhalation anesthetics (24 methylene blue rats, 24 saline rats).

The isolated lungs were challenged for 10 min with the hypoxic gas mixture (Phase I), and 5 min into the hypoxic challenge, acetylcholine was infused into the pulmonary artery to achieve a concentration of $0.5 \times 10^{-5} \text{ M}$. At this time, the saline and methylene blue groups of rats were each further subdivided into four groups to obtain a total of eight groups, each containing six rats. Group 1 (control) consisting of six saline and six MB rats, received no anesthetic. Groups 2, 3, and 4 received the halothane, isoflurane, or enflurane, respectively. The anesthetics were introduced into the ventilatory circuit by diverting the inspired gases through a calibrated Dräger® vaporizer set to deliver the percent anesthetic vapor that causes an approximate 50% depression of the hypoxic

response¹; halothane (0.57%), isoflurane (0.69%), and enflurane (1.1%).

After 20 min, to allow equilibration of the anesthetic vapor in the circuit, the isolated lungs were challenged for 10 min with the hypoxic gas mixture (Phase II) containing the same concentration of anesthetic, 5 min into the hypoxic challenge acetylcholine was added to the reservoir.

The anesthetic administration was discontinued and allowed to dissipate from the circuit over a 20-min period. The hypoxic challenge then was performed two more times (Phases III and IV) with acetylcholine added to the reservoir 5 min into the hypoxic challenge. Each of these hypoxic challenges was separated by 20 min of normoxic ventilation.

STUDY II

Study II concerned the effect of methylene blue on the action of Sin 1 (six methylene blue rats, six saline rats).

The lungs were ventilated with an hypoxic gas mixture, and 5 min into the hypoxic response, increasing doses of Sin 1 (3-morpholino-sydnominine-hydrochloride, the active principle of Molsidomine; Cassella AG, Frankfurt, FRG) ($1 \times 10^{-6} \text{ M}$; $1 \times 10^{-5} \text{ M}$; $5 \times 10^{-5} \text{ M}$; $1 \times 10^{-4} \text{ M}$; $1 \times 10^{-3} \text{ M}$) were injected into the pulmonary artery at 2-min intervals. Two minutes after the final Sin 1 injection, the ventilatory gas was returned to normoxia for 10 min. The lungs were finally rechallenged with the hypoxic gas mixture for 10 min, followed by the normoxic gas.

STUDY III

Study III concerned the influence of baseline tone on the hypoxic pulmonary pressor response (six rats).

Before the equilibration period, the thromboxane mimic, U46619, (Upjohn Co., Kalamazoo, MI) was injected into the pulmonary artery until a baseline pressure was obtained that was greater than the initial baseline pressure in the methylene-blue-treated animals (total U46619 injected was 0.3 µg). At the end of the stabilization period and after the three successive hypoxic responses, the study began. The lungs were challenged with the hypoxic gas mixture for 10 min followed by the normoxic gas mixture, after which the inhalational anesthetic halothane was introduced into the ventilatory circuit, in a similar manner to Study I. After 20 min, the isolated lungs were challenged with the hypoxic gas mixture for 10 min. The normoxic gas then was restored and the anesthetic was allowed to dissipate over a 20-min period. The lungs then were rechallenged with the hypoxic gas mixture, and then after 10 min of normoxia, the final hypoxic challenge was imposed.

In all studies, measurements of P_{O₂} and P_{CO₂} of the ventilated gases and of pulmonary artery pressure and

airway pressure were recorded on a Grass eight-channel recorder and the pressor responses derived as shown in figure 1 and explained in the legend. The concentration of inhalation anesthetics contained in the perfusate was measured by gas chromatography.¹¹ The concentration of anesthetics in the inspired gas mixture were measured on a Perkin Elmer[®] MGA 1100 Mass Spectrometer. The body weight, airway pressure, temperature, hematocrit, pH and final lung (wet-dry)/dry weight also were recorded. The data were analyzed by a two-way mixed design analysis of variance (ANOVA), and differences be-

tween means compared by Neuman-Keuls test, with significance at $P < 0.05$.¹² Additional statistical analysis are indicated in the text.

Results

For those conditions that did not differ between the studies, the following overall mean values (mean \pm SE) were derived: body weight, 298.0 ± 3.5 g; temperature, $38 \pm 0.10^\circ$ C; mean airway pressure, 5.6 ± 0.6 cmH₂O; hematocrit, $19.9 \pm 0.2\%$. For those groups pretreated with saline instead of methylene blue, the lung water content did not differ between the groups and the overall mean value was 4.27 ± 0.05 ml water/g dry weight. The lung water measurement for the groups receiving MB again did not differ between groups, but the overall mean value of 6.02 ± 0.2 ml water/g dry weight was significantly greater than that observed for the saline groups. The overall airway pressure for the saline group was 5.5 ± 0.3 cmH₂O, and for the methylene blue group was 5.6 ± 0.3 cmH₂O, and these were not significantly different.

STUDY I

Effect of Methylene Blue on Inhibition of Hypoxic Response by Inhalation Anesthetics

Table 1 shows the mean baseline pressure data and statistical analysis for each of the four phases for the eight groups of animals. The pattern of change between the individual groups receiving saline did not differ, but was different from those receiving MB. The individual saline groups and MB groups have therefore been combined. The combined initial baseline pressure for the saline groups ($n = 24$) at Phase I was 16.8 ± 0.3 cmH₂O, and by Phase IV there was a significant increase in the baseline pressure to 21.0 ± 0.4 cmH₂O. For the methylene blue group, the combined baseline pressure data from the four groups for Phase I was 21.7 ± 0.7 cmH₂O, and this significantly increased with time to 29.5 ± 2.0 cmH₂O. The baseline pressures were significantly greater for the MB group compared with the saline group at the beginning and end of the study.

The data and statistical analysis for the mean hypoxic pressor response before acetylcholine, with and without anesthetics, are presented in Table 2. The pressor response in the saline group progressively decreased (Phase I versus Phase IV), whereas that in the MB groups progressively increased so that by Phase IV, the combined pressor responses to hypoxia in the saline groups were significantly less than that of the MB groups (6.0 ± 0.1 cmH₂O and 12.6 ± 0.5 cmH₂O, respectively). This result was not dependent on the administration of an inhalational anesthetic agent, as the groups not receiving inhalational anesthetics had a similar pattern in their pressor

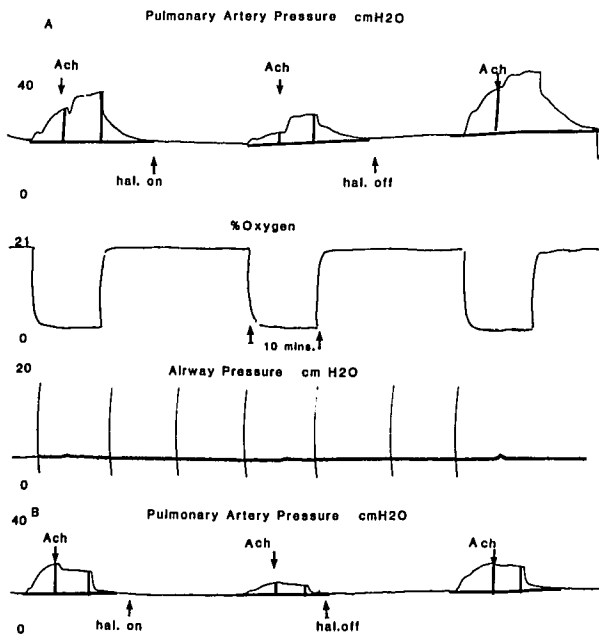


FIG. 1. Observation for representative animals from the saline:halothane group (B) and MB:halothane group (A). The middle two tracings are of the oxygen tension of the ventilatory gas and indicate the hypoxic and normoxic sequence and the airway pressure. The top tracing is of the pulmonary artery pressure from the MB:halothane group. The arrow marked "hal. on" and "hal. off" indicate when halothane was added to and removed from the ventilatory circuit. The arrow marked "Ach," indicates the time acetylcholine was infused into the pulmonary artery. The pressor response to hypoxia was measured after 5 min, immediately before Ach, and the response to Ach was measured 5 min later. The construction for deriving these pressor responses is indicated in the diagram and was performed as follows: a line was drawn connecting the baseline before and after the hypoxic challenge. The difference between this interpolated baseline and the observed pulmonary artery pressure at 5 min was recorded as the hypoxic pressor response. The measurement for the acetylcholine response was made at 10 min after commencement of the hypoxic challenge and was the difference between the pulmonary pressure at 5 min and at 10 min of hypoxia. The recording demonstrates the dilator response to Ach in the saline group and the reversal to a constrictor response in the MB group, and yet HPV response was equally present and well maintained in both groups. It also demonstrates a 50% depression of the HPV response with halothane with and without MB and also that the responses to Ach were still constrictor in the MB group and dilator in the saline group.

TABLE 1. Baseline Pulmonary Artery Pressure (cmH₂O)

Groups*	PHASES			
	1	2	3	4
Saline + no anesthetic	18.2	19.6	21.1	23.1†
	0.9	0.9	0.9	1.1
Saline + halothane	16.7	16.9	18.6	20.3†
	0.5	0.6	0.7	0.9
Saline + isoflurane	14.5	15.6	16.7	17.9†
	0.4	0.5	0.6	0.8
Saline + enflurane	18.0	19.0	20.2	22.7†
	0.4	0.4	0.5	0.7
MB + no anesthetic	23.0	23.2	25.6	28.9†
MB + halothane	2.3	1.9	3.0	5.8
	22.5	23.8	29.2	37.7†
MB + isoflurane	2.0	2.2	3.8	6.3
	18.5	19.4	21.7	26.3†
MB + enflurane	0.8	1.1	1.2	1.2
	22.6	23.2	23.9	25.2†
	0.6	0.8	0.7	0.5

n = 6 for each group; mean ± SE.

* Inhalation anesthetic was only administered during phase 2.

† Significantly different from phase 1.

responses to hypoxia (Table 2). In all the saline groups, the addition of acetylcholine resulted in dilatation, whereas for the MB groups the response was constrictor, and this difference was significant at all phases. The addition of anesthetic at Phase II caused a significant reduction in the pulmonary pressor response in all the groups. In the methylene blue groups, the increase in pressure due to acetylcholine was significantly reduced (fig. 1) in the presence of anesthetics, and in the saline

groups the dilator response to acetylcholine was significantly reduced compared with Phase I (by Student's *t* test).

The elimination of anesthetic from the circuit caused a return of the hypoxic pressor response. In the case of the methylene blue groups, the magnitude of the hypoxic pulmonary pressor response and the response to acetylcholine were restored to that of Phase I by 20 min (Phase III) and significantly exceeded that response by 30 min (Phase IV). In the saline groups, the hypoxic response at Phases III and IV did not return to the original pressor values (Phase I), and progressive reductions in responses were apparent although the response to acetylcholine remained dilator. The mean partial pressures for each of the inhalation anesthetics in the perfusate during Phase II were for halothane, 3.5 ± 0.1 mmHg; for isoflurane, 5.4 ± 0.3 mmHg; and for enflurane, 5.4 ± 0.2 mmHg. No significant differences in the effect of the different anesthetics on the hypoxic response or the response to acetylcholine were observed.

In summary, the combined data of Phase I for animals that subsequently received inhalational anesthetics during Phase II in the presence of saline (n = 18) was a significant decrease in the hypoxic pressor response from 11.6 ± 0.6 cmH₂O to 8.9 ± 0.4 cmH₂O (Phase I) when acetylcholine was added. The addition of inhalational anesthetic caused a 60% decrease (Phase I vs. Phase II) in the pressor response to hypoxia and a further decrease in the pressor response in the presence of acetylcholine (4.6 ± 0.3 cmH₂O to 3.9 ± 0.4 cmH₂O). In the MB groups, the combined data (n = 18) showed a significant increase in the pressor response Phase I (8.6 ± 1.0 cmH₂O to 11.0

TABLE 2. Hypoxic Pressor Response (cmH₂O) (pressure during hypoxia-base line pressure)

Groups*	Phases							
	1		2		3		4	
	Before ACH	With ACH	Before ACH	With ACH	Before ACH	With ACH	Before ACH	With ACH
Saline + no anesthetic	11.3	9.2	9.0	7.3	7.9	6.2	6.8†	5.7
	0.1	1.0	1.7	1.3	2.0	1.6	2.6	1.9
Saline + halothane	11.7	9.5	5.2†	4.2†	8.2†	6.7	6.2	5.7†
	1.1	0.9	0.8	0.7	1.1	1.0	1.8	1.5
Saline + isoflurane	11.3	8.0	2.7†	3.0†	7.8†	5.6	6.1†	4.8†
	0.6	0.3	0.5	0.3	0.9	0.5	1.4	0.8
Saline + enflurane	11.7	9.2	5.7†	4.5†	7.7†	5.7	4.8†	3.9†
	1.3	1.0	1.1	0.9	1.4	0.9	1.0	0.6
MB + no anesthetic	8.7	12.2	8.0	13.7	12.2	17.8†	15.8†	22.2†
	1.1	1.6	1.1	1.9	1.6	1.9	1.9	3.6
MB + halothane	9.9	13.1	3.3†	6.3†	8.3	15.1	13.2	21.2†
	1.1	1.2	0.5	0.3	1.6	1.6	2.5	2.1
MB + isoflurane	6.7	8.4	2.5†	4.3†	8.2	13.7†	12.0†	16.6†
	0.8	0.8	0.3	0.4	1.5	1.4	1.7	2.0
MB + enflurane	9.2	11.5	5.1†	8.5	8.7	12.7	9.3	16.3†
	1.7	2.0	0.9	1.6	2.5	2.2	1.7	2.4

* The anesthetics were administered only during phase 2.

† Significantly different from phase 1.

n = 6 for each group; mean ± SE.

± 0.8) when acetylcholine was added to the perfusate. The combined results for the addition of inhalational anesthetic showed a 58% decrease (Phase I *vs.* Phase II) in the hypoxic pressor response and a constrictor response to acetylcholine (3.6 ± 0.5 to 6.4 ± 0.5 cmH₂O).

STUDY II

Effect of Methylene Blue on the Action of Sin 1

The dose-response curve for Sin 1 in the presence and absence of methylene blue is shown in figure 2, and was analyzed by nonlinear regression analysis. In the presence of MB, the dose-response curve is shifted to the right by a factor of almost 2, so that the ED₅₀ for this group is 85 μ M, and for the saline group, 44 μ M. The final dose of Sin 1 was totally inhibitory and no pressor response was observed with the final hypoxic challenge.

STUDY III

Influence of Baseline Tone on the Hypoxic Pulmonary Pressor Response

Addition of U46619 increased the baseline pressure to 28.7 ± 1.9 cmH₂O, and in addition, the pressure tended to increase with time, to 30.1 ± 1.5 cmH₂O by the end of the study, but this was not statistically significant. The pressor response to hypoxia in Phase I was 28.9 ± 2.5

cmH₂O, and the addition of the inhalational anesthetic halothane caused a significant decrease in the pressor response to 11.0 ± 1.8 cmH₂O. After dissipation of the anesthetic, the pressor response to hypoxia increased to 18.8 ± 2.6 cmH₂O and 17.7 ± 2.4 cmH₂O (Phases III and IV, respectively), both of which were significantly less than that of Phase I.

Discussion

The present study has shown that in the presence of methylene blue the baseline pulmonary artery pressure is increased, the hypoxic pulmonary vasoconstrictor response is present and is potentiated with time, and the action of acetylcholine is converted from dilator to constrictor. Methylene blue inhibits the action of the enzyme guanylate cyclase and both the reversal of the normal vasodilator action of acetylcholine and the shift to the right of the Sin 1 dose-response curve are consistent with significant inactivation of soluble guanylate cyclase in the groups receiving MB. Inhalational anesthetics in equipotent concentrations of three different agents (halothane, enflurane, and isoflurane) decreased the hypoxic pulmonary pressor response approximately 60% both in the presence and absence of methylene blue. Finally, increased pulmonary vascular tone potentiated the initial pulmonary pressor responses to hypoxia, and these responses were not sustained with time. The small increase in lung water that we observed in the methylene blue groups is probably unimportant because the hypoxic pressor response is insensitive to moderate edema.¹³

Increased intracellular cyclic guanosine monophosphate (cGMP)⁹ is associated with relaxation of vascular smooth muscle. Increased cGMP concentration has been reported with agonists that are non-endothelial-dependent, such as nitroprusside, nitroglycerine, nitric oxide, and sodium nitrite, and with endothelium-dependent agents such as acetylcholine, methacholine, ADP, and the calcium ionophore, A23187.¹⁴ The latter group is believed to stimulate the synthesis or release of the relaxing factor from the endothelial cells (EDRF), which has now been suggested to be nitric oxide (NO).¹⁵ The nitrovasodilators and EDRF pass into the vascular smooth muscle and activate the soluble enzyme guanylate cyclase, the action of which is to convert guanosine triphosphate (GTP) to cGMP. The mechanism of the action of cGMP is still not completely known, but possibly it activates a cGMP-dependent kinase, which in turn phosphorylates cell proteins, resulting in vascular smooth muscle relaxation; a non-guanylate cyclase pathway for EDRF activity has not been established. Hemoglobin¹⁶ and MB¹⁷ both inhibit the action of EDRF, but by different mechanisms. The action of hemoglobin is presumably interstitial, as the hemoglobin molecule cannot enter the vascular

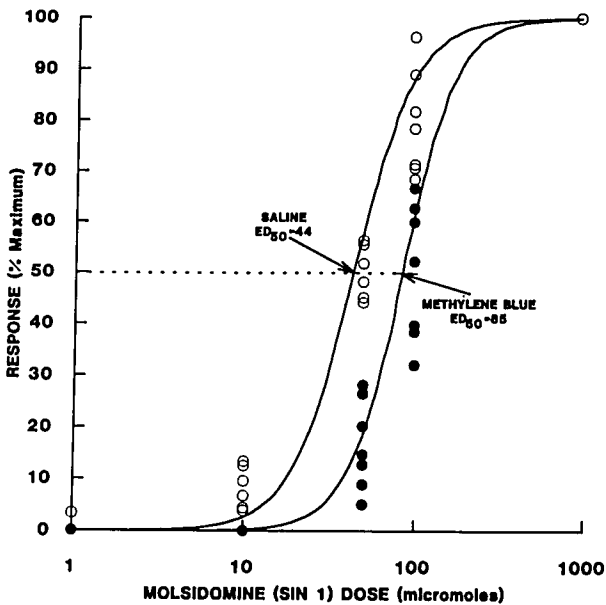


FIG. 2. The influence of methylene blue on the dilator effect of Sin 1. The open circles represent the effect of cumulative doses of Sin 1 on a hypoxic challenge in the presence of saline, the closed circles in the presence of methylene blue. ED₅₀ is the dose of Sin 1 that will cause a 50% decrease in a maximum hypoxic challenge.

smooth muscle cell. McMurtry *et al.*¹⁸ found that red blood cells (hemoglobin) prolonged the response to HPV, probably by reducing the availability of released EDRF. In contrast, MB combines with the heme-containing guanylate cyclase to prevent the activation of the enzyme by NO or EDRF, resulting in decreased synthesis of cGMP.¹⁶

In the pulmonary circulation, acetylcholine has both vasodilator and vasoconstrictor effects, and these actions have been shown to be tone-dependent.¹⁹ At low vascular tone, injection of acetylcholine causes pulmonary vasoconstriction, but, in the presence of increased pulmonary vascular tone, injections of acetylcholine stimulate the release of EDRF from endothelial cells, causing an increase of cGMP in the adjacent vascular smooth muscle cells, resulting in vasodilation. In the presence of methylene blue, although EDRF is released when acetylcholine is added, cGMP cannot increase and the vasoconstriction reported above is present even with pulmonary vascular tone¹⁷ increased by hypoxia.

The inhibition of the soluble form of the enzyme guanylate cyclase by MB was further shown in Study II. Sin 1, a vasoactive metabolite of molsidomine is a potent and direct stimulator of soluble guanylate cyclase. The dose-response curve for Sin 1 was shifted to the right in the presence of MB, indicating a competitive action between methylene blue and Sin 1 for the guanylate cyclase. Previous studies by Schmidt and Kukovetz²⁰ on bovine pulmonary arteries showed a 100-fold increase in the soluble guanylate cyclase activity when stimulated with Sin 1 and diminished activity when methylene blue was present. It also has been suggested by Martin *et al.*²¹ that methylene blue may interact directly with EDRF and prevent its vasodilatory action in vascular smooth muscle. They have suggested that MB may be a direct oxidant of and as such a direct inactivator of EDRF. This may account for the apparently more marked influence of methylene blue on the acetylcholine response compared with that of Sin 1.

In the present study, vascular tone was increased by hypoxia. The changes of response to acetylcholine and Sin 1 demonstrates that the dose of MB used had reduced the availability of guanylate cyclase and thus prevented EDRF from influencing vascular smooth muscle. Mazmanian *et al.*,²² in isolated perfused rat lungs, and Rodman *et al.*,²³ with isolated pulmonary vessels, showed a potentiation of HPV in the presence of MB, although they did not provide evidence as to whether the MB had inhibited EDRF. Brashers *et al.*²⁴ also demonstrated enhancement of HPV in isolated rat lungs after exposure to a variety of agents postulated to inhibit endothelial-derived relaxation, and this author used the response to cholinergic agonists to assess the effectiveness of this inhibition. These and the present study suggest that the fundamental mechanism of HPV is not dependent on changes in guanylate cyclase. Evidence that HPV is independent of en-

dothelial cells is supported by the demonstration by Murray *et al.*^{25,26} of reversible hypoxic constrictor activity in isolated pulmonary vascular smooth muscle cells. Further evidence that HPV is independent of endothelial cells was provided by Maxson *et al.*,²⁷ in isolated rat pulmonary arteries. In addition, Rodman *et al.*²⁸ and Johns *et al.*²⁹ found that the removal of the endothelium resulted in a decrease, not an abolition, of the hypoxic pressor response. The latter decreases are perhaps attributable to damage to the pulmonary vasculature rather than the change of EDRF, because 2 h after the pulmonary artery has been denuded by the usual mechanical means, transmission electron microscopy shows degenerative changes in the vascular smooth muscle³⁰ cells.

It has been observed in previous studies that a higher basal tone in the vessel allows a greater pressor response to hypoxia. To ensure that increased basal tone due to the presence of methylene blue was not the primary cause of the responsiveness with MB, the thromboxane mimetic U46619 was added to the lung to increase baseline pressure. The addition of U46619 caused a significant increase in the baseline pressure and greater initial responsiveness to hypoxia, but the response diminished with time, as did the control, unlike that of methylene blue groups, which increased with time. An explanation for this decreasing responsiveness with time in the absence of MB could be that, although endothelial-derived relaxing factor cannot accumulate,^{16,17} it may cause a progressive accumulation of cGMP in the vascular smooth muscle. The simplest explanation might be that there is a steady rate of metabolism of cGMP so that in the presence of MB, when no new cGMP is formed, the intracellular store is used up and the pulmonary artery baseline and hypoxic pressor response increase with time, while the reverse is true in the control and U46619 groups. The decrease in the responsiveness is not due to dilator effects of prostacyclin (PGI₃), as meclofenamate, the cyclooxygenase blocking agent, was present in the perfusate of all groups.

Blaise *et al.*,³ using coronary vessels with and without endothelium, demonstrated that inhibition of the constrictor responses to three different agonists involved different mechanisms of action. Thus, when prostaglandin F_{2 α} or serotonin were the agonists, the inhibition of the constriction by isoflurane observed when the endothelium was present was no longer observed when the endothelium was removed. This result suggests that the anesthetic action is exerted through the endothelium, via increasing release of or sensitivity to EDRF. In contrast, when the constriction was stimulated by phenylephrine, the inhibition by isoflurane was only partially decreased by removing the endothelium, suggesting that some of the mechanism of the anesthetic action lay with the vascular smooth muscle cell itself and was independent of endothelial effects. Muldoon *et al.*⁴ reported that halothane

caused constriction of canine femoral arteries and dilation of canine carotid arteries by a direct action on the vascular smooth muscle, because the effects were independent of the presence or absence of endothelium. When vasodilation was stimulated by exposing the endothelium-intact arteries to acetylcholine, Muldoon *et al.*⁴ reported that the magnitude of the vasodilation was inhibited by halothane.

In the present studies, the reduction of the hypoxic response in the presence of the inhalational anesthetics obscures the interpretation of changes in the vasodilator response to acetylcholine (in the absence of methylene blue). The small, and statistically not significant, reduction of the acetylcholine dilation reported here may represent inhibition of EDRF action, consistent with the conclusion of Blaise *et al.*³ and Muldoon *et al.*⁴ However, our observations of inhibition of both HPV and the constrictor effect of acetylcholine in the presence of methylene blue are consistent with a direct action of inhalation anesthetics on the pulmonary vascular smooth muscle cells. It is conceivable that the anesthetic effects might be exerted through reductions of endothelial-derived contracting factors, but the characteristics do not meet those of the best defined one, endothelin.³¹ In conclusion, HPV is not mediated by changes in guanylate cyclase, neither is the inhibitory action of inhalational anesthetics on HPV determined through changes in this enzyme. It follows, therefore, that EDRF maybe a modulator, but not a mediator, of HPV and that for pulmonary arteries the actions of the three inhalational anesthetics may be similar and are not primarily exerted through EDRF.

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References

1. Marshall C, Lindgren L, Marshall BE: Effects of halothane, enflurane and isoflurane on hypoxic pulmonary vasoconstriction in rat lungs *in vitro*. *ANESTHESIOLOGY* 60:304-308, 1984
2. Furchgott RF, Zawadzki JV: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288:373-376, 1980
3. Blaise G, Sille JG, Nugent M, Vandyke RA, Vanhoutte PM: Isoflurane causes endothelium-dependent inhibition of contractile responses of canine coronary arteries. *ANESTHESIOLOGY* 67: 513-517, 1987
4. Muldoon SM, Hart JL, Bowen KA, Freas W: Attenuation of endothelium-mediated vasodilation by halothane. *ANESTHESIOLOGY* 68:31-37, 1988
5. Furchgott RF: Role of endothelium in responses of vascular smooth muscle. *Circ Res* 53:557-573, 1983
6. Waldman SA, Murad F: Cyclic GMP synthesis and function. *Pharmacol Rev* 39:163-196, 1987
7. Griffith TM, Edwards DH, Lewis MJ, Henderson AH: Evidence that cyclic guanosine monophosphate (cGMP) mediates endothelium-dependent relaxation. *Eur J Pharmacol* 112:195-202, 1985
8. Murad F, Rapoport RM, Fiscus R: Role of cyclic-GMP in relaxations of vascular smooth muscle. *J Cardiovasc Pharmacol* 7: S111-S118, 1985
9. Ignarro LJ, Byrns RE, Wood KS: Endothelium-dependent modulation of cGMP levels and intrinsic smooth muscle tone in isolated bovine intrapulmonary artery and vein. *Circ Res* 60:82-92, 1987
10. Marshall C: A method for perfusing and ventilating rat lungs *in vitro*. *Methods Find Exp Clin Pharmacol* 6:281-285, 1984
11. Butler RA, Kelly AB, Zapp J: Determination of hydrocarbon anesthetic in blood by gas chromatography. *ANESTHESIOLOGY* 28:760-763, 1967
12. Snedecor GW, Cochran WG: *Statistical Methods*. Ames, Iowa State University Press, 1978
13. Wang CG, Hakim TS, Michel RP, Chang HK: Segmental pulmonary vascular resistance in progressive hydrostatic and permeability edema. *J Appl Physiol* 59:242-247, 1985
14. Griffith TM, Edwards DH, Lewis MJ, Henderson AH: Evidence that cyclic guanosine monophosphate (cGMP) mediates endothelium-dependent relaxation. *Eur J Pharmacol* 112:195-202, 1985
15. Moncada S, Herman AG, Vanhoutte PM: Endothelium-derived relaxing factor is identified as nitric oxide. *Trends Pharmacol Sci* 8:365-367, 1987
16. Martin W, Smith JA, Henderson AH, Furchgott RF: Blockade of endothelium-derived relaxing factor and inhibitory factor by hemoglobin. *Vasodilation: Vascular Smooth Muscle, Peptides, Autonomic Nerves and Endothelium*. Edited by Vanhoutte PM. New York, Raven Press, 1988, pp 443-446
17. Ignarro LJ, Byrns RE, Wood KS: Endothelium-dependent regulation of resting levels of cyclic GMP and cyclic AMP and tension in pulmonary arteries and veins. *Relaxing and Contracting Factors*. Edited by Vanhoutte PM. Clifton, The Humana Press, 1988, pp 309-345
18. McMurtry IF, Hookway BW, Roos SD: Red blood cells but not platelets prolong vascular reactivity of isolated rat lungs. *Am J Physiol* 234:H186-H191, 1978
19. Hyman AL, Kadowitz PJ: Tone-dependent responses to acetylcholine in the feline pulmonary vascular bed. *J Appl Physiol* 64:2002-2009, 1988
20. Schmidt K, Kukovetz, WR: Stimulation of soluble coronary arterial guanylate cyclase by Sin I. *Eur J Pharmacol* 122:75-79, 1986
21. Martin W, Villani GM, Jothianandan D, Furchgott RF: Selective blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation by hemoglobin and by methylene blue in the rabbit aorta. *J Pharmacol Exp Ther* 232:708-716, 1985
22. Mazmanian GM, Baudet B, Brink C, Cerrina, SK, Weiss M: Methylene blue potentiates vascular reactivity in isolated rat lungs. *J Appl Physiol* 66:1040-1045, 1989
23. Rodman DM, Yamaguchi T, O'Brien RF, McMurtry IF: Methylene blue enhances hypoxic contraction in isolated rat pulmonary arteries. *Chest* 93:935-945, 1988
24. Brashers VL, Peach MJ, Rose CE: Augmentation of hypoxic pulmonary vasoconstriction in the isolated perfused rat lung by *in vitro* antagonists of endothelium-dependent relaxation. *J Clin Invest* 82:1495-1502, 1988
25. Murray TR, Macarak EJ, Marshall C, Marshall BE: Contraction of isolated pulmonary artery smooth muscle cells in culture: A

- novel approach to the study of pulmonary artery tone (abstract). *ANESTHESIOLOGY* 67:A327, 1987
26. Murray TR, Macarak EJ, Marshall BE: Hypoxic pulmonary vasoconstriction in cell culture (abstract). *ANESTHESIOLOGY* 69: A19, 1988
 27. Maxson RE, Packer CS, Rhoades RA: Biphasic contractile response in rat pulmonary artery to hypoxia. *Am Rev Respir Dis* 139: A54, 1989
 28. Rodman DM, Yamaguchi T, O'Brien RF, McMurtry IF: Hypoxic contraction of isolated rat pulmonary artery. *J Pharmacol Exp Ther* 248:952-959, 1989
 29. Johns RA, Linden JM, Peach MJ: Endothelium-dependent relaxation and cyclic GMP accumulation in rabbit pulmonary artery are selectively impaired by moderate hypoxia. *Circ Res* 65:1508-1515, 1989
 30. Cosmi S, Dollahan N, Gardner R, Marshall C: A microscopic evaluation of endothelial cell removal techniques as applied to small intrapulmonary arteries (abstract). *Fed Am Soc Exp Biol J* 4: A909, 1990
 31. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 332:411-415, 1988