The Inhibitory Effect of Halothane on Mesenteric Venoconstriction and Related Reflex Responses during Acute Graded Hypoxia in Rabbits

Thomas A. Steckel, M.D.,* Masamune Tominaga, M.D.,† Zeljko J. Bosnjak, Ph.D.,‡ John P. Kampine, M.D., Ph.D.§

Systemic hypoxia is a common abnormality encountered frequently in the clinical setting that produces compensatory cardio-pulmonary changes affecting heart rate, blood pressure, peripheral vascular resistance, and respiratory drive. These changes are known to be inhibited or reversed by inhaled anesthetics. More recently, chemoreflex-mediated constriction of capacitance veins has been identified as a mechanism that contributes significantly to the hemodynamic adjustments during hypoxia. However, the effects of anesthetics on this response have not been clarified. The current study was designed to quantify sympathetically mediated mesenteric venoconstriction as well as heart rate and blood pressure responses to acute graded hypoxia; to identify the inhibitory effects of inhaled halothane on these responses; and to estimate the contribution of the peripheral chemoreceptors in mediating these changes. Changes in mesenteric vein diameter were measured in α-chloralose anesthetized rabbits in situ with simultaneous changes in heart rate and blood pressure during 40-s periods of 10%, 5%, 2.5%, and 0% inspired O2 administered sequentially before, during, and after 1% or 1.25% inhaled halothane. Sympathetic efferent nerve activity also was measured, and, in a separate group of animals, measurements were preceded by carotid chemoreceptor denervation. Hyoxia-mediated venoconstriction, bradycardia, and hypertension were attenuated almost equally by both 1% and 1.25% inhaled halothane (the higher dose produced only slightly greater inhibition). These responses were inhibited significantly in chemoreceptor-denervated animals, and the subsequent 1% inhaled halothane added only minimal additional attenuation. Increases in chemoreflex-mediated sympathetic efferent nerve activity also were reduced significantly by (1.25%) inhaled halothane. These results indicate that halothane impairs capacitance vein responses and other hemodynamic adjustments during hypoxia. Inhibition of these compensatory changes appears to be mediated, at least in part, via attenuation of peripheral chemoreflex responses and suppression of the resultant reflex increases in sympathetic efferent nerve activity. (Key words: Anesthesia; volatile halothane. Sympathetic nervous system; chemoreceptors; chemoreflex. Oxygen: hypoxia. Veins: capacitance vessels; mesenteric venoconstriction.)

RESPONSES TO HYPoxIA have been studied extensively because hypoxia is common to many disease processes, ranging from chronic conditions to acute events, any of which can be encountered in anesthetic practice. In addition to increased ventilatory drive, the physiologic responses to systemic hypoxia include a complex series of cardiovascular adjustments that result from the integration of reflex and direct effects.1 Chemoreflex-mediated bradycardia results from hypoxia during controlled ventilation, whereas hypoxia during spontaneous breathing causes hyperventilation and stimulates pulmonary stretch receptors, which decrease vagal tone and produce tachycardia; however, this is somewhat species specific.2-4 Hypoxia also causes selective local regional vasodilation, redistributing perfusion to coronary and cerebral tissue. Simultaneously, peripheral chemoreceptor activation causes a reflex increase in vascular resistance, thereby maintaining blood pressure and further redistributing blood flow by differential regional reflex vasoconstriction and vasodilation.5,6 In addition, central nervous system and chemoreceptor stimulation by hypoxia significantly reduces abdominal vascular capacitance by increasing sympathetic efferent nerve activity to the splanchic beds.7,8

During hypoxia, changes in capacitance may be as important in maintaining hemodynamic stability as those involving peripheral resistance or heart rate. Maximal constriction of splanchic capacitance vessels may increase effective blood volume by 5–7 ml/kg. This volume can increase central venous pressure by 2 mmHg and cardiac output by as much as 50%.9 Collectively, these cardiovascular responses during hypoxia constitute compensatory mechanisms for maintaining homeostasis.4,5 The administration of general anesthetics is known to interfere with many physiologic adjustments to hypoxia, including respiratory responses,9,10 heart rate and blood pressure changes,2,11,12 and alterations in peripheral vascular resistance.12,13 In extreme cases, this interference has led

* Clinical Instructor and Research Fellow, Department of Anesthesiology, Medical College of Wisconsin.
† Visiting Scientist, Department of Anesthesiology, Medical College of Wisconsin.
‡ Professor, Departments of Anesthesiology and Physiology, Medical College of Wisconsin.
§ Professor and Chairman, Department of Anesthesiology; Professor, Department of Physiology, Medical College of Wisconsin.

Received from the Departments of Anesthesiology and Physiology, Medical College of Wisconsin and the Zablocki Veterans Administration Medical Center, Milwaukee, Wisconsin. Accepted for publication May 19, 1992. Supported by Veterans Administration Medical Research Funds, United States Public Health Service grant HL 01901, and Anesthesiology Research Training Grant GM 08377. Presented in part at the Annual Meeting of the Federation of American Societies for Experimental Biology, Washington D.C., April 1990.

Address reprint requests to Dr. Steckel Medical College of Wisconsin, MFR, Room A1000, 8701 West Watertown Plank Road, Milwaukee, Wisconsin 53226.

to respiratory and circulatory arrest.\textsuperscript{13,14} However, the effects of anesthetics on hypoxia-mediated reflex changes in capacitance veins have not been clarified. Recently, we demonstrated an inhibitory effect of inhaled halothane on carotid sinus-mediated reflex mesenteric venoconstriction.\textsuperscript{15}

The objective of the current study was to use similar techniques to quantify simultaneous reflex changes in mesenteric vein diameter, heart rate, mean arterial pressure, and sympathetic efferent nerve activity during periods of acute graded hypoxia and to examine the inhibitory effects of inhaled halothane on these responses. In addition, the contribution of the peripheral chemoreceptors in mediating these responses before and during halothane administration was investigated.

Materials and Methods

EXPERIMENTAL PREPARATION

The preparation used in this study has been detailed in previous reports.\textsuperscript{15,16} After approval by the Animal Care Committee, a total of 40 New Zealand white rabbits (1–2 kg) were studied. After initial anesthetic induction with thiamylal (10–20 mg/kg) \textit{via} the ear vein, anesthesia was maintained with \textalpha-chloralose (12.5–37.5 mg/h). Surgical sites were infiltrated with a total of 3–5 ml 1% lidocaine.

After tracheotomy, one femoral artery and vein were cannulated for arterial pressure measurement and sampling and for continuous intravenous infusion, respectively. A midline laparotomy was performed in all animals. In eight rabbits in which sympathetic efferent nerve activity was to be recorded, a postganglionic splanchnic nerve was isolated in \textit{vivo}. Bipolar recording electrodes, composed of two single-strand coated stainless steel wires (0.25 mm OD) in Silastic tubing, were fixed to the nerve with Wagner-Sigel (Wacker-Chemie, Munich, Germany). In six other animals, the carotid arteries were dissected bilaterally, and a 30-s bilateral carotid occlusion was performed to produce the typical reflex tachycardia and hypertension.\textsuperscript{15,16} Subsequently, the entire carotid bifurcation area was denervated bilaterally with cautery and dissection. After this, bilateral carotid occlusion was repeated to demonstrate the absence of reflex tachycardia and hypertension, indicating effective elimination of the carotid sinus and, because of its close proximity, the carotid body as well. In earlier pilot studies, elimination of carotid sinus reflex responses, as described above, was associated with prevention of reflex bradycardia and hypertension in response to 40-s periods of hypoxia. This further supports the fact that denervation of the peripheral carotid sinus receptors by this technique was associated with denervation of peripheral chemoreceptors in this preparation.

In all rabbits studied, an approximately 13-cm loop of terminal ileum was externalized through a midline laparotomy and mounted in a temperature-regulated plastic tissue chamber that was placed on a movable microscope stage. The ileum and associated mesentery were superfused continuously with a physiologic salt solution originally formulated by Bohlen\textsuperscript{17} to simulate the environment of the peritoneal cavity. This solution was maintained at 37–38°C, and the \textit{pH} was kept between 7.35 and 7.45 by continuous slow bubbling with a gas mixture composed of 5% O\textsubscript{2}, 5% CO\textsubscript{2}, and 90% N\textsubscript{2}. The mesentery was pinned to a layer of clear Silastic rubber that coated the chamber floor, and short \textit{in situ} segments of 500–1,000 \textmu m mesenteric veins were cleared of excess fat tissue, when necessary, and used for diameter measurement.

Immediately after tracheotomy, ventilation was controlled with a Harvard animal respirator (model 655; Harvard Apparatus, South Natuk, MA). Vecuronium (0.1–0.2 mg/kg) was administered approximately every hour, as needed, to suppress spontaneous ventilation. Arterial blood was sampled periodically during the experiment to measure blood gases with a Model ABL I blood gas device (Radiometer Copenhagen, Copenhagen, Denmark). Normocarbia and normal \textit{pH} were maintained with ventilator adjustments and 1–2-meq boluses of NaHCO\textsubscript{3}. Additionally, a continuous baseline infusion of 0.5–1.5 meq/h NaHCO\textsubscript{3} was maintained to correct for the metabolic acidosis that typically occurs with the infusion of \textalpha-chloralose.\textsuperscript{18} The end-tidal CO\textsubscript{2} was monitored with a Perkin-Elmer medical gas analyzer mass spectrometer (model 1100; Perkin-Elmer Company, Norwalk, CT) and maintained between 30 and 40 mmHg, which was the approximate range observed for spontaneously breathing rabbits immediately after tracheotomy. Rectal temperature was measured intermittently with a thermistor probe and maintained between 36.5 and 37.5°C.

MEASUREMENTS

In each animal, arterial blood pressure was measured directly \textit{via} the femoral arterial cannula and heart rate was measured from the arterial pressure signal. An online videomicroimeter system was used to provide a continuous measurement of mesenteric vein diameter, and its calibration was verified at the beginning of each experiment. This system is capable of reproducibly measuring diameters ($r \geq 0.999$) as small as 200 \textmu m, and its use has been described in detail.\textsuperscript{15,16,19} When measured, sympathetic efferent nerve activity was recorded, with the bipolar electrodes described above, from a postganglionic splanchnic nerve, also as reported previously.\textsuperscript{15} All data were recorded on videocassette tapes with a Vetter digital videocassette recorder (model 820; A. R. Vetter Company, Rebersberg, PA) and printed subsequently on an
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Astro-Med eight-channel recorder (model 9500; Astro-Med, Inc., West Warwick, RI). Halothane concentrations in blood were measured with a Perkin-Elmer gas chromatography system (model Sigma 5B).

EXPERIMENTAL PROTOCOLS

Four groups of rabbits were studied. In each group, heart rate, mean arterial pressure, and mesenteric vein diameter were measured continuously. In the first group (n = 10), measurements were made continuously during sequentially administered 40-s periods of 10%, 5%, 2.5%, and 0% inspired O2, each separated by a 6-min reequilibration period at 21% inspired O2. During the reequilibration period, all measurements and arterial blood gas values tended to return to baseline values. When necessary, this period was extended to allow full recovery from the effects of hypoxia before the subsequent hypoxic episode was administered. Initial studies indicated that the order in which the levels of hypoxia were delivered did not affect the reflex responses. Accordingly, the same sequence was repeated after 45 min of 1% inhaled halothane, and finally it was repeated a third time after 45 min of a return to 0% inhaled halothane. In all cases, the carrier gas consisted of an O2-N2 mixture at a flow rate of 5 l/min. The nominal 1% and 0% inhaled halothane concentrations were confirmed by end-tidal mass spectrometry.

The second group (n = 17) was identical to the first group except that 1.25% halothane was administered. Also, in eight of these animals, sympathetic efferent nerve activity was measured continuously throughout the experiment, as described above, in addition to the other measurements. Both 1% and 1.25% inhaled halothane were used in the current study to allow examination of any possible dose-dependent inhibition of hypoxia-mediated responses. As such, they represented a relatively narrow concentration range. Nevertheless, pilot data indicated that, in the current preparation, inhaled halothane levels less than 1% had either inconsistent or very small effects on hypoxia-mediated reflexes, unlike baroreflex-mediated responses.\(^{15}\) Halothane concentrations greater than 1.25% frequently resulted in much greater hemodynamic depression than in the current study (mean arterial pressure < 20 mmHg) from which the animal was unable to recover.

The third group (n = 6) was identical to the first group (1% inhaled halothane); however, before measurements were obtained, rabbits in this group underwent carotid chemoreceptor and baroreceptor denervation, as described above, thus enabling an evaluation of locally and centrally mediated responses to hypoxia and the effects of halothane. In the current study, only a single dose of halothane was administered to any one animal studied. This was done to avoid any distortion of results due to residual halothane effects after the washout period.

The last group of animals studied (n = 7) served as a time control. Sequences of graded acute hypoxia was administered three times, with each sequence separated by a 45-min interval, as in the previous animals. However, no halothane was administered. This enabled the evaluation of any time-related decay or change in the preparation caused by factors independent of halothane inhalation. Subsequently, in one of the time control animals, after completion of the protocol, the vessel preparation was treated with 25 µg tetrodotoxin applied directly to the superfusate over the mesenteric vein being measured. Local application of tetrodotoxin, a blocker of neuronal conduction,\(^{29}\) enabled a comparison of hypoxia-mediated vein diameter change when local innervation was intact and then disrupted. Under both conditions (before and after local tetrodotoxin application), there was no systemic alteration in the animal other than the imposed hypoxia. The reflex responses to a 40-s period of 0% inspired O2, as described above, are shown in figure 1. The order in which the four experimental protocols were performed was not randomized specifically. However, additional studies, conducted after completion of the initial studies, indicated that the point in time at which a particular protocol was performed had no effect on the results.

Arterial blood could not be sampled each time hypoxia was administered to an animal because blood sampling from the arterial cannula interfered with heart rate, blood pressure, and mesenteric vein diameter measurements. Furthermore, repeated blood sampling would reduce hematocrit and total blood volume. Therefore, the same sequence of acute graded hypoxia was repeated at the end of each experiment, and arterial blood was sampled during each of the four levels of hypoxia to provide an estimate of the PaO2 and hemoglobin O2 saturation that existed at each level over the course of that experiment.

STATISTICS

Each time hypoxia was administered to an animal in the study, actual baseline measurements of heart rate, mean arterial pressure, mesenteric vein diameter, and sympathetic efferent nerve activity taken immediately before hypoxia were compared with corresponding measurements in response to hypoxia to determine whether the resultant changes were statistically significant. In each case, an analysis of variance with individual contrasts was used to compare the prehypoxia baseline measurement with the corresponding measurement during the imposed hypoxia. The mean of the prehypoxia measurements was considered to be statistically different from the mean of the measurements during hypoxia when the F values indicated significance at 95% confidence intervals. As illus-
trated in figure 1, reflex changes in heart rate, mean arterial pressure, mesenteric vein diameter, and sympathetic efferent nerve activity occurred transiently and at different times after the imposed period of hypoxia. For each individual measurement, the percentage change in reflex response to hypoxia was determined by comparing the measurement immediately before hypoxia with the peak change in the measurement that transiently occurred during or after the hypoxic period. Subsequently, the percentage change during hypoxia was calculated from the immediately preceding baseline for each measurement, and changes that were statistically significant were compared with each other.

In addition, for each group of rabbits studied, actual baseline (prehypoxia) measurements of heart rate, mean arterial pressure, mesenteric vein diameter, and sympathetic efferent nerve activity before halothane inhalation were compared with corresponding measurements during halothane administration. In the time control group, initial baseline measurements were compared with baseline measurements taken during the time that halothane would have been administered in the other groups. All data were analyzed by multiple analysis of variance for repeated measures with individual contrasts using the Super ANOVA statistical software produced by Abacus Corporation (Berkeley, CA) for Macintosh computers. Before analysis, arcsin transformations were performed on all percentage changes to ensure normal distribution of these values.

Results

Typical hypoxia-mediated changes in mesenteric vein diameter, heart rate, and sympathetic efferent nerve activity are illustrated in figures 1A and C. The application
of 25 μg tetrodotoxin directly to the superfusate over the mesenteric vein preparation abolished hypoxia-mediated reflex constriction without affecting mean arterial pressure or heart rate (i.e., systemic) responses to hypoxia (figs. 1A and B). A partial return of hypoxia-mediated venoconstriction was observed after 45 min of washout of the superfusate containing tetrodotoxin (this was not illustrated). During acute graded hypoxia in the absence of halothane, the magnitude of reflex mesenteric venoconstriction, bradycardia, hypertension, and increased sympathetic efferent nerve activity was proportional to the level of hypoxic stimulus (figs. 2-6, tables 1 and 2). Reflex changes resulting from 10% inspired O₂ were measurable but tended not to be statistically significant, whereas responses to 5%, 2.5%, and 0% inspired O₂ were all highly significant (figs. 2-6, tables 1 and 2). Average PₐO₂ and hemoglobin O₂ saturation values during each level of graded hypoxia are illustrated in figure 7. During the course of the time control experiment, little or no deterioration was observed in hypoxia-mediated venoconstriction or bradycardia (fig. 2, table 1). Reflex hypertension, however, did decrease after the first sequence of graded hypoxia, although it remained constant thereafter (table 2).

**Effects of 1% and 1.25% Halothane on Hypoxia-Mediated Responses**

Both 1% and 1.25% inhaled halothane significantly attenuated reflex mesenteric venoconstriction and bradycardia in response to graded hypoxia (figs. 3 and 4, table 1). At the low hypoxia levels (10% and 5%), reflex bradycardia was abolished entirely by both doses of halothane and actually reversed to tachycardia, although these resultant heart rate changes during halothane administration were not statistically significant (table 1). Reflex hypertension during administration of 2.5% and 0% inspired O₂ was attenuated significantly by 1.25% but not 1% inhaled halothane (table 2). Although 1.25% halothane tended to reduce most of the reflex responses to graded hypoxia more than 1% halothane, when they were compared directly, there were no significant differences between the two doses except at the 0% inspired O₂ level. In response to 0% inspired O₂, reflex mesenteric venoconstriction and mean arterial pressure increases during 1% halothane were 14.6% and 43.2%, respectively, compared with only 10.2% and 28.5%, respectively, during 1.25% halothane. Conversely, with 0% inspired O₂, the heart rate change during 1% halothane was not significant.

In both the 1% and 1.25% halothane studies, during the posthalothane condition, the changes in vein diameter, mean arterial pressure, and heart rate in response to graded hypoxia tended not to recover fully to initial levels (figs. 3B and 4B, table 2) despite very low blood halothane concentrations (table 3). Several of these studies were extended such that posthalothane responses were repeated for periods as long as an additional hour. However, no improvement in recovery was observed.

**Sympathetic Efferent Nerve Activity Studies**

Reflex increases in sympathetic efferent nerve activity at each level of hypoxia were attenuated significantly during inhalation of 1.25% halothane such that none of the increases was statistically significant (fig. 6). Unlike the other hypoxia-mediated responses, reflex increases in sympathetic efferent nerve activity after elimination of
0% inspired O_2 produced a significant reflex venoconstriction in denervated animals, and this was not changed by the administration of 1% halothane (fig. 5); it also was not different from the corresponding response in intact animals under the same conditions as illustrated in figure 3. Only 2.5% and 0% inspired O_2 produced a reflex bradycardia in denervated animals. During administration of 1% halothane, these changes were eliminated, and there were no significant hypoxia-mediated heart rate changes, as was the case during administration of 1% halothane in the intact animals (table 1). Mean arterial blood pressure in denervated animals decreased significantly in response to 5% and 2.5% inspired O_2. These changes and the smaller and not statistically significant hypotensive response during 0% O_2 were different from the hypertensive responses observed during corresponding levels of hypoxia returned to levels that were not different from those during the prehalothane condition. Representative recordings of halothane-induced inhibition of increased sympathetic efferent nerve activity, as well as diameter, heart rate, and arterial pressure responses during hypoxia, are illustrated in figures 1C and D.

**CAROTID DENERVATION STUDIES**

Reflex changes in mesenteric vein diameter and heart rate, in response to graded hypoxia in the prehalothane condition, were reduced significantly after carotid chemoreceptor denervation (fig. 5A, table 1). Similarly, hypoxia-mediated hypertension was observed in the intact animals, whereas either no significant change or hypotension occurred in denervated animals (table 2). Only 0% inspired O_2 produced a significant reflex venoconstriction in denervated animals, and this was not changed by the administration of 1% halothane (fig. 5); it also was not different from the corresponding response in intact animals under the same conditions as illustrated in figure 3. Only 2.5% and 0% inspired O_2 produced a reflex bradycardia in denervated animals. During administration of 1% halothane, these changes were eliminated, and there were no significant hypoxia-mediated heart rate changes, as was the case during administration of 1% halothane in the intact animals (table 1). Mean arterial blood pressure in denervated animals decreased significantly in response to 5% and 2.5% inspired O_2. These changes and the smaller and not statistically significant hypotensive response during 0% O_2 were different from the hypertensive responses observed during corresponding levels of hypoxia returned to levels that were not different from those during the prehalothane condition. Representative recordings of halothane-induced inhibition of increased sympathetic efferent nerve activity, as well as diameter, heart rate, and arterial pressure responses during hypoxia, are illustrated in figures 1C and D.

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Fig. 5. Hypoxia-mediated responses in carotid chemoreceptor denervated rabbits. A: Effect of graded hypoxia on mesenteric vein diameters before, during, and after 1% inhaled halothane. B: Attenuation of hypoxia-mediated mesenteric vasoconstriction during and after 1% inhaled halothane. Calculation of percentage change, columns, and symbols as in figures 2 and 3, n = 6.

oxia in intact animals (table 2). After 1% halothane administration in denervated animals, none of the mean arterial blood pressure responses to hypoxia was significant, in contrast to those in the intact animals, in which a diminished but significant hypertensive response to 2.5% and 0% O2 persisted (table 2). In denervated animals, as in the intact animals, hypoxia-mediated heart rate and blood pressure responses that were attenuated by halothane did not recover completely to initial levels during the posthalothane conditions (tables 1 and 2).

EFFECT OF HALOTHANE ON PREHYPOXIA BASELINE MEASUREMENTS

The effects of halothane alone on baseline measurements of mesenteric vein diameters, heart rate, and mean arterial pressure (i.e., measurements before each episode of hypoxia) are reported in table 4. The effects of halothane on baseline sympathetic efferent nerve activity are illustrated in figure 6C. In intact animals, neither 1% nor

Fig. 6. A: Increase in splanchnic sympathetic efferent nerve activity in response to sequentially administered graded hypoxia before, during, and after 1.25% inhaled halothane. B: Attenuation of hypoxia-mediated sympathetic efferent nerve activity increase during and after 1.25% inhaled halothane. C: Attenuation of pooled baseline (prehypoxia) measurements of sympathetic efferent nerve activity during 1.25% inhaled halothane. In A and B, calculation of percentage changes, columns, and symbols as in figure 5, n = 8. Columns in C represent mean ± SEM of pooled prehypoxia sympathetic efferent nerve activity measurements before and during 1.25% halothane. *<Prehalothane, n = 32.
TABLE 1. Percent Change in Heart Rate Responses to Acute Graded Hypoxia

<table>
<thead>
<tr>
<th>Protocol</th>
<th>1% Halothane (n = 9-10)</th>
<th>1.25% Halothane (n = 16-17)</th>
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<td></td>
<td>Prehalothane</td>
<td>1% Halothane</td>
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<tr>
<td>Prehypoxia heart rate (beats/min)</td>
<td>292 ± 4</td>
<td>273 ± 5</td>
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<tr>
<td>Inspired oxygen (%)</td>
<td>10</td>
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<td>5</td>
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<td>−66.3 ± 4.8</td>
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Protocol | Chemoreceptor Denervation (n = 5-6) | Time Control (n = 7)
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<td>241 ± 11</td>
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<tr>
<td>Inspired oxygen (%)</td>
<td>10</td>
<td>−4.0 ± 2.3 NS</td>
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</table>
|          | 5 | −1.9 ± 10 NS | −1.5 ± 2.8 NS | −10.0 ± 4.3 NS | −55.0 ± 7.1 | −45.0 ± 6.4 | −34.2 ± 6.0 *
|          | 2.5 | −25.5 ± 7.8 | −2.7 ± 2.8 NS | −15.7 ± 5.7 † | −63.5 ± 7.8 | −61.8 ± 4.9 | −58.8 ± 6.1 |
|          | 0 | −31.6 ± 4.9 | −5.0 ± 4.8 NS | −26.5 ± 6.8 † | −77.4 ± 5.2 | −75.5 ± 4.0 | −73.5 ± 2.6 |

Mean ± SEM percent changes in heart rate in response to sequential reductions in inspired oxygen for each protocol in the study. Prehypoxia heart rates are room air control heart rates (mean ± SEM) for which the percentage changes are described.

NS = not a statistically significant change (i.e., not different from the prehypoxia condition). All changes not designated NS are significant.

* Different from prehalothane (or initial), P ≤ 0.05.
† Different from prehalothane and 1% halothane, P ≤ 0.05.

1.25% inhaled halothane significantly dilated mesenteric capacitance veins. However, in carotid chemoreceptor denervated animals, 1% halothane did produce significant baseline venodilation. The baseline heart rate was reduced significantly by 1% halothane in both intact and denervated animals; however, the reduction during administration of 1.25% halothane, administered to intact animals, was not significant. The baseline mean arterial pressure was reduced significantly in all groups of animals during halothane administration.

TABLE 2. Mean Arterial Blood Pressure Responses to Acute Graded Hypoxia

<table>
<thead>
<tr>
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<th>1.25% Halothane (n = 16-17)</th>
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<td>Prehalothane</td>
<td>1% Halothane</td>
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<tr>
<td>Prehypoxia MAP (mmHg)</td>
<td>66 ± 1</td>
<td>39 ± 1</td>
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<tr>
<td>Inspired oxygen (%)</td>
<td>10</td>
<td>1.6 ± 2.0 NS</td>
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Protocol | Chemoreceptor Denervation (n = 5-6) | Time Control (n = 7)
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<td>1% Halothane</td>
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<td>Prehypoxia MAP (mmHg)</td>
<td>76 ± 5</td>
<td>32 ± 3</td>
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<tr>
<td>Inspired oxygen (%)</td>
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Mean ± SEM percent changes in mean arterial blood pressure in response to sequential reductions in inspired oxygen for each protocol in the study.

MAP = mean arterial blood pressure. NS = not statistically significant change (i.e., not different from the baseline prehypoxia condition). All changes not designated NS are significant.

* Different from prehalothane (or initial), P ≤ 0.05.
† Different from 1% halothane, P ≤ 0.05.
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![Graph showing changes in PaO2 and O2 SAT vs % inspired oxygen](image)

Fig. 7. Changes in PaO₂ (left ordinate, solid line) and arterial blood oxygen saturation (right ordinate, dashed line) as a function of acute graded hypoxia. Each point represents mean ± SEM, n = 40.

**Discussion**

In the current study, hypoxia-mediated changes in heart rate, blood pressure, mesenteric vein diameter, and sympathetic efferent nerve activity were proportional to the level of hypoxic stimulus. Both 1% and 1.25% inhaled halothane equally attenuated hypoxia-mediated responses, except at the most severe O₂ level (0% inspired) (mean PaO₂ = 17.1 ± 0.58). Recovery from the attenuation after elimination of halothane tended to be incomplete. However, during time control studies, in the absence of halothane, the magnitude of heart rate, mesenteric vein diameter, and (after a slight initial reduction) blood pressure responses to graded hypoxia was preserved over the course of the experiment. This indicates that a deterioration of the preparation with time probably was not the cause of incomplete recovery after halothane administration. After carotid chemoreceptor and baroreceptor denervation, the hypoxia-mediated heart rate, blood pressure, and mesenteric vein diameter changes that had been observed in intact animals were reduced significantly or reversed. Only minimal additional attenuation of these responses resulted in denervated animals after administration of 1% inhaled halothane. In denervated animals, 1% inhaled halothane caused significant (prehypoxia) baseline mesenteric venodilation, whereas neither 1% nor 1.25% inhaled halothane significantly dilated mesenteric veins in intact animals. Baseline arterial pressure, however, was reduced in all rabbits that received halothane.

All of the responses to acute graded hypoxia were measured in rabbits that were anesthetized with α-chloralose. Therefore, all halothane-mediated changes in the responses were superimposed on the basal level of α-chloralose anesthesia. Nevertheless, although α-chloralose was found to depress chemoreflex-mediated cardiovascular responses slightly, the degree of depression was much less than that with halothane or barbiturates. Furthermore, in other reports, α-chloralose has provided adequate anesthesia for acute studies, while preserving or exaggerating cardiopulmonary reflexes. In addition, all hypoxia-mediated responses that were compared in the current study, including control responses, were measured during baseline α-chloralose anesthesia. Thus, any changes in these responses resulting from halothane administration or other interventions were considered to be independent of baseline anesthetic effects.

The hypoxia-mediated responses of bradycardia, hypertension, and mesenteric vasoconstriction observed in the current study are in agreement with previous observations. Collectively, these responses represent an integration of adjustments that result from activation of the peripheral chemoreflex mechanism, and direct effects of hypoxia on peripheral tissues and the central nervous system. The relative contribution of peripheral chemoreceptor activation to these responses, before and during halothane administration, can be examined in the current study by comparison of carotid chemoreceptor denervated animals with intact animals. Ablation of carotid bodies eliminates chemoreflex activity in rabbits because they have no other functional peripheral chemoreceptors.

In the current study, carotid body denervation was associated with elimination of the baroreceptors in the carotid sinus as well. Although baroreceptor and chemoreceptor reflexes are known to interact, this should not have altered the results in this study. Withdrawal of baroreceptor activation (i.e., decreasing carotid sinus pressure) enhances chemoreceptor responses; conversely, increased carotid sinus pressure attenuates chemoreflex responses. Therefore, elimination of baroreceptor activity would be expected to enhance, not reduce, any residual chemoreceptor activity. This was not observed in the current study.

Vascular beds dilate in response to hypoxia; these changes are offset by peripheral chemoreflex activation, resulting in splanchnic vasoconstriction and reduced abdominal vascular capacitance, which redistribute blood flow and support arterial pressure, presumably via increased sympathetic efferent nerve activity. The results of the current study were consistent with such a mechanism. When measured, postganglionic splanchnic sympathetic efferent nerve activity increased simultaneously with systemic hypertension and mesenteric vasoconstrictive-
TABLE 4. Effect of Halothane on Baseline (prehypoxia) Measurements

<table>
<thead>
<tr>
<th>Protocol</th>
<th>1% Halothane (n = 59)</th>
<th>1.25% Halothane (n = 67-68)</th>
<th>Chemoreceptor Denervation (n = 21-24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condition</td>
<td>Prehalothane</td>
<td>1% Halothane</td>
<td>Prehalothane</td>
</tr>
<tr>
<td>Mesenteric vein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>diameter (mm)</td>
<td>683 ± 15</td>
<td>686 ± 11</td>
<td>739 ± 16.5</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>292 ± 4.0</td>
<td>273 ± 11*</td>
<td>265 ± 4.2</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td>66.3 ± 1.3</td>
<td>38.8 ± 1.2*</td>
<td>73.8 ± 1.3</td>
</tr>
</tbody>
</table>

Mean ± SEM of baseline (prehypoxia) measurements taken before and during inhaled halothane.

* Different from corresponding prehalothane mean, P ≤ 0.05.

Also, locally applied tetrodotoxin blocked neurally mediated constriction of one mesenteric vein in response to hypoxia, without altering systemic reflex hypertension or, presumably, constriction in other mesenteric veins. Furthermore, elimination of the chemoreflex response in the denervation study abolished this sympathetically mediated mesenteric vеноconstriction, except at 0% inspired O2. Simultaneously, reflex hypertension either was abolished or reversed to hypotension. That may have been the result of residual bradycardia coupled with unopposed hypoxia-mediated local vasodilation.

Similar to the hypoxia-mediated peripheral vascular effects, several mechanisms interact to determine heart rate changes during hypoxia, and there are substantial species differences. Typically, during controlled ventilation there is a predominant chemoreflex-mediated vagal bradycardia. During spontaneous ventilation in response to hypoxia, hyperventilation and pulmonary reflexes produce tachycardia as a result of reduced vagal tone and possibly via a sympathetic activating mechanism as well. In addition, a hypoxia-mediated vagal tachycardic mechanism that is independent of pulmonary reflexes and subordinate to the vagal bradycardia mechanism also has been reported.

In the isolated heart model, in the absence of exogenous neural and humoral control mechanisms, the direct effects of hypoxia are depressive and result in significant bradycardia. In the current study, ventilation was controlled, and increasing levels of acute hypoxia produced graded bradycardia in intact animals that was consistent with the chemoreflex mechanism described above. After carotid body and sinus denervation, hypoxia-mediated bradycardia was eliminated except for residual small significant responses at the lower 2.5% and 0% O2 levels. These residual responses may have represented the direct depression of the myocardium by hypoxia, as described by Marshall.

Hypoxia-mediated cardiovascular and respiratory responses are known to be inhibited by halothane administration. In the current study, halothane not only attenuated hypoxia-mediated hypertension and bradycardia, but also significantly reduced increases in sympathetic efferent nerve activity and reflex mesenteric vеноconstriction. Inhibition of these responses is similar to inhibition of carotid sinus–mediated responses, which was reported previously. In addition, most of the hypoxia-mediated responses that had been attenuated by halothane in intact animals were abolished before halothane administration in the denervated animals. These observations suggest that the inhibition of circulatory adjustments to hypoxia during halothane is mediated, at least in part, through an interruption of the chemoreflex-induced sympathetic and vagal response. The reduction in arterial pressure during 1% and 1.25% halothane illustrated in table 4 is less than the autoregulation range (60–140 mmHg in rabbits). As such, an inhibition of sympathetic outflow on the basis of pressure-passive hypoperfusion of autonomic regulatory centers cannot be excluded.

With the available data in our study, it cannot be established whether halothane interferes with other mechanisms contributing to these circulatory adjustments to hypoxia, such as local tissue responses or central pressor effects. It is possible that, in the current study, the residual hypoxia-mediated blood pressure and heart rate changes that persisted after carotid body denervation and were inhibited subsequently by 1% halothane may have been the result of local tissue effects of hypoxia. Similarly, in the same animals, the residual mesenteric vеноconstriction during 0% inspired O2, which was not affected by halothane, may have represented a central response to severe hypoxia.

The current study was not designed to identify the specific locations in the chemoreflex mechanism that were inhibited by halothane. Previous studies of hypoxia-mediated ventilatory and carotid body afferent responses have identified the carotid bodies themselves as one site of inhibition by halothane. Other data have suggested that halothane disrupts chemoreflex-mediated bradycardia in the diencephalic region. Furthermore, multiple sites of inhibition by halothane along the baroreflex path-
way have been identified. With the current data available, it is reasonable to consider the same to be true for the chemoreflex.

Cardiovascular dose-responses of anesthetics tend to be dependent on dose, based on different levels of minimum alveolar concentration. In the current study, the lack of difference between 1% and 1.25% halothane, except at the most severe O$_2$ levels (2.5 and 0% inspired), is consistent with the fact that these two doses represent minimum alveolar concentration fractions of only 0.7 and 0.9, respectively, for halothane in rabbits. As mentioned previously, pilot data indicated that these halothane concentrations represented the most practical dose range to study using the current preparation. Furthermore, although 1.25% inhaled halothane resulted in a greater blood halothane concentration than 1% halothane, the two concentrations were not statistically different from each other.

Most of the responses in the current study that were inhibited by halothane did not recover completely after halothane concentrations in blood returned to nearly 0. In addition, little or no change in the same responses occurred in the time control studies, indicating that there was no significant deterioration in the preparation itself, in the absence of halothane, over the time course of the experiment. These observations suggest that some residual inhibitory effect of halothane persisted after its initial elimination from the circulation. As stated in the Results, no improvement in recovery was observed when the post-halothane period was extended in several of these experiments. Nevertheless, the value of extending these studies for longer periods is questionable, given the invasive nature of the in situ preparation and the likelihood that the animal condition eventually will deteriorate. The fact that sympathetic efferent nerve activity responses recovered completely after the elimination of halothane suggests that the persistent inhibition may affect the cardiovascular tissues themselves rather than the (neural) chemoreflex control mechanisms. This is supported further by the observation of similar prolonged inhibition of aortic and coronary vascular smooth muscle responses by halothane in vitro.

In the current study, neither 1% nor 1.25% halothane produced a significant baseline (prehypoxia) mesenteric venodilation, although a tendency was present at both doses. These observations are similar to halothane effects on baseline diameter in a previous study of baroreflex mechanisms. Additional venodilation may have been offset by mechanisms compensating for the significant baseline arterial pressure decrease that resulted from both halothane doses, although baseline sympathetic efferent nerve activity also was reduced by 1.25% halothane. In the denervated animals in this study, baseline mesenteric vein diameters were increased significantly by 1% halothane. This may have been the result of an attenuation by the halothane of increased resting sympathetic tone to these vessels that resulted from ablation of the carotid bodies and carotid sinus baroreceptor denervation.

In summary, in this study inhaled halothane inhibited chemoreflex-mediated constriction of mesenteric capacitance veins as well as reflex bradycardia and hypertension resulting from acute graded hypoxia. Other systemic responses to hypoxia also may have been inhibited by halothane. These observations provide additional evidence that halothane may affect hemodynamic stability significantly during hypoxic episodes that still are encountered commonly in the clinical setting.

The authors thank Mimi Mick, Suzanne Emmrich, Mary Ziebell, and Edith Sulzer for their assistance in preparing the manuscript.

References

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