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Halothane Impairs the Hemodynamic Influence of Endothelium-derived Nitric Oxide

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Background: **The endogenous vasodilator endothelium-derived nitric oxide (EDNO) contributes to the regulation of vascular tone and organ perfusion. It has been suggested that some volatile anesthetics may diminish the influence of EDNO and thereby decrease regional blood flow.**

Methods: **Radioactive microspheres were used to determine regional hemodynamics in rats. The authors tested the hypothesis that halothane inhibits EDNO and, therefore, should diminish the response to nitric oxide synthesis inhibition by N^W-nitro-L-arginine methyl ester (L-NAME) compared with either conscious or barbiturate-anesthetized rats.**

Results: **N^W-nitro-L-arginine methyl ester decreased blood flow to the brain by 23% ($P < 0.005$) in conscious rats to a level similar to that seen with either anesthetic agent. In both conscious and barbiturate-anesthetized rats, L-NAME increased blood pressure (BP) by 24 ± 2 ($P < 0.001$) and 20 ± 1 ($P < 0.001$) mmHg and total peripheral resistance (TPR) by 132% ($P < 0.001$) and 105% ($P < 0.001$), respectively. In contrast, during halothane anesthesia, both the pressor response (only 7 ± 1 mmHg) and the increase in TPR (only 22%) were greatly diminished ($P < 0.001$). N^W-nitro-L-arginine methyl ester decreased cardiac output (CO) by 47% ($P < 0.001$) and heart rate (HR) by 28% ($P < 0.001$) in conscious rats. In barbiturate-anesthetized rats, L-NAME decreased CO by 38% ($P < 0.005$) and HR by 13% ($P < 0.001$). In halothane-anesthetized rats, L-NAME changed neither CO nor HR. Thus halothane anesthesia largely eliminated the systemic response to EDNO synthesis inhibition. In conscious rats, L-NAME decreased blood flow to the heart (30%) and kidneys (47%). In barbiturate-anesthetized rats, L-NAME did not alter blood flow to the heart but decreased renal blood flow by 35% ($P < 0.005$). In halothane-anesthetized rats, L-NAME did not alter blood flow to either the heart or the kidneys. Overall, halothane blunted or blocked the systemic and regional hemodynamic responses to EDNO synthesis inhibition seen in conscious and barbiturate-anesthetized rats.**

Conclusions: **Halothane anesthesia greatly diminished or eliminated all systemic and regional hemodynamic responses to L-NAME. These data indicate that halothane anesthesia inhibits EDNO-mediated regulation of systemic and organ hemodynamics. (Key words: Anesthetics, volatile: barbiturate; halothane. Endothelium: endothelium-derived nitric oxide. Hemodynamics: blood flow; vascular resistance.)**

ALTHOUGH the endothelium produces a number of vasoactive factors, the potent vasodilator endothelium-derived nitric oxide (EDNO) appears to have a major influence on vascular resistance and tone.[1,2] In the endothelium, EDNO is derived from L-arginine and exerts its action in the underlying smooth muscle cells through activation of soluble guanylate cyclase,[1,3,4] resulting in generation of the "second messenger" cyclic 3-5′-guanosine monophosphate (cGMP).[3] Release of EDNO is reportedly regulated by flow-induced shear stress,[5,6] as well as by certain vasoactive factors, including acetylcholine, bradykinin, and histamine.[7,8] The importance of basal release of EDNO in maintaining normal organ perfusion has been demonstrated by a number of investigators. Inhibition of EDNO synthesis with L-arginine analog antagonists, such as N^W-nitro-L-arginine methyl ester (L-NAME) or N^G-monomethyl-L-arginine (L-NMMA), has been shown to increase blood pressure (BP), decrease blood flow, and increase vascular resistance in a number of vascular beds, including the renal, mesenteric, cerebral, and gastric circulations.[9–12]

Halothane, a volatile anesthetic, has a variety of systemic and regional hemodynamic effects. It decreases BP by decreasing cardiac output, but has little effect on systemic vascular resistance.[13–15] Halothane also alters basal blood flow to a variety of vascular beds; it increases cerebral blood flow while decreasing blood flow to such organs as the heart, kidney, liver, and muscle.[13,16,17] Using *in vitro* models, a number of investigators found that volatile anesthetics, including halo-

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thane, enflurane, and isoflurane, attenuate endothelium-dependent vasodilation. This can be reversed by either removing the anesthetic[18,19] or pretreating with the free radical scavenger superoxide dismutase.[19] However, *in vivo* data on the interaction between volatile anesthetics and EDNO are less clear. Wang *et al.*[14] reported that, in spontaneously breathing halothane-anesthetized rats, inhibiting EDNO synthesis had no effect on BP, indicating that the systemic influence of EDNO has been blocked by the anesthetic agent. However, another report in rats anesthetized with either urethane or enflurane found that L-NMMA caused a marked pressor response, indicating that halothane, but not urethane or enflurane, interferes with the influence of EDNO.[14] In contrast, Greenblatt *et al.*[15] reported that, in rats in which the lungs are mechanically ventilated, halothane or isoflurane had little effect on either the systemic or the regional hemodynamic response to EDNO synthesis inhibition. Therefore, the current study was designed to test the hypothesis that, in spontaneously breathing rats, contrary to barbiturate anesthesia, halothane interrupts the influence of EDNO. If we are correct, halothane should diminish both the systemic and the local hemodynamic responses to inhibition of EDNO synthesis when compared with the response to L-NAME in either conscious or barbiturate-anesthetized rats.

Materials and Methods

Male Sprague-Dawley rats (Charles River Laboratory, Wilmington, MA) weighing 225–325 g were used in all experiments. Rats fasted overnight but were allowed free access to water. Cardiac output and regional blood flow were determined using radiolabeled microspheres.

In all experiments, a PE 10 catheter (Fisher Scientific, Chicago, IL) was passed through the right common carotid artery into the left ventricle for infusion of microspheres. The catheter tip was adjusted until pulse pressure in the left ventricle could be read without artifacts. In addition, the right femoral vein and artery were catheterized with PE 50 tubing; the venous catheter was used for constant infusion of saline (40 μl/min) in anesthetized rats and for drug infusion and blood replacement in all rats. The arterial catheter was used to monitor BP and for reference blood sampling. Blood pressure and heart rate (HR) were monitored using a Statham pressure transducer (Viggo-Spectramed, Oxnard, CA) connected to a Gould chart recorder (Gould, Valley View, OH). Heart rate in beats per minute (beats/min) was derived electronically from the arterial pressure wave.

The effect of EDNO synthesis inhibition on systemic and regional hemodynamics was measured using paired injections of radioactive microspheres (DuPont-New England Nuclear, Boston, MA), 15 ± 1.5 μm in diameter, labeled with either Ce^{141} or Sr^{85}, as described previously.[20] Because of rats' severe hypotensive reaction to the commercial dextran vehicle,[21] microspheres were suspended in 3.5 M glucose using 0.01% Tween-80 as an antiaggregant. We have found that this concentration has no effect on systemic pressure. Microspheres at a concentration of 400,000/ml were agitated mechanically for approximately 15 min. Then 0.2 ml of the suspension, corresponding to about 80,000 microspheres, was withdrawn into a syringe that was counted to obtain the preinjection dose. The microspheres, together with 0.2 ml saline, were infused into the left ventricle over 20 s; at the same time, arterial blood was withdrawn at a rate of 0.48 ml/min over 75 s. The sampled blood was replaced with blood from a donor rat nephrectomized 16–24 h earlier. After microsphere injection, the syringe was again counted to obtain the postinjection dose. The injection count was obtained by subtracting the preinjection from the postinjection dose.

The first injection of microspheres was given under stable control conditions. Fifteen minutes after EDNO synthesis inhibition, the second set of microspheres was injected. At the conclusion of the experiment, the animals were killed with an overdose of pentobarbital sodium (Nembutal, Abbott Laboratories, Chicago, IL, 150 mg/kg intravenously) and the target tissues excised for analysis. Samples were counted in a Packard gamma counter using dual window settings of 10–250 and 400–700 MeV at a sample level of 0.5 cm.

A bolus dose of 10 mg/kg body weight (bw) of L-NAME (Sigma, St Louis, MO) was used to inhibit EDNO synthesis. We have previously shown that this dose induces sustained EDNO synthesis inhibition in the systemic and renal vasculature.[22] We used the response to L-NAME as an index of the degree of participation of endogenous EDNO in regulating systemic and regional hemodynamics. All experimental protocols were approved by the Institutional Animal Care and Use Committee and are consistent with the United States Public Health Services Guidelines on Care and Use of Laboratory Animals.

In conscious rats, the catheters were implanted the day before as described above, using diethyl ether

anesthesia and antiseptic technique. The catheters were led subcutaneously and exteriorized at the nape of the neck. After fasting overnight, rats were isolated in restraining cages for 60 min, during which BP and HR were monitored. A separate group of rats were instrumented and blood *p*H, p_{CO_2}, and p_{O_2} were measured before and after L-NAME.

In the barbiturate-anesthesia group, rats fasted overnight were anesthetized by an intraperitoneal injection of 125 mg/kg bw thiobutabarbital (Inactin, Andrew Lockwood Co., Milwaukee, WI) and placed on a heating pad to maintain constant body temperature. Rats were instrumented as described above and allowed a 60-min stabilization period, during which BP and HR were monitored. A separate group of rats were instrumented and blood *p*H, p_{CO_2}, and p_{O_2} were measured before and after L-NAME. All rats spontaneously breathed room air without the aid of mechanical ventilation.

In halothane-anesthetized rats, the catheters were implanted the day before as described above and exteriorized at the nape of the neck. After fasting overnight, halothane was administered in an air-tight, 5-l Plexiglas chamber using a calibrated vaporizer which delivered 1% halothane with 21% O_2 at a gas flow of 4 l. After anesthesia, rats were allowed a 60-min stabilization period, during which BP and HR were monitored. A separate group of rats were instrumented and blood pH, p_{CO_2}, and p_{O_2} were measured before and after L-NAME. All experiments were performed on spontaneously breathing rats.

Analysis

Cardiac output (CO; $ml \cdot min^{-1} \cdot 100^{-1} \cdot g$ bw), organ blood flow ($ml \cdot min^{-1} \cdot g$ tissue $weight^{-1}$), total peripheral resistance (TPR; $mmHg \cdot ml^{-1} \cdot min^{-1} \cdot 100^{-1} g$ bw, referred to as resistance units or RU), and organ vascular resistance ($mmHg \cdot ml^{-1} \cdot min^{-1} \cdot g$ tissue $weight^{-1}$, referred to as resistance units or RU) were determined from the measured counts per minute (CPM) as follows: 1) CO = cpm injected × pump speed · cpm blood per 100 $g\ bw^{-1}$, 2) organ blood flow = cpm organ × pump speed/(cpm blood × tissue weight $[g])^{-1}$, 3) TPR = mean BP/CO, and 4) organ vascular resistance = mean BP/organ blood flow. The right and left kidneys were analyzed separately to establish a control for the technique. To accept the results of the experiment, blood flow in each kidney had to be within 10% of the mean calculated value of both kidneys.

Data were evaluated in several statistical ways. To assess the influence of L-NAME, Student's paired *t* test was run on the changes resulting from treatment; $P < 0.05$ was considered significant. Comparisons among the three groups (conscious and barbiturate- and halothane-anesthetized) were analyzed using ANOVA and the Bonferroni's *t* test; an adjusted *P* value of $P < 0.05$ was considered significant.

Results

Effect of EDNO Synthesis Inhibition on Systemic and Regional Hemodynamic and Blood Gases in Conscious Rats

Systemic Hemodynamics. Changes in BP, HR, CO, and TPR in conscious rats in response to L-NAME are

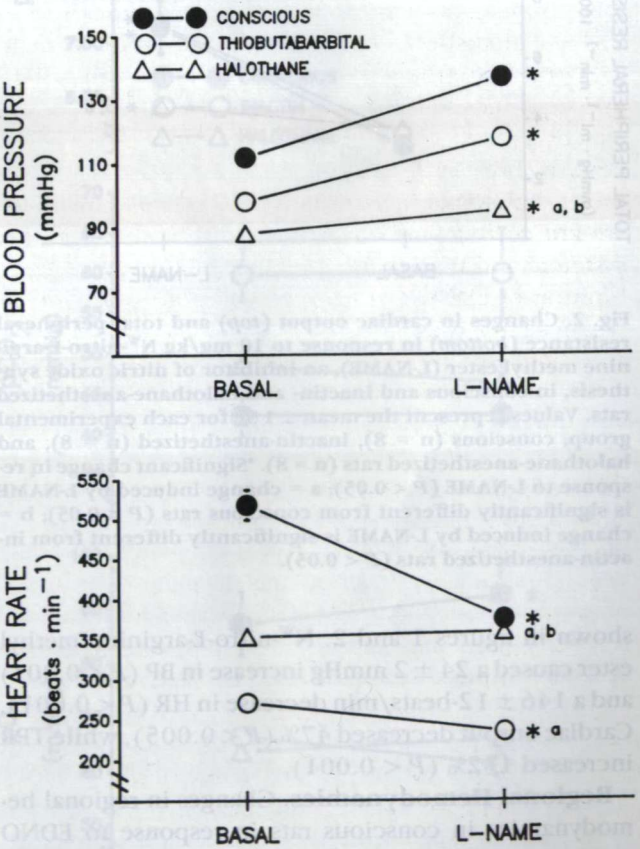

Fig. 1. Changes in BP (*top*) and HR (*bottom*) in response to 10 mg/kg N^{ω}-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthesis, in conscious rats and inactin- and halothane-anesthetized rats. Values represent the mean ± 1 SE for each experimental group; conscious (n = 8), inactin-anesthetized (n = 8), and halothane-anesthetized rats (n = 8). *Significant change in response to L-NAME ($P < 0.05$); a = change induced by L-NAME is significantly different from conscious rats ($P < 0.05$); b = change induced by L-NAME is significantly different from inactin-anesthetized rats ($P < 0.05$).

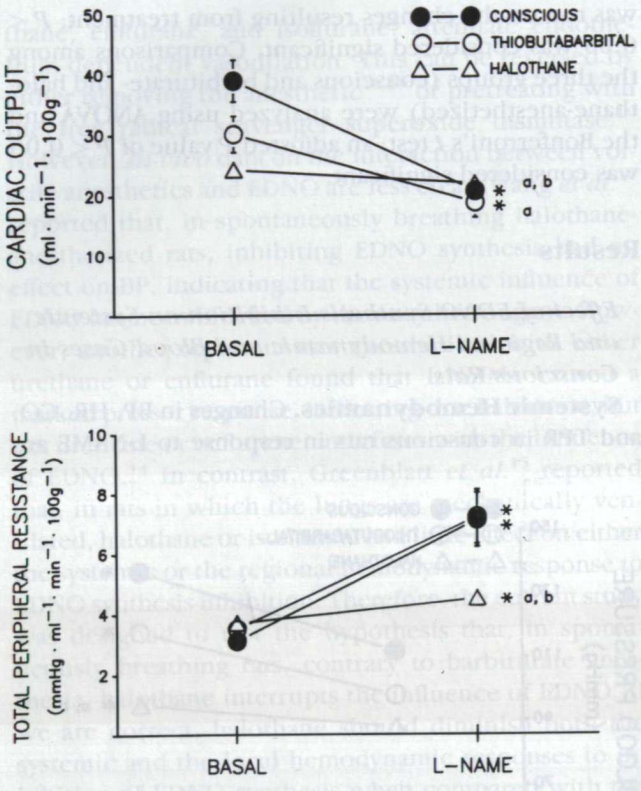

Fig. 2. Changes in cardiac output (*top*) and total peripheral resistance (*bottom*) in response to 10 mg/kg N^W-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthesis, in conscious and inactin- and halothane-anesthetized rats. Values represent the mean ± 1 SE for each experimental group; conscious (n = 8), inactin-anesthetized (n = 8), and halothane-anesthetized rats (n = 8). *Significant change in response to L-NAME ($P < 0.05$); a = change induced by L-NAME is significantly different from conscious rats ($P < 0.05$); b = change induced by L-NAME is significantly different from inactin-anesthetized rats ($P < 0.05$).

shown in figures 1 and 2. N^W-nitro-L-arginine methyl ester caused a 24 ± 2 mmHg increase in BP ($P < 0.001$) and a 146 ± 12-beats/min decrease in HR ($P < 0.001$). Cardiac output decreased 47% ($P < 0.005$), while TPR increased 132% ($P < 0.001$).

Regional Hemodynamics. Changes in regional hemodynamics in conscious rats in response to EDNO synthesis inhibition are shown in figure 3.

Basal cerebral blood flow was 1.2 ± 0.10 ml · min^{-1} · g tissue $weight^{-1}$ and vascular resistance was 96 ± 9 RU. N^W-nitro-L-arginine methyl ester decreased blood flow by 23% ($P < 0.005$) and increased vascular resistance by 67% ($P < 0.02$).

Basal cardiac blood flow was 5.7 ± 0.6 ml · min^{-1} · g tissue $weight^{-1}$, while vascular resistance was 22 ± 3 RU. N^W-nitro-L-arginine methyl ester decreased blood flow by 30% ($P < 0.02$) and increased vascular resistance by 95% ($P < 0.001$).

Basal hepatic arterial blood flow was 0.4 ± 0.1 ml · min^{-1} · g tissue $weight^{-1}$, while vascular resistance was 391 ± 59 RU. N^W-nitro-L-arginine methyl ester had no significant effect on either blood flow or vascular resistance.

Basal renal blood flow was 8.4 ± 0.9 ml · min^{-1} · g tissue $weight^{-1}$, while vascular resistance was 15 ± 2 RU. N^W-nitro-L-arginine methyl ester decreased blood flow by 47% ($P < 0.001$) and increased vascular resistance by 145% ($P < 0.001$).

Basal mesenteric blood flow was 2 ± 0.2 ml · min^{-1} · g tissue $weight^{-1}$, while vascular resistance was 60 ± 6 RU. N^W-nitro-L-arginine methyl ester decreased blood flow by 53% ($P < 0.001$) and increased vascular resistance by 167% ($P < 0.001$).

Blood Gases. Blood *p*H, p_{CO_2}, and p_{O_2} in conscious rats (n = 3) before and after L-NAME are shown in figure 4. Under basal conditions, conscious rats had a blood *p*H of 7.46 ± 0.01, a p_{CO_2} of 42 ± 2.6 mmHg, and a p_{O_2} of 87 ± 6 mmHg. Administration of L-NAME had no effect on blood *p*H or p_{CO_2} levels,while causing a slight but significant 6% ($P < 0.05$) increase in p_{O_2} levels.

Effect of EDNO Synthesis Inhibition on Systemic and Regional Hemodynamics and Blood Gases in Rats Anesthetized with the Barbiturate Anesthetic Inactin

Systemic Hemodynamics. Changes in BP, HR, CO,and TPR in inactin anesthetized rats in response to L-NAME are shown in figures 1 and 2. N^W-nitro-L-arginine methyl ester caused a 20 ± 1-mmHg increase in BP ($P < 0.001$) and a 36 ± 6-beats/min decrease in HR ($P < 0.001$). Cardiac output decreased by 38% ($P < 0.005$), while TPR increased by 106% ($P < 0.001$). The decrease in HR was significantly less than in conscious rats ($P < 0.05$).

Regional Hemodynamics. Changes in regional hemodynamics in inactin-anesthetized rats in response to EDNO synthesis inhibition are shown in figure 3.

Basal cerebral blood flow was 0.6 ± 0.1 ml · min^{-1} · g tissue $weight^{-1}$ and vascular resistance was 179 ± 20 RU. N^W-nitro-L-arginine methyl ester had no effect on blood flow, but increased vascular resistance by 44% ($P < 0.05$). The decrease in blood flow was significantly less than that seen after L-NAME in conscious rats ($P < 0.05$).

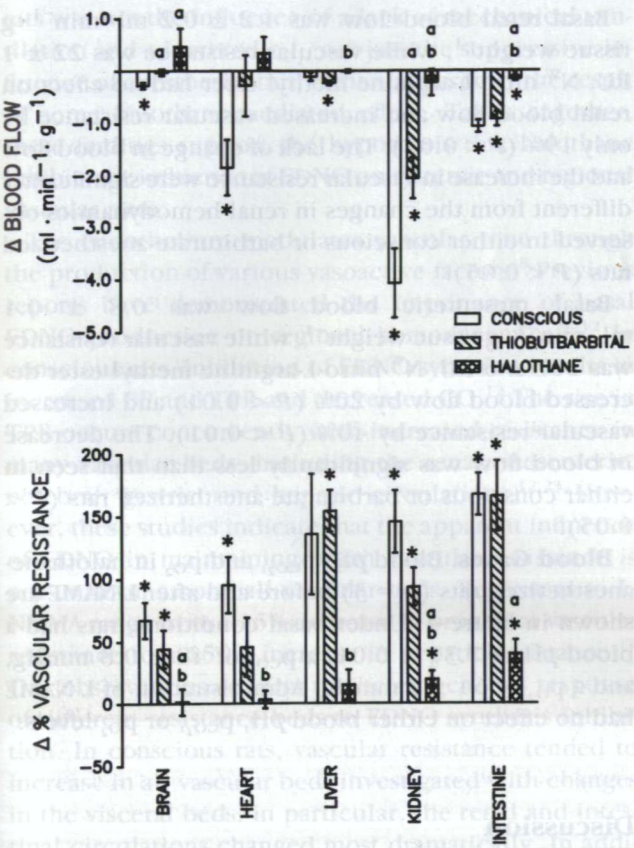

Fig. 3. Changes in organ blood flow (*top*) and vascular resistance (*bottom*) in response to 10 mg/kg N^W-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthesis, in conscious and inactin- and halothane-anesthetized rats. Values represent the mean ± 1 SE for each experimental group; conscious (n = 8), inactin-anesthetized (n = 8), and halothane-anesthetized rats (n = 8). *Significant change in response to L-NAME ($P < 0.05$); a = change induced by L-NAME is significantly different from conscious rats ($P < 0.05$); b = change induced by L-NAME is significantly different from inactin-anesthetized rats ($P < 0.05$).

Basal cardiac blood flow was 4.2 ± 0.4 ml · min^{-1} · g tissue weight^{-1}, while vascular resistance was 25 ± 3 RU. N^W-nitro-L-arginine methyl ester had no effect on either blood flow or vascular resistance, although there was a nonsignificant trend for L-NAME to increase resistance.

Basal hepatic arterial blood flow was 0.4 ± 0.05 ml · min^{-1} · g tissue weight^{-1}, while vascular resistance was 281 ± 42 RU. N^W-nitro-L-arginine methyl ester decreased blood flow by 48% ($P < 0.005$) and increased vascular resistance by 146% ($P < 0.001$). Although the decrease in blood flow after L-NAME was only about one-half that observed in conscious rats, the variability was such that this difference was not statistically significant.

Basal renal blood flow was 5.6 ± 0.4 ml · min^{-1} · g tissue weight^{-1}, while vascular resistance was 18 ± 1 RU. N^W-nitro-L-arginine methyl ester decreased blood flow by 35% ($P < 0.001$) and increased vascular resistance by 93% ($P < 0.001$). The decrease in blood flow

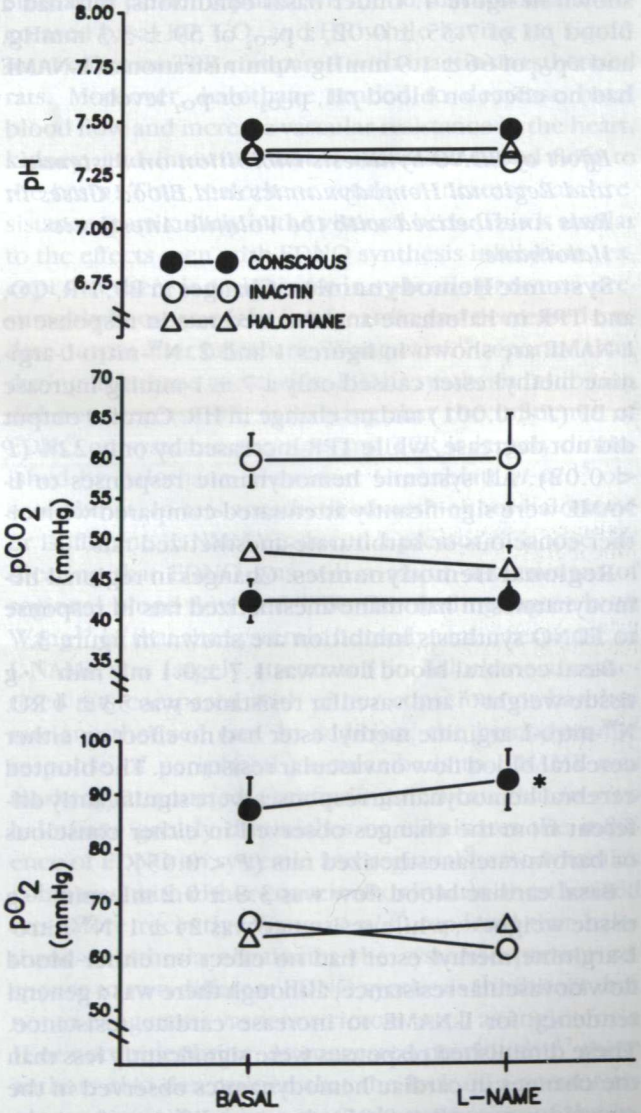

Fig. 4. Changes in blood *p*H, p_{CO_2} and p_{O_2} in response to 10 mg/kg N^W-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthesis, in conscious (n = 3) and inactin- (n = 3) and halothane-anesthetized rats (n = 3). Values represent the mean ± 1 SE for each experimental group; conscious, inactin-anesthetized, and halothane-anesthetized rats. *Significant change in response to L-NAME ($P < 0.05$).

was significantly less than that observed in conscious rats ($P < 0.05$).

Basal mesenteric blood flow was 1.8 ± 0.2 ml · min^{-1} · g tissue weight^{-1}, while vascular resistance was 59 ± 5 RU. N^{W}-nitro-L-arginine methyl ester decreased blood flow by 51% ($P < 0.001$) and increased vascular resistance by 166% ($P < 0.005$).

Blood Gases. Blood *p*H, p_{CO_2}, and p_{O_2} in inactin-anesthetized rats (n = 3) before and after L-NAME are shown in figure 4. Under basal conditions, rats had a blood *p*H of 7.35 ± 0.02, a p_{CO_2} of 59 ± 3.3 mmHg, and a p_{O_2} of 66 ± 1.9 mmHg. Administration of L-NAME had no effect on blood *p*H, p_{CO_2}, or p_{O_2} levels.

Effect of EDNO Synthesis Inhibition on Systemic and Regional Hemodynamics and Blood Gases in Rats Anesthetized with the Volatile Anesthetic Halothane

Systemic Hemodynamics. Changes in BP, HR, CO, and TPR in halothane-anesthetized rats in response to L-NAME are shown in figures 1 and 2. N^{W}-nitro-L-arginine methyl ester caused only a 7 ± 1-mmHg increase in BP ($P < 0.001$) and no change in HR. Cardiac output did not decrease, while TPR increased by only 22% ($P < 0.02$). All systemic hemodynamic responses to L-NAME were significantly attenuated compared with either conscious or barbiturate-anesthetized rats.

Regional Hemodynamics. Changes in regional hemodynamics in halothane-anesthetized rats in response to EDNO synthesis inhibition are shown in figure 3.

Basal cerebral blood flow was 1.7 ± 0.1 ml · min^{-1} · g tissue weight^{-1} and vascular resistance was 53 ± 4 RU. N^{W}-nitro-L-arginine methyl ester had no effect on either cerebral blood flow or vascular resistance. The blunted cerebral hemodynamic responses were significantly different from the changes observed in either conscious or barbiturate-anesthetized rats ($P < 0.05$).

Basal cardiac blood flow was 3.8 ± 0.2 ml · min^{-1} · g tissue weight^{-1}, while resistance was 24 ± 1. N^{W}-nitro-L-arginine methyl ester had no effect on either blood flow or vascular resistance, although there was a general tendency for L-NAME to increase cardiac resistance. These diminished responses were significantly less than the changes in cardiac hemodynamics observed in the conscious rats ($P < 0.05$), but not different from the response in barbiturate-anesthetized rats.

Basal hepatic arterial blood flow was 0.3 ± 0.01 ml · min^{-1} · g tissue weight^{-1}, while vascular resistance was 304 ± 14 RU. N^{W}-nitro-L-arginine methyl ester had no effect on either blood flow or vascular resistance.

Basal renal blood flow was 4.2 ± 0.2 ml · min^{-1} · g tissue weight^{-1}, while vascular resistance was 22 ± 1 RU. N^{W}-nitro-L-arginine methyl ester had no effect on renal blood flow and increased vascular resistance by only 19% ($P < 0.02$). The lack of change in blood flow and the increase in vascular resistance were significantly different from the changes in renal hemodynamics observed in either conscious or barbiturate-anesthetized rats ($P < 0.05$).

Basal mesenteric blood flow was 0.8 ± 0.1 ml · min^{-1} · g tissue weight^{-1}, while vascular resistance was 107 ± 6 RU. N^{W}-nitro-L-arginine methyl ester decreased blood flow by 20% ($P < 0.01$) and increased vascular resistance by 40% ($P < 0.01$). The decrease in blood flow was significantly less than that seen in either conscious or barbiturate-anesthetized rats ($P < 0.05$).

Blood Gases. Blood *p*H, p_{CO_2}, and p_{O_2} in halothane-anesthetized rats (n = 3) before and after L-NAME are shown in figure 4. Under basal conditions, rats had a blood *p*H of 7.37 ± 0.01, a p_{CO_2} of 48 ± 0.8 mmHg, and a p_{O_2} of 64 ± 1 mmHg. Administration of L-NAME had no effect on either blood *p*H, p_{CO_2}, or p_{O_2} levels.

Discussion

The tonic vasodilator EDNO is an important factor in the regulation of vascular tone and organ perfusion.[1] *In vivo* and *in vitro* data have indicated that some volatile anesthetics may inhibit the vasodilator influence of EDNO, thereby increasing vascular tone.[14,18,19] Our study was designed to test the hypothesis that halothane inhibits EDNO-mediated vasodilation and its regulation of regional blood flow. To test this hypothesis, we inhibited EDNO synthesis in halothane-anesthetized rats and compared systemic and regional hemodynamic responses with conscious and inactin (barbiturate)-anesthetized rats. Regional hemodynamics were measured before and after inhibiting EDNO synthesis with L-NAME. Our results demonstrate that, although EDNO synthesis inhibition increased BP, TPR, and local vascular resistance and decreased CO and HR in both conscious and inactin-anesthetized rats, halothane anesthesia greatly blunted these responses. The observation that blood *p*H was similar in all three groups and that blood p_{O_2} was not different between inactin- and halothane-anesthetized rats indicates that the highly attenuated systemic and regional hemodynamics responses to EDNO synthesis inhibition in halothane-anesthetized

rats was not the influence of a lack of mechanical ventilation and a lowered p_{O_2}, nor just the suppressive influence of anesthesia, but, rather, apparently the result of some halothane-mediated effect. Taken together, these findings support the hypothesis that halothane inhibits the influence of EDNO on systemic and regional vascular tone.

The endothelium modulates vascular tone through the production of various vasoactive factors.[8] Previous reports have demonstrated the importance of basal EDNO production on regional hemodynamics.[9–12] In conscious rats, inhibition of EDNO synthesis results in increased BP and TPR and decreased CO.[12] The rise in TPR occurs concurrently with increased resistance in many vascular beds, including the renal, mesenteric, cerebral, gastric, and hepatic circulations.[12,23] However, these studies indicate that the apparent influence of EDNO in maintaining organ vascular resistance is not uniform among all vascular beds. Responses to L-NMMA range from a 15% decrease in bronchial vascular resistance to a 150% increase in hepatic resistance.[12] Our observations confirm the heterogeneous response of different resistance beds to EDNO synthesis inhibition. In conscious rats, vascular resistance tended to increase in all vascular beds investigated with changes in the visceral beds; in particular, the renal and intestinal circulations changed most dramatically. In addition, we found that EDNO synthesis inhibition caused no changes in either blood *p*H or p_{CO_2} and only a slight increase in p_{O_2}. This indicates that, in conscious rats, EDNO is an important regulator of blood flow, and the hemodynamic changes are not related to changes in blood gases.

It has been reported that, in barbiturate-anesthetized animals, as in conscious rats, EDNO synthesis inhibition increases BP and TPR while decreasing CO and HR.[10,12,24] Loeb and Longnecker[10] demonstrated that L-NMMA increased vascular resistance from 45% in the cerebral circulation to more than 200% in brown fat. Again, the current study confirms the heterogeneous response of different vascular beds to EDNO synthesis inhibition in barbiturate-anesthetized rats. Although L-NAME tended to increase resistance in all vascular beds, blood flow to the brain and heart were not changed by L-NAME, consistent with the study by Wang *et al.*[12] Thus, blood flow to these beds may be under more profound central control and, therefore, sensitive to the general influence of anesthesia. Under such conditions, suppression of the central nervous system (by anesthesia) will reduce the effects of other regulatory systems, such as EDNO.

Halothane exerts a variety of influences on systemic and regional hemodynamics. It has been shown to decrease BP by decreasing cardiac output without altering systemic vascular resistance.[17] Halothane has also been shown to alter basal blood flow to a variety of vascular beds; it increased cerebral blood flow while decreasing blood flow to the heart, kidney, liver, and muscle.[13,16,17] Similar to earlier reports, we found that halothane decreased basal BP, CO, and HR while having no significant effect on TPR compared with inactin-anesthetized rats. Moreover, halothane tended to decrease basal blood flow and increase vascular resistance in the heart, kidney, and intestine, but it increased blood flow to the brain. Thus, halothane tends to increase basal resistance, particularly in the visceral beds. This is similar to the effects seen with EDNO synthesis inhibition, except that there is no increase in systemic pressure, presumably because of the bradycardia and decreased cardiac output after halothane. Wang *et al.*[14] reported that, during halothane anesthesia, EDNO synthesis inhibition did not increase BP, indicating that the influence of EDNO on systemic pressure and TPR is largely diminished by halothane. However, Greenblatt *et al.*[15] observed that, in rats anesthetized with either halothane or isoflurane, L-NMMA induced a pressor response, indicating that EDNO was still a major determinant of regional blood flow and TPR. Our findings agree with Wang[14] in that the systemic hemodynamic response to L-NAME was largely attenuated in halothane-anesthetized rats compared with either conscious or barbiturate-anesthetized rats. In addition, the hemodynamic response of peripheral vascular beds to L-NAME was markedly attenuated. Our findings indicate that *in vivo* halothane greatly diminishes or eliminates the influence of EDNO on systemic and regional hemodynamics compared with either conscious or inactin-anesthetized rats. More recently, Wang *et al.*[25] reported that halothane anesthesia eliminates the systemic pressor response to two different EDNO synthesis inhibitors, but not to exogenous vasoconstrictors, such as angiotensin II or norepinephrine. As suggested previously,[17] these authors also determined that the lack of a pressor response was caused by a marked attenuation of the increase in TPR. As in our study, they found that halothane diminished the local increase in vascular resistance in a variety of organ beds, except for the liver and spleen. They concluded that halothane suppresses the constrictor effect of EDNO synthesis inhibitors by releasing

NO or potentiating its vasodilator action. Although our results are similar, we interpret them quite differently. If the hemodynamic responses to L-NAME reflect the contribution of EDNO to systemic or local vascular tone, dissipating these responses indicates that halothane directly or indirectly blocks either the synthesis of EDNO or its targets, rather than potentiating its effect. Clearly, if halothane potentiated EDNO, blocking its synthesis and removing its influence would amplify, rather than eliminate, systemic or local hemodynamic responses.

Both barbiturate and halothane anesthesia (without ventilation) resulted in similar decreases in the p_{O_2}, and these were not changed further by EDNO synthesis inhibition. Because the response to L-NAME was so different with halothane compared with inactin, despite having similar p_{O_2}, these findings indicate that anesthesia-induced changes in arterial blood gases cannot account for the different responses to EDNO synthesis reported, nor the apparent lack of influence of L-NAME on systemic and regional tone in halothane-anesthetized rats. It should be noted that, in anesthetized rats breathing room air, a p_{O_2} of 60–70 represents a significant desaturation of arterial hemoglobin and, therefore, may not represent what would be observed in a controlled clinical setting.

The mechanism by which halothane interferes with the influence of EDNO on vascular tone is not yet defined. Several possibilities have emerged in the recent literature. Both *in vivo* and *in vitro* studies have demonstrated that volatile anesthetics, including halothane, may cause a marked decrease in the activity of nitric oxide synthase[26] or of soluble guanylate cyclase,[27,28] the second messenger target of EDNO in vascular smooth muscle. Interruption of either of these steps in the cascade of endothelium-derived relaxation could account for the halothane-mediated decreases in basal cGMP levels.[29] Some volatile anesthetics may inhibit EDNO by generating superoxide anions, closely related oxygen-free radicals,[19] which are known inhibitors of EDNO, or both.[30,31] Finally, it has been demonstrated that halothane increases calcium efflux and decreases calcium influx through receptor-operated channels in vascular smooth muscle.[32] Therefore, halothane could interfere with the action of EDNO by altering calcium fluxes, because calcium is a cofactor for EDNO synthesis.

Our studies demonstrate that, in both conscious and barbiturate-anesthetized rats, EDNO is an important regulator of systemic and regional hemodynamics. Also, although the response to EDNO synthesis inhibition was qualitatively similar in both conscious and barbiturate-anesthetized rats, the changes were generally greater in conscious animals, particularly in the cardiac and cerebral circulations. By comparison, halothane decreased BP and CO and virtually eliminated the systemic and regional hemodynamic responses to inhibition of EDNO synthesis. These observations indicate that halothane has some negative influence over the phenomenon of endothelium-derived vasodilation, either directly on synthesis of EDNO or indirectly on some step in its physiologic pathway. The implications for an anesthetic agent suppressing or eliminating an important regulator of vascular tone in certain patients, especially those undergoing vascular surgery, may not yet be appreciated. However, it is clear that the efficacy or appreciation of certain anesthetics in different types of procedures may be influenced by how these agents interact with the production or the effect of nitric oxide from the endothelium.

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