Role of nitric oxide-containing factors in the ventilatory and cardiovascular responses elicited by hypoxic challenge in isoflurane-anesthetized rats

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Mendoza JP, Passafaro RJ, Baby SM, Young AP, Bates JN, Gaston B, Lewis SJ. Role of nitric oxide-containing factors in the ventilatory and cardiovascular responses elicited by hypoxic challenge in isoflurane-anesthetized rats. J Appl Physiol 116: 1371–1381, 2014. First published April 17, 2014; doi:10.1152/japplphysiol.00842.2013.—Exposure to a hypoxic environment elicits increases in minute ventilation in humans and animals (26, 55). This process involves direct relaxation of vascular smooth muscle via activation of ATP-sensitive K⁺-channels and a decrease in voltage-gated Ca²⁺-channel (Ca²⁺-VS-channel) activity (10), the release of NO or related species (e.g., S-nitrosothiols) from the vascular endothelium (44, 45), and generation of plasma S-nitrosothiols via hemoglobin-based redox chemistry in red blood cells (2). Systemic injections of NO synthase (NOS) inhibitors blunt hypoxia-induced hypotension and vasodilation (44); microinjections of NOS inhibitors into the caudal NTS attenuate the ventilatory responses elicited by hypoxia (35) and peripheral chemoreceptor stimulation (14) whereas they do not affect hypoxia-induced hypotension (14). Taken together, it is tempting to assume that the hypoxia-induced changes in hemodynamic function are due predominantly to peripheral mechanisms involving NO and/or S-nitrosothiols. This may also pertain to cardiac function since NOS exists in cardiac neurons and muscle cells (6), and NO and S-nitrosothiols impact virtually every facet of cardiac function (28).

Most studies examining the mechanisms underlying hypoxia-induced changes in minute ventilation used between 5–30 min of acute hypoxia (5, 37, 38). However, episodes of naturally occurring apnea in humans and animals are substantially shorter in duration (7, 26). We are interested in the mechanisms that are involved in the ventilatory and cardiovascular response to such brief episodes of hypoxia. As such, we chose to examine the responses elicited by 90-s exposures to hypoxia (10% O₂, 90% N₂) in rats. These brief episodes of hypoxia were chosen specifically to limit the recruitment of central pathways responsible for ventilatory roll-off, the decrease in minute ventilation observed during sustained exposure to hypoxia (13).

Despite considerable investigation into the role of NO and S-nitrosothiols in the ventilatory and cardiovascular effects of hypoxia, few have focused on their roles in the responses elicited by brief episodes of hypoxia (e.g., 12). We decided to perform our studies in isoflurane-anesthetized rats since abrupt exposure of conscious subjects to an hypoxic environment elicits a transient but strong behavioral reaction that complicates interpretation of the nonbehavioral responses and therefore the cellular mechanisms underlying them (36). Since isoflurane is widely used (37–39), understanding how it affects hypoxia-driven responses is of clinical importance. In preliminary studies, we found that exposure to brief episodes of hypoxia (90 s) elicited robust changes in respiratory frequency (fr) and cardiovascular parameters in rats anesthetized with 1.25% isoflurane. The rat is an ideal model species to investigate the effects of volatile anesthetics on ventilatory function because humans and animals show similar sensitivities to these

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EXPOSURE to a hypoxic environment elicits increases in minute ventilation in humans and animals (26, 55). This process involves activation of primary glomus cells in the carotid bodies with release of “neurotransmitters” that activate carotid body chemoreceptors, which relay their information to the nucleus of the tractus solitarius (NTS) in the brain stem (26, 55). The generation of nitric oxide (NO) and/or S-nitrosothiols in the blood (29), brain (3, 14, 33), and carotid bodies (19, 26) plays vital roles in the hypoxia-induced changes in ventilation (36).

Exposure to a hypoxic challenge can reduce mean arterial blood pressure (MAP) in rats (12, 18, 44, 59), via decreases in cardiac output and regional vascular resistances (18, 44) whereas heart rate usually increases (59). Hypoxia-induced vasodilation

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agents with the efficacy of ventilatory depression being halothane > isoflurane > sevoflurane (23, 37). In addition, the effects of volatile anesthetics on ventilatory responses to hypoxia are species related with the rat being least susceptible (23, 30). Indeed, robust increases in minute ventilation occur in rats anesthetized with isoflurane (1.4%) or halothane (1.1%), which are accompanied by pronounced increases in carotid body chemoreceptor discharge (23, 30).

The first aim of the present study was to establish the contribution of the carotid bodies and carotid sinus nerves (CSNs) in the ventilatory and cardiovascular responses elicited by a 90-s episode of hypoxia. The second aim was to examine the role of NO/S-nitrosothiols in the initiation of these hypoxic responses. In these studies, the effects of the NOS inhibitor, N(G)-nitro-L-arginine methyl ester (L-NAME), on the ventilatory and cardiovascular responses elicited by exposure to four 90-s episodes of hypoxia (10% O2, 90% N2) were determined. Four episodes of hypoxia were examined to determine whether blockade of NOS elicits temporal changes in the hypoxic-induced responses. Finally, the data were recorded every second and averaged into 15-s bins to allow for detailed analyses of the rapid changes in ventilatory and cardiovascular parameters associated with the hypoxic exposures.

**MATERIALS AND METHODS**

**Rats**

All studies were carried out in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 80-23) revised in 1996. The protocols were approved by the University of Virginia Animal Care and Use Committee. Male Sprague-Dawley rats of 14–16 wk of age (*study 1*: 319 ± 4 g body wt; *study 2*: 321 ± 6 g; *study 3*: 326 ± 5 g) at the time of experimentation were purchased from Harlan (Madison, WI).

**Surgical Procedures**

Rats were anesthetized via continuous administration of 2% isoflurane in 95% O2:5% CO2 via a facemask at a flow rate of 2 l/min to help maintain the subsequent ventilatory responses to hypoxia. A rectal probe connected to a thermostatically controlled heating pad (Homeothermic Monitor, Harvard Apparatus, Holliston, MA) was used to maintain the body temperature of each rat at 37.0 ± 0.2°C. Each rat was given a 200-μl subcutaneous injection of bupivacaine (0.25%) at the site of incision for subsequent placement of the femoral artery catheter. A catheter (PE-50, Braintree Scientific, Braintree, MA) was placed in the left femoral artery to monitor MAP, and another was placed in the left femoral vein for drug injection. A 1-in., 23-gauge needle catheter (Becton Dickinson, Franklin Lakes, NJ) was placed through the thoracic wall to measure changes in thoracic cavity pressure. After surgery, each rat was maintained under anesthesia via continuous administration of 1.25% isoflurane in room air (compressed medical air) via a facemask at a flow rate of 2 l/min. This level of isoflurane was sufficient to eliminate responses to tail-pin and audiovisual stimuli. This is important since such stimuli can antagonize the effects of isoflurane on ventilatory responses to hypoxia (23).

**Data Recording**

MAP and thoracic pressure were measured by pressure transducers (SP 844) connected to a Bridge AMP (ADInstruments, Colorado Springs, CO) and ADInstruments Power Lab, and recorded with ADInstruments Lab Chart 7.0 viewing software. Heart rate was determined from the pulsatile pressure waveform. ft was determined continuously from the intrathoracic pressure waveform. Data were collected into 15-s bins to establish one data point for analyses.

**Experimental Protocol**

*Study 1*: hypoxia-induced responses before and after CSN transection. Each rat was given 20–25 min for MAP, heart rate, and ft to stabilize after placement of catheters before beginning the recordings. Baseline values were recorded for 5 min. Changes in MAP, heart rate, and ft elicited by a single episode of hypoxia (10% O2, 90% N2) for 90 s were given before and beginning 30 min after bilateral transection of the CSNs (n = 9 rats) or sham transection of these nerves (n = 6 rats). The principal reason for exposing rats to 90-s challenges with 10% O2 was that this allowed examination of the maximal (plateau) responses resulting from exposure to the hypoxic gas. This length of exposure to 10% O2 was not designed to have a practical medical context since patients would not be expected to have apneas of such duration (7).

*Study 2*: pharmacological studies. Each rat was given 20–25 min for MAP, heart rate, and ft to stabilize following placement of all catheters. Baseline values for statistical analyses were recorded for 5 min. Each rat then received four consecutive episodes of hypoxia (10% O2, 90% N2) of 90 s in duration given 10 min apart. Ten minutes after episode 4 of hypoxia, rats were given bolus injections of vehicle (saline, n = 6 rats), L-NAME (50 μmol/kg, n = 6 rats), or the inactive enantiomer, D-NAME (50 μmol/kg, n = 5 rats). Ten minutes later, the rats again received four episodes of hypoxia (10% O2, 90% N2) of 90 s in duration given 10 min apart. This dose of L-NAME was chosen because it impair central as well as peripheral NOS-dependent mechanisms (42), thereby allowing for the global assessment of the roles of NOS in the hypoxia-induced responses.

*Study 3*: repeat L-NAME study. The above protocols left a somewhat unresolved question as to the exact temporal changes in ft following injection of vehicle or L-NAME. To resolve this issue, the temporal changes in baseline ft were determined before and after injection of vehicle (n = 6 rats) or L-NAME (50 μmol/kg, n = 6 rats). These rats were not subjected to episodes of hypoxia.

**Drugs and Hypoxic Gas**

L- and D-NAME were purchased from Sigma-Aldrich (St. Louis, MO). Bupivacaine was purchased from Hospira (Lake Forest, IL). Isoflurane liquid was purchased from Butler Schein (North Dublin, OH). Tanks containing compressed hypoxic gas mixture (10% O2, 90% N2) were purchased from GTS-WELCO (Charlottesville, VA).

**Data Analysis**

Periodically, arterial line saline flushes or intrathoracic needle dislodging caused erroneous “peaks” in the data. These were removed and replaced by the average of the two data points immediately before and after the peak. The area under the curves for the decreases in MAP recorded during the episodes of hypoxia were calculated using LabChart 7.0 (ADInstruments, Colorado Springs, CO), taking the integral relative to baseline beginning at the start of each hypoxic episode and ending exactly 4 min later. Resting values before each episode of hypoxia were determined by averaging the values recorded over the 5-min period immediately preceding exposure to hypoxia.

**Statistics**

All data are presented as means ± SE. Data were analyzed by one-way or repeated-measures analysis of variance (BMDP Statistical Package, Statistical Solutions, Boston, MA) followed by Student’s modified t-test with Bonferroni correction for multiple comparisons between means using the error mean square terms from the analyses of variance (58). A value of P < 0.05 was taken to denote statistical significance.
RESULTS

CSN Transections

The changes in heart rate, MAP, and fr elicited by a single episode of hypoxia and upon return to room air (posthypoxia) before (Pre) and after bilateral transection of the CSNs (CSNX) are summarized in Fig. 1. Hypoxia elicited a decrease in MAP that was similar before and after CSNX. Reintroduction of room air resulted in a similar return to prehypoxia levels (within 60 s) before and after CSNX. The hypoxia-induced decreases in MAP were associated with increases in heart rate that were similar before and after CSNX. Upon return to room air, heart rate decreased to below prehypoxia levels equally before and after CSNX. This bradycardia was resolved after 5–6 min (data not shown). Prior to CSNX, hypoxia elicited increases in fr. In contrast, fr values fell below prehypoxia levels for 90 s upon reintroduction of room air. These hypoxia and posthypoxia changes in fr were absent following CSNX.

Pharmacology Studies: MAP

The changes in baseline MAP elicited by the drug injections are summarized in Fig. 2, top. Neither vehicle nor d-NAME affected MAP whereas L-NAME elicited a sustained hypertension. The effects of hypoxia on MAP before and after injection of vehicle, d-NAME, or L-NAME are summarized in Fig. 3. Prior to drug injection, each episode of hypoxia elicited decreases in MAP that were similar in magnitude and duration in all three groups. Neither saline nor d-NAME affected the hypoxia responses whereas L-NAME markedly diminished the
decreases in MAP. The maximal (Fig. 3, middle) and total decreases in MAP (Fig. 3, bottom) elicited by hypoxia were attenuated in L-NAME-treated rats but not in vehicle- or D-NAME-treated rats.

Pharmacology Studies: Heart Rate

The changes in baseline heart rates (as measured before each exposure to hypoxia) elicited by the drug injections are summarized in Fig. 2. Heart rates were similar before and after administration of vehicle (n = 6 rats), N\textsuperscript{\textgamma}-nitro-D-arginine methyl ester (\textgamma-NAME; 50 \textmu mol/kg iv, n = 5 rats) or N\textsuperscript{\textgamma}-nitro-L-arginine methyl ester (L-NAME; 50 \textmu mol/kg iv, n = 6 rats). Data are presented as means ± SE of the average of values recorded 5 min prior to exposure to hypoxia. *P < 0.05, significant difference from average of Pre values.

Pharmacology Studies: fr

The changes in baseline fr values elicited by the drug injections are summarized in Fig. 2. fr values after vehicle or \textgamma-NAME were slightly below but not significantly less than those before injection. Resting fr values were slightly diminished after injection of L-NAME. However, the possibility that L-NAME diminishes baseline fr was not confirmed in other groups of rats. More specifically, resting fr values recorded before and after injection of vehicle (plateau value at 30 min postinjection) were 66 ± 3 vs. 61 ± 4 breaths/min (~5 ± 2 breaths/min, P < 0.05, n = 6 rats)
whereas resting fR values recorded before and after L-NAME (50 μmol/kg iv) were 64 ± 3 vs. 58 ± 4, arithmetic difference of −6 ± 2 breaths/min (P < 0.05, n = 6 rats). The arithmetic decreases in both groups were similar to one another (P > 0.05) and the actual postvehicle and post-L-NAME values were equal throughout the 60-min recording period (P > 0.05) and to those in the initial studies summarized above. These findings suggest that baseline fR decays gradually but minimally in rats breathing isoflurane (1.25%) and room air. The effects of hypoxia on fR before and after injection of vehicle, D-NAME, or L-NAME are summarized in Fig. 5. Prior to drug injection, each episode of hypoxia elicited similar initial increases in fR that resolved before the next episode of hypoxia was given (Fig. 5, top). The maximum hypoxia-induced increases in fR were similar before and after administration of vehicle, D-NAME, or L-NAME (Fig. 5, bottom).

**DISCUSSION**

**Baseline Parameters in Isoflurane-Anesthetized Rats**

The rats maintained on 1.25% isoflurane presented with a stable ventilatory and cardiovascular profile. Resting MAP values were 10–15 mmHg below those usually observed in conscious rats whereas resting heart rates were similar to those of conscious rats (15). The mechanisms by which isoflurane and related volatile anesthetics affect hemodynamic status have been investigated (9, 61). These anesthetics lower MAP by reducing peripheral vascular resistance due principally to diminished sympathetic outflow (9, 61) although an increase in vascular NOS activity and thereby enhanced NO-mediated vasodilation has also been suggested to be involved in the early but not later phase of isoflurane-induced hypotension (9). The direct effects of ISO on the vasculature are complicated since
it enhances the contractile activity of the major sympathetic neurotransmitter, norepinephrine, in endothelium-intact arteries whereas it suppresses the contractile effects in endothelium-denuded arteries (61). The resting fR of these isoflurane-anesthetized rats were also less (−10 breaths/min) than those usually seen in conscious rats (62). The mechanisms by which isoflurane and related volatile anesthetics affect ventilatory function have also been widely investigated. These agents suppress ventilation by actions within the central nervous system and peripheral structures including the carotid bodies (5, 37–39).

**Hypoxic Responses in Isoflurane Rats**

The changes in MAP and heart rate elicited by the hypoxic challenge (10% O2, 90% N2) in the isoflurane-anesthetized rats were substantially different from those observed in conscious rats (12, 48, 59). For example, the hypoxic challenge in isoflurane-anesthetized rats elicited a prompt and robust fall in MAP (e.g., episode 1 of hypoxia in vehicle-treated rats of −27 ± 3 mmHg) whereas falls of less than 5 mmHg occur in conscious rats (12, 48, 59). Moreover, the hypoxic challenge in isoflurane-anesthetized rats elicited a relatively minor increase in heart rate that was followed by relatively minor decrease (e.g., episode 1 of hypoxia in vehicle-treated rats of +17 ± 2 beats/min and −16 ± 3 beats/min, respectively) compared with a robust and sustained tachycardia of at least 60 beats/min in conscious rats (12, 48). In our own laboratory (unpublished observations), we have found that hypoxic challenge elicits a minor change in MAP (−3 ± 2 mmHg) in conscious Sprague-Dawley rats (n = 8) that was accompanied by a robust increase in heart rate (+68 ± 8 beats/min) as measured at 90 s, the time the peak changes in isoflurane-anesthetized rats were observed. These findings are consistent with evidence that isoflurane disturbs the mechanisms by which the heart responds to the
challenges (37–39). Studies in humans (5) and animals (37–39) have demonstrated that volatile anesthetics blunt the ventilatory responses to hypoxia by actions within the carotid bodies. Studies in carotid body preparations have also provided compelling evidence that volatile anesthetics suppress the hypoxia-induced increases in intracellular Ca\textsuperscript{2+}-concentrations in primary glomus cells by activation of background TASK-like K\textsuperscript{+} channels (39) although isoflurane was substantially weaker than halothane in suppressing the hypoxic response. This observation is supported by in vivo studies, which demonstrated that isoflurane is substantially weaker than halothane with respect to inhibition of the hypoxic ventilatory response in humans (37) and rats (23) and that 1.0% end-tidal concentration of isoflurane does not depress the hypoxic chemosensitivity of peripheral chemoreceptors (21). Moreover, Teppema et al. (54) provided evidence that the generation of free radicals may also play an essential role in depression of the acute hypoxic ventilatory response by subanesthetic doses of halothane in humans. The brief episodes of hypoxia elicited rapid and robust increases in \( f_R \) that were substantially smaller in magnitude than those observed in conscious rats (12, 31, 48). For example, we reported that acute exposure to a hypoxic challenge elicited a maximal increase in \( f_R \) of \( +123 \pm 15 \) breaths/min in conscious Sprague-Dawley rats (31), which is substantially greater than the maximal responses (e.g., episode 1 of hypoxia in vehicle-treated rats of \( +24 \pm 3 \) breaths/min) observed in the isoflurane-anesthetized Sprague-Dawley rats.

**Carotid Sinus Nerve Transection Studies**

The finding that the hypoxia-induced decreases in MAP and presumably baroreceptor reflex-mediated increases in heart rate were similar before and after bilateral CSNX suggests that CSN chemoafferents/baroafferents are not essential for the expression of these responses. Indeed, increases in heart rate in response to the hypoxia-induced falls in MAP in the CSNX rats would be expected because of the presence of aortic baroafferents, which travel in the aortic depressor nerves (42), the integrity of which was not affected by the CSNX procedure. Upon reintroduction to room air, MAP recovered to prehypoxia levels. A bradycardia, which was sustained for several minutes, was also observed. That these post-hypoxia changes in MAP and heart rate were similar before and after CSNX suggests that the recovery of MAP is due to the loss of the hypoxic-vasodilator stimulus, and that the bradycardia is independent of the carotid body complex. Our findings that this bradycardia was absent in L-NAME-treated rats suggests a primary role of NOS in this phenomenon.

The ventilatory responses to hypoxia are mediated principally via carotid body chemoafferent input to the NTS, which elicits signaling cascades that increase motor output to the chest wall and diaphragm (55). The vital role of the carotid body complex in tonic regulation of ventilation is supported by evidence that upon recovery from surgery, \( f_R \) in conscious rats is depressed for several days following bilateral CSNX (46). In contrast, bilateral CSNX does usually suppress ventilation in animals anesthetized with volatile anesthetics (in which ventilation is already suppressed) possibly because the anesthetics inhibit resting carotid body chemoafferent activity (37–39). Our finding that bilateral CSNX did not depress \( f_R \) in our isoflurane-anesthetized rats is consistent with the above possibility. That the hypoxia increases in \( f_R \) were eliminated after bilateral CSNX in these isoflurane rats is consistent with evidence that hypoxia stimulates ventilation via activation...
of the carotid body/chemoafferent complex in humans (56) and rats (46).

Minute ventilation falls below resting levels following recovery from brief hypoxia (i.e., upon return to room air) via reductions in tidal volume and fr. This posthypoxia depression of fr, which is referred to as posthypoxia frequency decline (PHFD), is an active neural process that depends on the integrity of the ventrolateral pons (16). Two key findings of the present study were that the isoflurane rats displayed PHFD, and that this decline was absent following bilateral CSNX. These novel findings suggest that CSN input is essential for the expression of PHFD and specifically that CSN activity falls below normal activity, thereby promoting hypoventilation. This possibility is directly supported by studies in an in vitro rat carotid body preparation, which found that carotid body chemoafferent activity was transiently but substantially diminished (sensory posthypoxia decline) after recovery from mild hypoxia challenge (4). A perhaps equally compelling possibility is that input from carotid body chemoafferents (or indeed baroafferents) within the CSN play an obligatory role in allowing neuronal, and especially those within the ventrolateral pons, to promote the decrease in fr (enhanced expiratory duration). This would be analogous to the permissive role of the carotid body chemoafferent input in the direct depressive effects of hypoxia on central neuronal activity (4, 16).

Pharmacology Studies: MAP

Exposure to 90-s episodes of hypoxia (10% O2, 90% N2) elicited decreases in MAP of about 40 mmHg in our rats. Although we did not determine the mechanisms responsible for the decreases in MAP, Huang et al. (18) found that the hypotensive responses elicited by brief episodes of hypoxia (10% O2, 90% N2 for 5 min) in pentobarbital-anesthetized rats were due to reductions in total peripheral resistance and in cardiac output. The hypoxia-induced changes in MAP during and following each hypoxia episode in our isoflurane-anesthetized rats were similar before and after injection of vehicle or t-NAME. This suggests that there were no substantial time-dependent impairments in the mechanisms by which hypoxia elicited its hemodynamic effects. The sustained increases in MAP elicited by t-NAME (50 μmol/kg iv) in our isoflurane-anesthetized rats are probably due to sustained increases in peripheral vascular resistance that are offset by a decrease in cardiac output (15, 18, 42). The robust and sustained nature of the t-NAME-induced hypertension in these rats certainly suggests that the volatile anesthetic did not obviously impair the activity of NOS or the dependence of the vascular system on NO factors. Our findings contrast substantially from those of Sigmon et al. (51), who reported that halothane markedly attenuated the hemodynamic effects of t-NAME. This difference between isoflurane and halothane is another example of the differing pharmacology of these two volatile anesthetics (37–39).

The finding that the arithmetic and percent changes in MAP elicited by hypoxia were markedly attenuated following injection of t-NAME contrasts somewhat with those of Huang et al. (18), who found that the arithmetic decreases in MAP elicited by hypoxia were not decreased in t-NAME-treated rats, but because of the elevated baseline, the arithmetic response computed to a smaller percent decrease. Although this may argue against a role of NO in hypoxia-induced hypotension, Huang et al. (18) found that the hypoxia-induced reductions in peripheral vascular resistance were diminished after administration of L-NAME. Moreover, other studies have certainly provided evidence that the maintenance of hypoxia-induced decreases in MAP are attenuated by NOS inhibitors. For example, Ray and Marshall (45) found that hypoxia elicited hypotension, a decrease in femoral artery resistance (i.e., vasodilation), and a substantial increase in plasma NO (based on conversion of NO3 to NO2 and conversion of NO2 to NO) in anesthetized rats. The hypoxia-induced decreases in MAP and femoral artery vasodilation were attenuated after blockade of NOS (45). As such, it is tempting to assume that a principal mechanism by which hypoxia exerts its hypotensive and vasodilator effects is via the release of endothelium-derived NO and/or S-nitrosothiols (15). However, it is also distinctly possible that the vasodilator effects of hypoxia involve neurogenic (NO factor-mediated) vasodilator mechanisms (42), and the direct hypoxia-induced formation of blood-borne S-nitrosothiols (2). The question therefore arises as to whether NO and/or S-nitrosothiols are involved in mediating the hypotensive/vasodilator actions of hypoxia. The possible role of S-nitrosothiols is supported by evidence that exposure to hypoxia increases the S-nitrosylation status and activities of many functional proteins including Ca2+Vs-channels (36).

Pharmacology Studies: Heart Rate

NOS is also localized to cardiac neurons and muscle cells (6). The primary role of NO appears to include inhibition of the positive inotropic and chronotropic responses elicited by β-adrenergic receptor stimulation, and facilitation of parasympathetic (vagal) nerve-mediated slowing of heart rate and inhibition of β-adrenergic receptor-mediated increases in cardiac contractility (6). In addition, S-nitrosylation is a ubiquitous signaling modality impacting virtually every facet of cardiac function (28). The precise effects of S-nitrosylation are complex but include inhibition of Ca2+Vs-channel activity and sarcoplasmic-localized Ca2+-ATPase (28), effects that would promote bradycardia and a decrease in cardiac contractility. In agreement with a variety of studies (12), hypoxia elicited an increase in heart rate, most likely due to activation of the baroreflex system in response to the decrease in MAP (12). Upon reinduction of room air, heart rate fell to below prehypoxia levels. This bradycardia was unaffected by prior transection of the CSNs and could not have been due to activation of the baroreflex system since MAP remained depressed. Although a variety of mechanisms could be involved, it is tempting to assume that the generation of NO/S-nitrosothiols in the heart during hypoxia limited the initial presumably baroreflex-mediated increase in heart rate and as time progresses directly suppresses cardiac function. Indeed, a key finding was that the initial tachycardia elicited by hypoxia was augmented in t-NAME-treated rats whereas the subsequent bradycardia upon return to room air was virtually absent in these rats. These findings suggest that hypoxia directly generated NO factors that suppress pacemaker activity/conductance in the heart and that blockade of NOS allows for fuller baroreceptor reflex-mediated changes in autonomic nerve activity to affect heart rate.
Pharmacology Studies: fr

The systemic administration of L-NAME elicits a pronounced increase in fr in conscious rats (12, 52). Moreover, application of NO inhibitors to carotid body preparations increases resting activity of chemoreceptors and augments the sensitivity of these afferents to hypoxia (43). These and other data suggest that NO is primarily inhibitory in the carotid body complex whereas S-nitrosothiols may have a facilitatory role (19, 20, 26, 36). In contrast to conscious animals, systemic injection of these NO inhibitors elicits minimal effects on ventilatory parameters in anesthetized animals (17, 53), including fr in rats (40). As such, it appears that anesthetics interfere with NO-dependent regulation of ventilatory drive in the carotid bodies and/or brain. The finding that L-NAME did not elicit an increase in fr in isoflurane-anesthetized rats suggests that the potential decrease in cerebral blood flow is not sufficient to drive ventilation or more likely because NO inhibitors depress ventilatory responses to hypercapnia by actions in the brain stem (53). Moreover, McPherson et al. (32) demonstrated that whereas L-NAME diminished cerebral blood flow it did not interfere with hypoxia-induced vasodilation in isoflurane-anesthetized dogs. As such, an appropriate cerebral vasodilation in response to hypoxia in L-NAME-treated animals would not represent a ventilatory stimulus.

A key finding of this study was that the increases in fr elicited by hypoxia were unaffected by L-NAME despite the propensity of NO inhibitors to promote the depth of anesthesia (and possibly respiratory depression) elicited by volatile anesthetics including isoflurane (22). L-NAME did not affect the peak magnitude of the ventilatory response during hypoxic challenge nor did it affect the posthypoxia changes in fr. These findings contrast to those in conscious rats in which blockade of endothelial NOS, the form of NOS in the primary glomus cells of the carotid bodies (19, 26, 60), inhibited the increases in fr (12), although the substantial increase in baseline fr in the L-NAME-treated rats makes interpretation somewhat difficult. Our data also contrast with evidence that the ventilatory responses to hypoxia are augmented in mice deficient in neuronal NOS (24) whereas mice deficient in endothelial NOS display enhanced ventilatory responses to hypoxia challenge (25). Taken together, it appears that despite displaying a robust increase in fr to hypoxia, isoflurane-anesthetized rats are devoid of a central and/or peripheral NOS-dependent component in the hypoxia response.

Despite direct biochemical evidence that NOS activity decreases under hypoxia (1), it appears that (in the carotid body at least) NOS activity increases during brief hypoxia challenges because carotid body O2 tension does not reach levels that inhibit NOS activity and because hypoxia increases intracellular Ca2+ levels, which promotes NOS activity (33, 60). There is also substantial evidence that hypoxic challenges increase NOS activity in other tissues with concomitant increases in NO and S-nitrosothiol bioavailability (2, 11, 29, 36). Although inhibition of NOS activity and blockade of NO neurotransmitter function are thought to be key steps in the mechanism of action of volatile anesthetics, there is evidence that these anesthetics have little effect or markedly increase NOS activity and NO production (27, 50). The interaction of isoflurane-induced and hypoxia-induced changes in NOS activity are likely to be complex although our results raise the possibilities that 1) isoflurane prevents the hypoxia-induced activation of NOS and therefore the de novo synthesis of NO factors in the brain, carotid body, or blood; and/or 2) isoflurane impairs the mechanisms by which NO and especially S-nitrosothiols exert their effects on these structures. Indeed, numerous studies have demonstrated potential mechanisms by which volatile anesthetics could impair the ability of hypoxia to stimulate NO, including activation of TASK-like K+ channels, which would lead to diminished Ca2+ entry into primary glomus cells and inhibition of a variety of other Ca2+ channels and intracellular Ca2+-mobilizing processes (39, 41).

Study Limitations

A limitation of this study is that we did not verify that the isoflurane-anesthetized rats had comparable blood gases at baseline, and that they achieved comparable changes in blood oxygen during the acute hypoxic challenges. However, our findings that each hypoxic episode elicited similar temporal increases in fr in the two groups of rats prior to administration of vehicle or L-NAME suggests that each hypoxic challenge elicited similar changes in arterial blood-gas chemistry. Exposure to hypoxic challenges elicits profound falls in PO2 and PCO2 in isoflurane-anesthetized rats (8, 49). These reductions in PCO2 are in stark contrast to the increases in PCO2 that occur during episodes of apnea (7). As such, our study does not address the potent effects of blood PCO2 on ventilatory drive and therefore cannot be considered as a model of apnea in which to examine the effects of isoflurane or the roles of NOS. Another limitation of the present study is that we did not determine the effects of hypoxia on tidal volumes or the effects of L-NAME on these hypoxia-induced responses. Although we determined that the increase in fr elicited by hypoxic gas challenge is markedly suppressed in isoflurane-anesthetized rats and that these responses were not blunted by L-NAME, it is possible these effects on fr may not be mirrored by the effects on tidal volume. Nonetheless, the clear dependency of the hypoxia-induced changes in fr on the integrity of the carotid sinus nerves in the isoflurane-anesthetized rats lends strength to the argument that isoflurane diminishes NOS-dependent processes within the carotid bodies.

Summary and Clinical Implications

Our key findings were that whereas L-NAME markedly attenuated the falls in MAP during hypoxic challenges in isoflurane-anesthetized rats, it had minimal impact on the ventilatory responses. As discussed above, there is substantial evidence that NO and S-nitrosothiols play pivotal roles in the ventilatory responses to hypoxia challenges in conscious animals. Our findings raise the possibility that isoflurane blocks the actions of NO-containing factors within the carotid bodies or brain sites processing ventilatory information rather than the de novo formation of NO and S-nitrosothiols in these structures (26, 28, 29) or the blood (2, 57) or from the release of preformed pools of NO-containing factors (15). Considering evidence that NO itself plays an inhibitory role in the carotid body (34), the findings that L-NAME diminishes the hypoxic ventilatory response in conscious rats raises the possibility that other products of NOS, namely S-nitrosothiols, may be involved in hypoxic signaling in the brain and carotid bodies as...
well as gas exchange. Indeed, hypoxic challenge elicits increases in circulating S-nitrosothiols in conscious rats (29); the microinjection of S-nitrosothiols such as l-S-nitrosoglutathione and L-S-nitrosocysteine into the NTS of conscious rats increases minute ventilation (29); S-nitroso-N-acetyl-penicillamine increases CSN chemoafferent activity in isolated carotid body-CSN preparations from cats (19, 20, 34), and S-nitrosothiols exert positive effects on ventilatory function and pulmonary gas-exchange mechanisms in a variety of species (11). As suggested by Rubanyi and Vanhoutte (47), decreased O2 availability likely exerts positive effects on ventilatory function and pulmonary gas-exchange mechanisms in a variety of species (11). As suggested by Rubanyi and Vanhoutte (47), decreased O2 availability in tissues could increase NO radical bioactivity by decreasing tissue superoxide anions, which directly interact with NO. This could be a mechanism involved in the vascular effects of hypoxia, and may be relevant to the ventilatory effects with increased NO bioavailability being inhibitory but enhanced S-nitrosothiol bioavailability being excitatory (29).

Although isoflurane is likely to attenuate the hypoxic ventilatory response by a multiplicity of mechanisms including blockade of hypoxia-induced increases in NOS activity, we are currently examining whether this anesthetic inhibits the hypoxic ventilatory response via alterations in the biological activities of NO and S-nitrosothiols within the carotid body and brain stem.

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REFERENCES


