Effect of nitric oxide synthase inhibition on cardiorespiratory responses in the conscious rat

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Gozal, David, José E. Torres, Yair M. Gozal, and Sanford M. Littwin. Effect of nitric oxide synthase inhibition on cardiorespiratory responses in the conscious rat. *J. Appl. Physiol.* 81(5): 2068–2077, 1996.—Nitric oxide synthase (NOS) blockade was used to test the cardioventilatory responses to hypercapnia and hypoxia in freely behaving animals. Chronically instrumented adult Sprague-Dawley rats were studied before and after intravenous administration of either 100 mg/kg of N^G-nitro-L-arginine methyl ester (L-NAME), a nonspecific NOS blocker, or 10 mg/kg of *S*-methyl-L-thiocitrulline (SMTC), a selective neural NOS inhibitor. L-NAME injection induced sustained blood pressure (BP) elevation with transient tachycardia and increased minute ventilation ($\dot{V}_E$), which returned to baseline within minutes. SMTC elicited similar, although transient, BP increases; however, heart rate and $\dot{V}_E$ decreased. L-NAME and SMTC did not modify overall steady-state hypercapnic responses. In control conditions, hypoxia induced early $\dot{V}_E$ increases with further $\dot{V}_E$ enhancements at 30 min. L-NAME increased the early $\dot{V}_E$ response to 10% O_2 but induced late $\dot{V}_E$ reductions in hypoxia. SMTC did not change early $\dot{V}_E$ responses but induced marked reductions in the later $\dot{V}_E$ hypoxic responses. In control animals, hypoxia induced a significant heart rate increase. This increase was absent during the early response after SMTC and was followed in both L-NAME- and SMTC-treated animals by significant heart rate reductions to values below room air. Similarly, the sustained BP response to hypoxia in control animals was absent after administration of NOS inhibitors. These findings suggest that NOS activity exerts excitatory influences on respiration and cardiac chronotropy and sustained vasomotor tone during hypoxia. We speculate that NOS-mediated mechanisms may play an important role in hypoxia-induced ventilatory roll-off during wakefulness.

control of breathing; blood pressure; baroreceptor; chemoreceptor; whole body plethysmography; hypercapnia; hypoxia

NITRIC OXIDE (NO) is an ubiquitous mediator of biological processes and a potent vasodilator. In the brain, NO production mediates both resting and cerebral blood flow (CBF) increases to a variety of stimuli (14). NADPH diaphorase-containing neurons are found within several pontomedullary regions that are known to mediate important functions of cardiac and respiratory regulation in the rat (34). Such topographic distribution suggests that NO may play a role in respiratory control. Indeed, NO enhances the excitability and spontaneous discharge rates of neurons within the nucleus tractus solitarius (NTS; 19, 31), in the dorsal motor nucleus of the vagus (33), and in the pontine respiratory group (17). Furthermore, NO blockade with the potent nonspecific NOS inhibitor N^ω-nitro-L-arginine (L-NNA) is associated with significant phrenic nerve output reductions during hypoxia in anesthetized cats (26). In a more recent study, Ogawa et al. (24) further demonstrated that when NO donors are microinjected into the NTS, enhanced ventilation ensues, whereas NO-blocker microinjections attenuated L-glutamate and ventilatory increases during hypoxia (24). In addition, NO attenuates the chemosensory function of the carotid body (5, 27), suggesting that the same molecule subserves both inhibitory and excitatory functions at different sites of the hypoxic chemotransduction pathway.

Recent development of compounds with selective-isoform NO synthase (NOS) inhibitory properties allowed us to further explore the possibility that NO derived from constitutive endothelial NOS (eNOS) and neural NOS (nNOS) may differently modulate the cardiorespiratory responses to hypoxia and hypercapnia in an awake unrestrained rat preparation. More specifically, we hypothesized that nNOS could play an important role in sustaining central drive during hypoxia-induced roll-off.

METHODS

Animals

The experimental protocols were approved by the Institutional Animal Use and Care Committee. Survival experiments were performed on male Sprague-Dawley adult rats (300–400 g). In a preliminary stage, anesthesia was induced by pentobarbital sodium (Nembutal, 50 mg/kg ip). Rectal temperature was monitored by a Harvard thermal probe, and core temperature was maintained at 37.5°C by a servo-controlled heating pad. After a 1-cm incision of the skin was performed, indwelling polyethylene catheters (PE-50; 0.56-mm ID, 0.88-mm OD) were surgically placed in the femoral artery and vein and advanced ~5–7 cm to reach the abdominal aorta and inferior vena cava for subsequent blood pressure (BP) measurements, arterial blood sampling, and fluid or drug administration. After the catheters were secured, they were tunneled subcutaneously and exteriorized in the dorsal aspect of the neck, flushed with a heparin-containing solution (1,000 U/ml saline), sealed with heat, and stored in a cap sutured to the skin. Animals were then allowed to recover for at least 48–72 h, as demonstrated by return to normal feeding and sleep-waking schedules. Animals were provided with water and rat chow ad libitum and were kept on a light-dark cycle of 12:12-h (light onset at 0630) and at 22 ± 1°C ambient temperature for at least 1 wk of habituation before surgery and during the postsurgical recovery period. For habituation purposes, animals spent at least 1–2 h each day in a whole body plethysmograph chamber.

Ventilatory and Cardiovascular Recordings

Cardiorespiratory measurements were continuously acquired in the freely behaving unrestrained animal placed in a

previously calibrated 3-liter barometric chamber (Buxco Electronics, Troy, NY) by using the methods described by Bartlett and Tenney (1) and Pappenheimer (25). To minimize the effect of signal drift due to temperature and pressure changes outside the chamber, a reference chamber of equal size was used in which temperature was measured by using a T-type thermocouple. Environmental temperature was maintained within the thermoneutral range (24–28°C). A calibration volume of 0.5 ml of air was repeatedly introduced into the chamber before and on completion of recordings. At least 60 min before the start of each protocol, animals were allowed to acclimate to the chamber, in which humidified air (90% relative humidity) was passed through at a rate of 8 l/min by using a precision-flow pump-reservoir system. Pressure changes in the chamber due to the inspiratory and expiratory temperature changes were measured by using a high-gain differential pressure transducer (model MP45-1, Validyne; Ref. 9). Analog signals were continuously digitized and analyzed on-line by a microcomputer software program (Buxco-Electronics, Troy, NY). A rejection algorithm was included in the breath-by-breath analysis routine and allowed for accurate rejection of motion-induced artifacts. Inspiratory time (T_I), expiratory time (T_E), tidal volume (V_T), respiratory frequency (f), and minute ventilation ($\dot{V}_E$) were computed and stored for subsequent off-line analysis.

Systemic arterial pressure was measured from the arterial femoral catheter connected to a calibrated pressure transducer via a custom-designed swivel apparatus in the recording chamber (BuxcoElectronics). Physiological signals were digitized, and a beat-to-beat peak-trough analysis routine allowed computation of heart rate (HR) and systolic and diastolic BP values.

Protocol

Animals received 2 ml normal saline intravenously and underwent hypoxic and hypercapnic ventilatory responses, after which either the nonspecific NOS blocker N^G-nitro-L-arginine methyl ester (L-NAME; 100 mg/kg in 2 ml normal saline; $n = 29$) or 10 mg/kg *S*-methyl-L-thiocitrulline (SMTC; $n = 19$), a selective nNOS inhibitor (10), was slowly administered intravenously over a period of 1 min. Thirty minutes after L-NAME or SMTC administration, ventilatory challenges were repeated. The adequacy of NOS-inhibitor dosages and experimental time frames was previously validated (13). In brief, changes in brain tissue NO concentration during mild hypoxia (10% O_2) were determined in five anesthetized, paralyzed, and mechanically ventilated rats with a microporphyrinic NO sensor before and after increasing doses of SMTC and L-NAME (Fig. 1). For NOS-blocker doses employed in the present study, attenuation of NO response remained unaltered up to 3 h after drug administration.

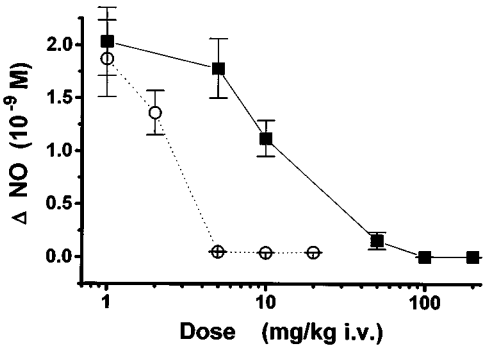

Fig. 1. Peak changes in brain nitric oxide (ΔNO) tissue concentration during mild hypoxia 30 min after intravenous administration of increasing doses of N^G-nitro-L-arginine methyl ester (L-NAME; ■) and *S*-methyl-L-thiocitrulline (SMTC; ○). Values are means ± SE.

Ventilatory challenges of 30-min duration were performed with 5% CO_2-balance room air and with 10% O_2-90% N_2 for both L-NAME- and SMTC-treated groups by rapidly bleeding the premixed gas mixture into the recording chamber. Hypercapnic or hypoxic gases were administered in random order.

Measurement of Blood-Gas Values

Arterial blood samples were obtained from the implanted catheter, and the absence of visible behavioral disturbances during the procedure was ascertained. After withdrawal of 75–100 μl of blood from the dead space of the catheter, another 150 μl were sampled for immediate analysis of P_{O_2}, P_{CO_2}, and pH with a blood-gas analyzer (model 178, Ciba Corning). Measurements were always performed in room air and during the last minute of each ventilatory challenge.

Data Analysis

Values are reported as means ± SE. For ventilatory challenges, we defined early response as the response measured during the initial 30 s after introduction of gas mixture. Steady-state responses were assessed during the last minute of each challenge. Statistical significance of the difference in data before and after each drug administration was assessed within each group by paired *t*-tests. Differences in data from the two treatment groups were compared by analysis of variance (2-way ANOVA) and the Newman-Keuls test (36). A P value of < 0.05 was considered to achieve statistical significance.

RESULTS

Baseline Measurements

Administration of 100 mg/kg L-NAME induced within 15–30 s a marked and sustained BP increase (Fig. 2*A*). Initial increases occurred in systolic BP (~17%) and were followed within several minutes by decreases to stable values ~10% above baseline (Table 1). Similarly, diastolic BP increased by 7% ($P < 0.01$). In contrast, an initial 25% elevation in HR that followed L-NAME injection lasted only several minutes and was followed by cardiac frequencies below preinjection values that persisted for at least 5 h, the time at which recordings were stopped (Table 1, Fig. 2).

A marked and transient ventilatory increase occurred within 15–25 s of L-NAME injection (Table 1, Fig. 3*A*). The $\dot{V}_E$ increase was due to a marked increase in f resulting from decreases in both T_I and T_E that persisted beyond $\dot{V}_E$ return to baseline. Such changes in ventilatory pattern were associated with concomitant and persistent V_T decreases (Table 1, Fig. 3).

SMTC injection was associated with similar early BP changes as with L-NAME (Table 1, Fig. 2*B*). However, BP values returned to preinjection values within 20 min. Systolic and diastolic BP values initially increased by 35 and 18% respectively ($P < 0.001$) and then returned to stable values within baseline range (Table 1, Fig. 2). On SMTC injection, no significant HR increases occurred (Fig. 2). Instead, several minutes after SMTC administration, HR decreased by 17% below baseline values and remained lower for at least 5 h (Table 1; $P < 0.01$).

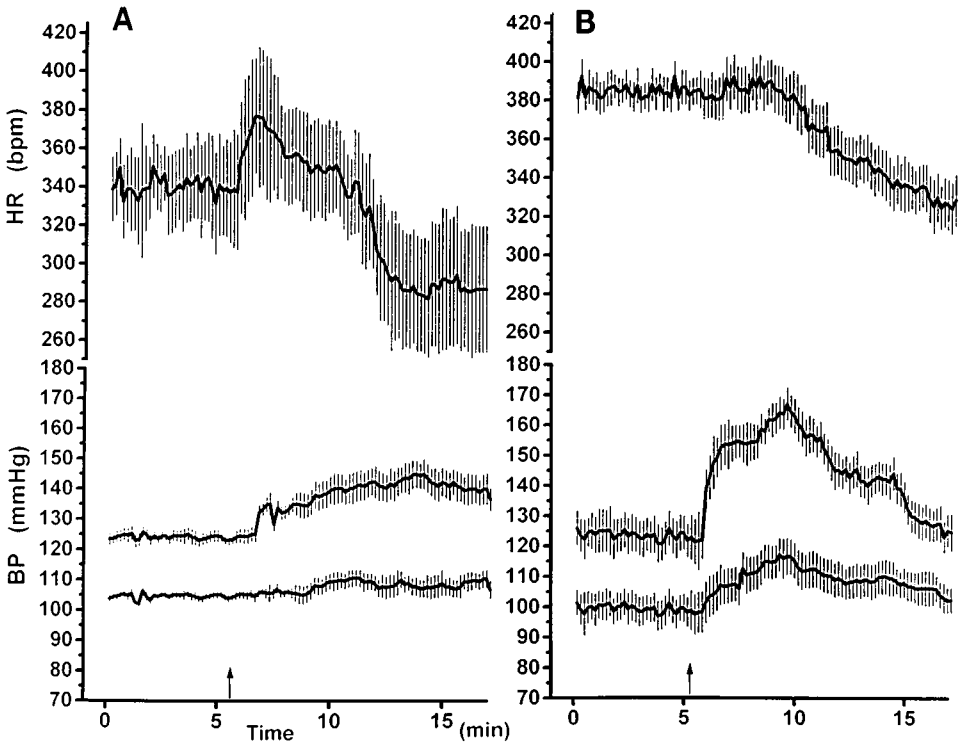

Fig. 2. Changes in heart rate (HR) and in systolic and diastolic arterial blood pressure (BP) before and after intravenous administration of 100 mg/kg L-NAME (*A*) or 10 mg/kg SMTC (*B*). Values are means ± SE. bpm, Beats/min. Arrows, time of injection.

The marked ventilatory increase associated with L-NAME injection did not occur with SMTC administration (Fig. 3*B*; L-NAME vs. SMTC, $P < 0.02$ by ANOVA). A very mild and transient $\dot{V}_E$ increase was measured during the 60 s after injection (Table 1; $P < 0.05$) and was followed by stable $\dot{V}_E$ values similar to preinjection $\dot{V}_E$ measurements (Table 1, Fig. 3). However, the ventilatory pattern associated with such mild $\dot{V}_E$ changes was markedly affected, such that f increased ($P < 0.001$) and V_T decreased (Table 1, Fig. 3). These changes in ventilatory strategy persisted for ~10 min and gradually returned to pre-SMTC injection values (Table 1).

Hypercapnic Ventilatory Challenges

L-NAME. Similar increases in $\dot{V}_E$ accompanied the introduction of 5% CO_2 in the recording chamber before and after L-NAME administration. The response after L-NAME administration was characterized by more profound f increases in the L-NAME group, whereas V_T, although increased with CO_2, remained at levels below those found in pretreated animals (Fig. 4). Thus $\dot{V}_E$ was similar in pretreated and L-NAME-treated animals (Fig. 4). Arterial blood gases in room air and hypercapnia are shown in Table 2. Although BP values during room air conditions were higher in the L-NAME group, no significant differences in HR and BP changes were identified in pre- and post-L-NAME groups undergoing hypercapnic challenges.

SMTC. Hypercapnic challenges in six SMTC-treated rats were associated with similar $\dot{V}_E$ increases to pretreated animals (Fig. 4). As with L-NAME, SMTC was associated with increased f and diminished V_T responses (Fig. 4). Cardiovascular responses and arterial blood gases were also similar (Table 2).

Hypoxic Ventilatory Challenges

L-NAME. $\dot{V}_E$ was slightly elevated in the L-NAME group before hypoxic gas administration (Table 3, Fig. 5*A*; $P < 0.05$). During the early phase of the hypoxic response, $\dot{V}_E$ increases were more pronounced

Table 1. *Mean arterial blood pressure, heart rate, and ventilatory measurements in waking unrestrained rats before and after administration of L-NAME or SMTC*

	L-NAME ($n = 29$)			SMTC ($n = 19$)		
	Baseline	Peak response	Steady-state response	Baseline	Peak response	Steady-state response
Systolic BP, mmHg	124.2 ± 2.5	144.9 ± 2.7‡	138.5 ± 2.6†	123.0 ± 3.1	167.1 ± 3.5‡	124.2 ± 3.2
Diastolic BP, mmHg	103.8 ± 2.3	115.4 ± 3.0‡	110.4 ± 2.6†	99.7 ± 2.0	117.7 ± 2.5‡	101.0 ± 2.6
Heart rate, beats/min	342.4 ± 2.7	426.3 ± 4.2†	290.2 ± 3.1*	384.4 ± 2.4	317.7 ± 3.3†	322.1 ± 3.1†
f, breaths/min	105.7 ± 3.1	168.7 ± 4.0‡	162.4 ± 3.7‡	97.6 ± 2.3	158.6 ± 3.8‡	106.4 ± 2.8
V_T, ml	3.74 ± 0.11	3.53 ± 0.18	2.25 ± 0.11†	2.60 ± 0.12	1.95 ± 0.11†	2.54 ± 0.09
$\dot{V}_E$, ml/min	382.2 ± 8.9	522.7 ± 16.6‡	365.8 ± 11.6	276.6 ± 6.4	307.4 ± 5.1*	269.0 ± 4.4

Values are means ± SE; *n*, no. of rats. L-NAME, N^G-nitro-L-arginine methyl ester; SMTC, *S*-methyl-L-thiocitrulline; BP, blood pressure; f, respiratory frequency; V_T, tidal volume; $\dot{V}_E$, minute ventilation. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$.

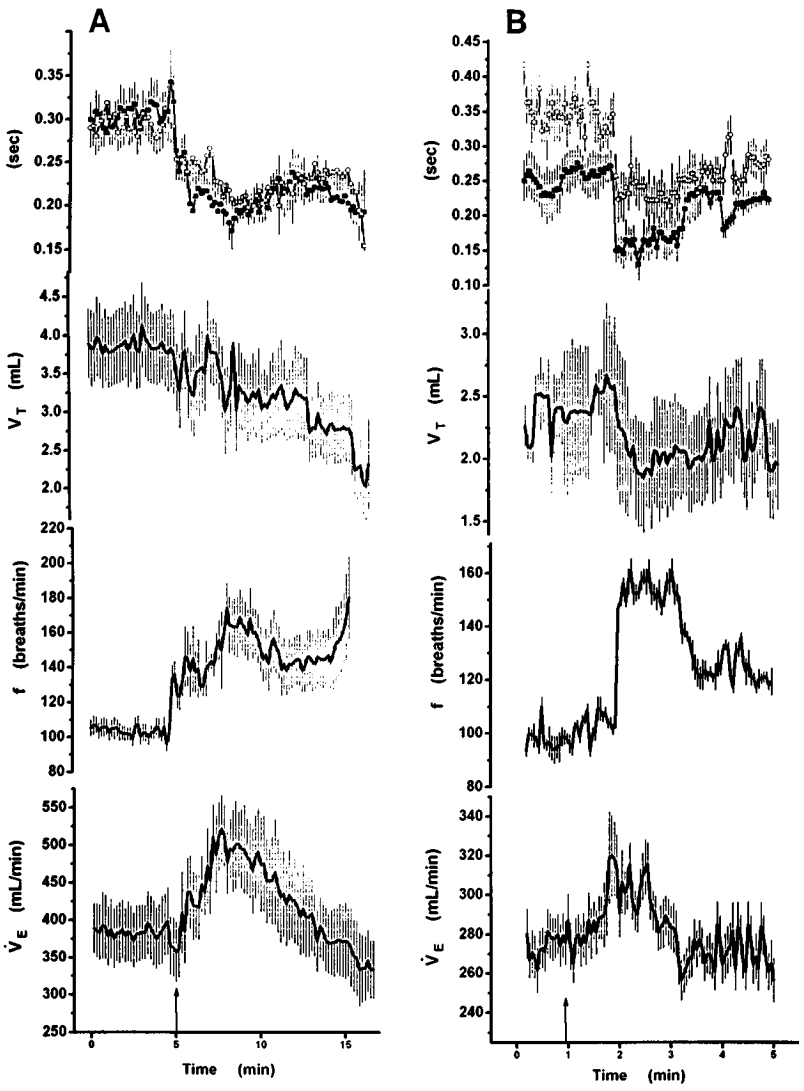

Fig. 3. Changes in inspiratory time (TI; *top panels*; ■), expiratory time (TE; *top panels*; ○), tidal volume (V_T), respiratory frequency (f), and minute ventilation ($\dot{V}_E$) before and after intravenous administration of 100 mg/kg L-NAME (*A*) or 10 mg/kg SMTC (*B*). Values are means ± SE. Arrows, time of injection.

in L-NAME-treated rats (control vs. L-NAME, $P < 0.002$). The enhanced ventilatory response after L-NAME was primarily due to f contributions (Table 3, Fig. 5). Early HR increases during hypoxia occurred before and after L-NAME administration and were more pronounced after L-NAME (Fig. 6). Although BP was already elevated after L-NAME, it was sustained during early hypoxia, whereas it increased in pretreated animals (Fig. 6).

After 30 min of hypoxic challenge, $\dot{V}_E$ was further increased in untreated animals, whereas significant decreases occurred in the L-NAME group ($P < 0.001$; Table 3, Fig. 5). This $\dot{V}_E$ decrease was due to significant f reductions compared with early hypoxia (Fig. 5). Arterial blood gases drawn in room air and late hypoxic conditions are shown in Table 4 for pre-L-NAME and post-L-NAME groups.

HR elevation during hypoxia was sustained in the pre-L-NAME conditions (Fig. 6). In L-NAME-treated rats, HR reductions in late hypoxia were more pronounced (Fig. 6; $P < 0.001$; L-NAME vs. control, $P < 0.004$ by ANOVA). In control rats, slight early BP elevations followed by late reductions occurred (Fig. 6). However, after L-NAME, whereas BP values remained stable in early hypoxia, marked BP decreases occurred in late hypoxia ($P < 0.001$; L-NAME vs. control, $P < 0.003$ by ANOVA; Fig. 6).

SMTC. The early ventilatory response to 10% hypoxia was similar in pre- and post-SMTC-treated animals (Table 3, Fig. 5). After 30-min in hypoxia, $\dot{V}_E$ in pretreated animals remained elevated. However, in SMTC-treated rats, $\dot{V}_E$ was significantly decreased (Table 3, Fig. 5). The decrease in $\dot{V}_E$ was primarily due to reductions in f and to mild V_T decreases (Fig. 5). Blood gases in pre- and post-SMTC groups in room air and late hypoxia are shown in Table 4.

HR was lower before hypoxia in the SMTC group (see *Baseline Measurements*) but increased similarly during early hypoxia in both groups (Fig. 6). However, in SMTC-treated animals, hypoxia-induced tachycardia was not sustained and HR decreased ($P < 0.01$), whereas no changes from early to steady-state hypoxia occurred in pretreated animals (Fig. 6). In early and late hypoxia, both systolic and diastolic BP significantly decreased after SMTC ($P < 0.01$), whereas an early, mild BP increase occurred in control conditions

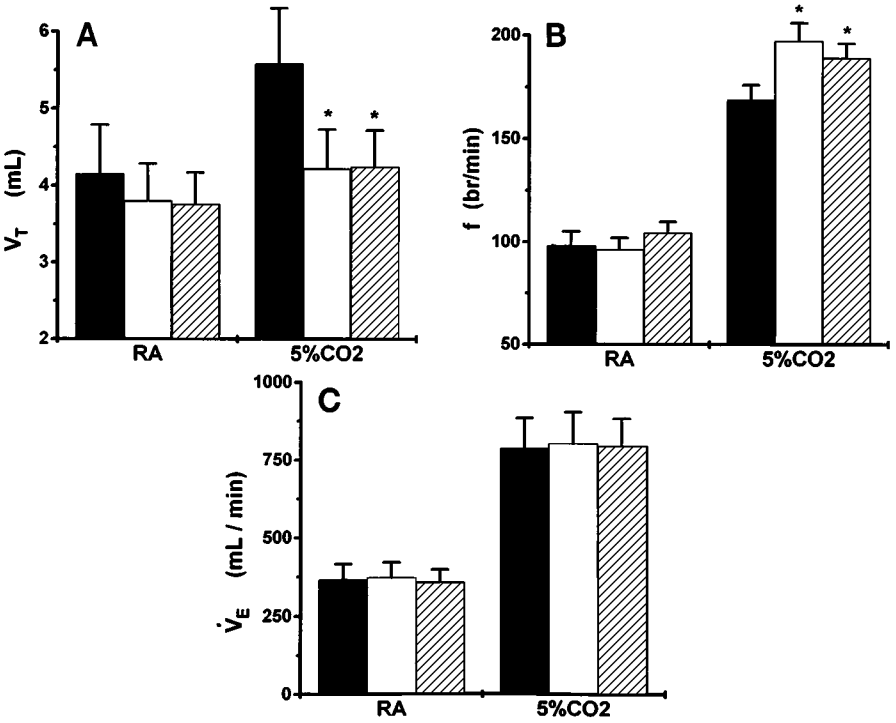

Fig. 4. VT (*A*), f (*B*), and $\dot{V}_E$ (*C*) before (solid bars) and after intravenous administration of either 10 mg/kg SMTC (open bars; $n = 6$) or 100 mg/kg L-NAME (hatched bars; $n = 16$) in room air (RA) and during last 2-min period of 30-min-duration 5% CO_2 challenge. br/min, breaths/min. Values are means ± SE. *$P <$ 0.01 vs. control conditions.

and was followed by small BP reductions in late hypoxia (Fig. 6).

DISCUSSION

In this study, the responses to NOS blockers suggest that NO plays a modulatory role in ventilatory responses to hypoxia in the awake unrestrained rat. NO, possibly derived from nNOS, exerts an excitatory effect to sustain central ventilatory drive and prevents full expression of hypoxia-induced depression. However, NO does not appear to play a major role in the steady-state ventilatory response to hypercapnia. NO may also exert an excitatory effect on chronotropic cardiac regulation, and NO blockade is associated with a diminished pressor response during sustained hypoxic conditions.

Ventilatory Responses to Hypercapnia

NOS inhibition did not modify the overall ventilatory response to a mild hypercapnic stimulus. However, both NOS blockers elicited significant changes in ventilatory strategy consisting of VT decreases with parallel f increases. The mechanisms underlying these changes are unclear. One possible mechanism could involve changes in regional CBF (rCBF) responses in response to hypercapnia. However, SMTC administration does not modify rCBF increases induced by CO_2, whereas L-NAME attenuates rCBF hypercapnic response by ~45% (13). A second possibility could reside in the muscarinic antagonist properties of certain NOS inhibitors. Indeed, L-NAME demonstrates significant, dose-dependent competitive binding with muscarinic receptors, which are dependent on alkyl ester modifications of the carboxyl end of the molecule (4). It remains unclear whether SMTC has similar antimuscarinic effects. However, muscarinic antagonists would be expected to diminish the ventilatory response to hypercapnia (23), an effect that was not observed. Instead, only changes in ventilatory strategy rather than changes in overall ventilatory output resulted from NOS blockade, suggesting that NO is not important to chemosensitivity per se but may play a role in neurons underlying

Table 2. *Arterial blood gases in room air and after 30 min in 5% CO_2 in waking unrestrained rats before and after either L-NAME or SMTC*

	Room Air			5% CO_2		
Group	pH	P_{CO_2}, Torr	P_{O_2}, Torr	pH	P_{CO_2}, Torr	P_{O_2}, Torr
Pre-L-NAME	7.414 ± 0.009	29.8 ± 1.8	95.2 ± 1.9	7.408 ± 0.023	34.5 ± 2.5	121.0 ± 11.8
L-NAME	7.481 ± 0.014	25.4 ± 1.5	98.7 ± 4.1	7.392 ± 0.011	36.2 ± 1.7	124.6 ± 8.8
P value	<0.01	<0.01	NS	NS	NS	NS
Pre-SMTC	7.405 ± 0.020	31.5 ± 1.9	94.6 ± 7.5	7.374 ± 0.026	36.5 ± 2.5	117.8 ± 11.8
SMTC	7.405 ± 0.017	33.1 ± 2.1	95.5 ± 8.3	7.388 ± 0.013	35.7 ± 2.1	120.3 ± 7.9
P value	NS	NS	NS	NS	NS	NS

Values are means ± SE for 13 rats in L-NAME group and 6 rats in SMTC group. NS, not significant.

Table 3. *Ventilatory measurements in waking unrestrained rats in room air and after 1 min (early) and 30 min (late) in 10% O_2 before and after either L-NAME or SMTC*

Group	Room Air			10% O_2 (Early)			10% O_2 (Late)		
	$\dot{V}_E$, ml/min	f, breaths/min	V_T, ml	$\dot{V}_E$, ml/min	f, breaths/min	V_T, ml	$\dot{V}_E$, ml/min	f, breaths/min	V_T, ml
Pre-L-NAME	344.8 ± 10.5	93.8 ± 2.3	4.21 ± 0.11	411.7 ± 12.2	112.6 ± 3.1	3.91 ± 0.16	558.1 ± 22.2	162.4 ± 4.2	3.7 ± 0.12
L-NAME	389.8 ± 11.3	116.4 ± 2.7	3.50 ± 0.09	533.8 ± 13.5	130.0 ± 2.0	4.11 ± 0.14	427.9 ± 20.3	103.8 ± 2.1	3.9 ± 0.10
P value	<0.05	<0.01	<0.05	<0.001	<0.001	NS	<0.001	<0.0001	NS
Pre-SMTC	223.0 ± 17.1	103.2 ± 3.3	2.27 ± 0.18	353.7 ± 27.4	171.4 ± 4.9	2.22 ± 0.21	380.4 ± 26.1	169.7 ± 4.6	2.24 ± 0.21
SMTC	218.4 ± 17.6	107.3 ± 3.5	2.12 ± 0.19	365.4 ± 25.1	173.7 ± 4.6	2.11 ± 0.19	301.0 ± 20.4	142.4 ± 3.6	2.01 ± 0.23
P value	NS	NS	NS	NS	NS	NS	<0.01	<0.01	NS

Values are means ± SE for 16 rats in L-NAME group and 9 rats in SMTC group.

functions of respiratory timing and amplitude (17, 19, 31, 33).

Ventilatory Responses to Hypoxia

It is now well established that NOS is present in the carotid body. By using NADPH diaphorase histochemistry, Prabhakar and colleagues (26) demonstrated that many nerve plexuses innervating the glomus tissue of the carotid body in cats stained positive for NADPH diaphorase, whereas glomus cells showed no intrinsic staining. Similarly, other investigators have described NOS-like immunoreactive staining localized in nerve fibers and ganglion cells distributed across the carotid body structure, within tissue septa, and in close proximity to blood vessels (6, 35). Small populations of NOS-positive axons were also identified in the glossopharyngeal nerve distal to the petrosal ganglion, the latter showing no evidence of NOS immunoreactivity (6). Sodium nitroprusside, a NO donor, induces significant attenuation of chemosensory activity of superfused carotid bodies, and nonspecific NOS inhibition enhances this response (5, 26). With acute hypoxia, NOS

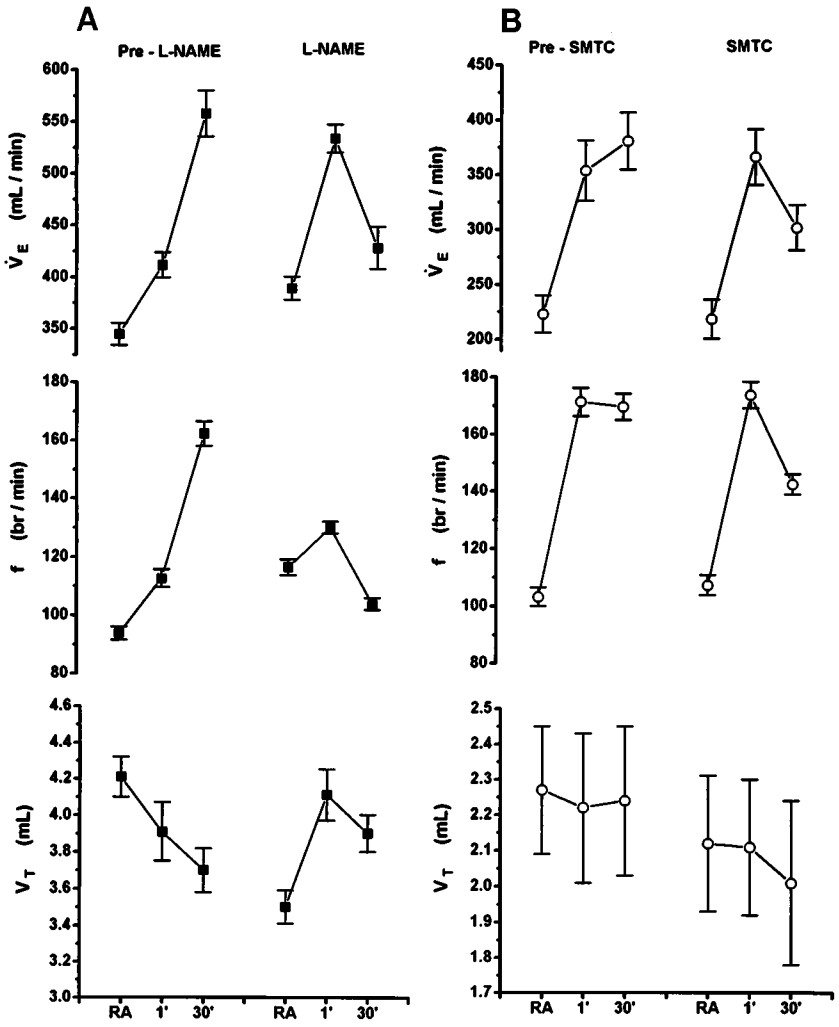

Fig. 5. $\dot{V}_E$, f, and V_T in RA, initial minute of 10% O_2 challenge, and last minute of 30-min-duration 10% O_2 challenge. *A*: ventilatory measurements for hypoxic challenges performed in 16 rats before and after intravenous 100 mg/kg L-NAME. *B*: ventilatory measurements for 9 animals before and after intravenous 10 mg/kg SMTC. Values are means ± SE.

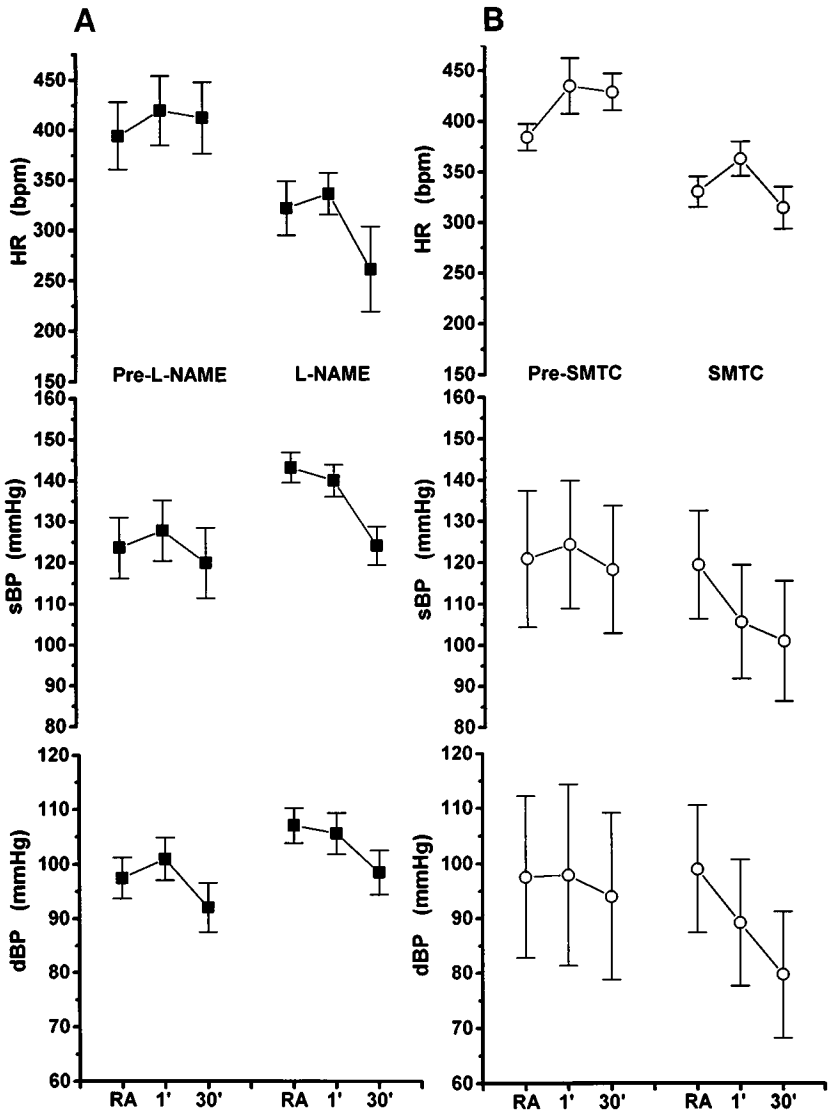

Fig. 6. HR, systolic (sBP), and diastolic (dBP) arterial BP in RA, initial minute of 10% O_2 challenge, and last minute of 30-min-duration 10% O_2 challenge. *A*: cardiovascular measurements of hypoxic challenges performed in 16 rats before and after intravenous 100 mg/kg L-NAME. *B*: cardiovascular measurements for 9 animals before and after intravenous 10 mg/kg SMTC are shown. Values are means ± SE.

activity within the carotid body is probably inhibited, leading to diminished NO concentrations and resultant increases in afferent peripheral chemoreceptor activity (5, 27). Present results suggest that nonspecific NOS inhibition achieved by in vivo administration of L-NAME induces a short-lived (~2.5 min) and marked $\dot{V}_E$ increase, whereas such effect was minimal after injection of SMTC, a putative nNOS blocker. In addition, nonspecific NOS blockade enhanced the early response to mild hypoxia, and such effect was absent when SMTC was used. Thus, although more specific studies are necessary, we postulate that nNOS inhibition has minimal or at least no discernible effect on peripheral chemoreceptor afferent activity. We further suggest that NO derived from endothelial sources may be the primary NOS enzyme isoform that modulates carotid

Table 4. *Arterial blood gases in room air and after 30 min in 10% O_2 in waking unrestrained rats before and after either L-NAME or SMTC*

	Room Air			10% O_2		
Group	pH	P_{CO_2}, Torr	P_{O_2}, Torr	pH	P_{CO_2}, Torr	P_{O_2}, Torr
Pre-L-NAME	7.430 ± 0.008	30.8 ± 1.7	92.4 ± 2.0	7.560 ± 0.009	21.8 ± 0.8	35.8 ± 0.8
L-NAME	7.471 ± 0.012	27.4 ± 1.3	99.2 ± 3.9	7.482 ± 0.013	28.7 ± 1.4	39.6 ± 1.8
P value	<0.01	<0.01	NS	<0.002	<0.002	NS
Pre-SMTC	7.411 ± 0.020	30.7 ± 2.4	98.8 ± 9.5	7.540 ± 0.031	22.8 ± 0.9	36.7 ± 4.2
SMTC	7.405 ± 0.007	32.1 ± 1.5	94.5 ± 6.8	7.489 ± 0.008	27.8 ± 1.0	35.3 ± 3.7
P value	NS	NS	NS	<0.01	<0.01	NS

Values are means ± SE for 16 rats in L-NAME group and 9 rats in SMTC group.

body tonic activity. Interestingly, the enhancement of the ventilatory response to transient hypoxia after L-NAME was primarily mediated via increases in f. It remains unclear whether this effect is due to NO-tonically mediated inhibition of centrally located rhythm generating neurons or NO-induced excitatory effects on chest wall afferents or central volume-related neurons.

Preliminary evidence from anesthetized vagotomized cats indicated that phrenic nerve output was markedly diminished after 5 min of an hypoxic challenge with 12% O_2 after systemic administration a potent nonspecific NOS inhibitor (26). Furthermore, phrenic nerve activity in hypoxia was silenced if carotid sinus nerves were sectioned after NOS inhibition (26). One possible mechanism for this effect could be that NOS blockade may have exacerbated the degree of central hypoxia by preventing CBF increases through vasoconstriction. Such mechanism is unlikely because neither basal CBF, CBF autoregulation, nor rCBF responses to hypoxia appear to be modified when NO production is blocked (3, 13). A second, more likely, mechanism assigns an excitatory role to NO within the central nervous system in general (2) and within structures underlying the ventilatory response to hypoxia in particular (20, 26). Such a mechanism is probably mediated either directly or via glutamate receptors, such as *N*-methyl-D-aspartate receptors (11, 12), to increase guanosine 3′,5′-cyclic monophosphate (cGMP) formation and neuronal activity (15). Hypoxia could prolong the half-life of NO and other endogenous NO-containing substances such as nitrosothiols, because oxidation is one of the primary processes of in vivo NO removal (21). On the other hand, molecular oxygen is a substrate for NO synthesis (16, 28), which could lead to reduced NO production in hypoxia. Thus the net effect of these two phenomena will principally dictate tissue NO levels. Thus it is possible that some component of the ventilatory roll-off that occurs during hypoxia is due to the downregulatory effect that hypoxia exerts on NOS activity.

Our data concur with those of both Prabhakar et al. (26) and Ogawa et al. (24) to support a central excitatory role of NO during hypoxia because significant ventilatory depression occurred in all animals after NOS inhibition. However, because ventilatory depression in hypoxia occurred after both L-NAME and SMTC, we postulate that nNOS is the more likely source of NO-derived hypoxic ventilatory enhancement. Of note, the primary ventilatory strategy during sustained hypoxia after nNOS inhibition was based on increases in f, suggesting that NO may modulate the activity of neurons involved in volume control or, alternatively, could downregulate neural input arising from slowly adapting pulmonary and chest wall stretch receptors. Our data somewhat conflict with those reported by Ling et al. (17), who found that microinjections of the nonspecific NOS inhibitor L-NNA into the pontine respiratory group induced significant prolongations of inspiration when lung inflation was withheld, thus suggesting that NO formation was involved in mechanisms associated with respiratory rhythm generation (17). In contrast to Ling et al., we found increases rather than decreases in f, and these changes were due to similar reductions in inspiratory and expiratory duration. It is possible that the apneustic effect of NO blockade observed by Ling and colleagues under rigidly controlled conditions in an anesthetized preparation may have been overcome by other respiratory drives that are operative during wakefulness. Thus, although current studies do not elucidate which neuronal populations underlie nNOS inhibition-mediated effects, they indicate that NO plays an important role in respiratory control, primarily linked to central hypoxic defense mechanisms.

Cardiovascular Responses

Administration of L-NAME induced an early and transient increase in HR that was followed by a stable relative bradycardia. The early tachycardic effect was absent after SMTC injection, although similarly sustained HR reductions occurred. As expected, L-NAME induced a sustained BP elevation that was only transient after SMTC and could reflect a mild rapidly resolving nonspecific competitive eNOS effect by the latter. These results suggest that NOS plays an important role in maintaining the tonic excitability of autonomic centers associated with cardiac chronotropy and may possibly have a role in neurons mediating vascular motor tone response to hypoxia.

Our data concur with previous studies whereby pretreatment with NO inhibitors or cGMP synthesis blockers abolished the baroreflex- and glutamate-induced responses (8, 29, 32). In contradistinction, microinjections of nitroglycerin, a NO donor, elicited both excitatory and inhibitory effects to the spontaneous activity of NTS neurons (20). The NTS provides the primary site of reflex integration from the majority of cardiovascular and respiratory afferents (18). This region also demonstrates intense positive NOS staining (32), suggesting that NOS-containing neurons are involved in cardiorespiratory regulation. Indeed, Ma et al. (19) recently showed that when BP was maintained constant in anesthetized rats, L-NAME decreased neuronal discharge in 12 of 14 medial NTS neurons, whereas in 2 neurons discharge firing was increased (20). These effects of L-NAME were reversed by either L-arginine or nitroglycerin (19). Many additional pontomedullary regions such as the lateral and medial parabrachial nuclei, ventral tegmental nucleus, pontine reticular nuclei, trapezoid body, and medullary raphe, as well as many other rostral brain structures, are important to autonomic regulation (30). All these regions stain densely for NOS-positive neurons (34). The exact role of NO within each of these regions awaits further studies.

Intravenous administration of reversible NOS inhibitors, including SMTC, produces marked increases in arterial BP (7, 22). Most likely, the early component of this SMTC pressor effect is due to a direct eNOS inhibitory effect on the vascular smooth muscle tone. Indeed, although SMTC is at least 10 times more

specific of constitutive nNOS than of constitutive eNOS (10), the latter may be temporarily inhibited by SMTC.

During early hypoxia, BP was sustained in L-NAME-treated rats but significantly decreased in SMTC-treated animals (Fig. 6). However, during late hypoxia, BP was reduced in both NOS blocker-treated rats. If it is assumed that NO plays an excitatory role within neurons underlying vasomotor regulation, the discrepant responses between L-NAME and SMTC during early hypoxia could be related to L-NAME-related peripheral eNOS or to muscarinic antagonist effects (4). The similar late hypoxia-induced BP reductions suggest that NO mediates components pertaining to the sustained vasomotor responses to hypoxia.

Conclusions

Pharmacological manipulation of NOS isoforms and NO production in awake rats suggests that NO exerts excitatory cardiac chronotropic and respiratory effects and also appears to modulate components of BP regulation during hypoxia. Thus NO emerges as an important modulator of the biphasic hypoxic response. The role of NO in the integrative response to central chemoreceptor stimulation appears to be minimal at most.

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