■ LABORATORY INVESTIGATIONS

Anesthesiology
1998; 88:1240–8
© 1998 American Society of Anesthesiologists, Inc.
Lippincott–Raven Publishers

Nitric Oxide Synthase Inhibitors Alter Ventilation in Isoflurane Anesthetized Rats

Gaurav M. Patel, Damian J. Horstman, M.S.,† J. Milton Adams, Ph.D.,‡ George F. Rich, M.D., Ph.D. §*

Background: **Nitric oxide (NO) is present in medullary structures and can modulate respiratory rhythm. The authors determined if spontaneous ventilation at rest and in response to increased carbon dioxide is altered by selective neuronal NO synthase (NOS; 7-nitro-indazole, 7-NI) or nonselective (neuronal plus endothelial) NOS (N^G-L-arginine methyl ester [L-NAME] and N^G-monomethyl L-arginine [L-NMMA]) inhibitors in rats anesthetized with isoflurane.**

Methods: **Fifty-four rats received either L-NAME or L-NMMA (1, 10, and 30 mg/kg) or 7-NI (20, 80, and 400 mg/kg) and were compared with time controls (isoflurane = 1.4%), with isoflurane concentrations (1.6%, 1.8%, and 2%) increased consistent with the increased anesthetic depth caused by NOS inhibitors, or with L-arginine (300 mg/kg). Tidal volume (V_T), respiratory frequency (f), minute ventilation ($\dot{V}_E$), and ventilatory responses to increasing carbon dioxide were determined.**

Results: **L-NAME and L-NMMA decreased resting V_T and $\dot{V}_E$, whereas 7-NI had no effect. Increasing concentrations of isoflurane decreased resting f, V_T, and $\dot{V}_E$. L-NAME and L-NMMA decreased V_T and $\dot{V}_E$, whereas 7-NI had no effect at 8%, 9%, and 10% end-tidal carbon dioxide ($ETCO_2$). Increasing concentrations of isoflurane decreased f, V_T, and $\dot{V}_E$ at 8%, 9%, and 10% $ETCO_2$. The slope of $\dot{V}_E$ *versus* $ETCO_2$ was decreased by isoflurane but was unaffected by L-NAME, L-NMMA, or 7-NI. L-arginine alone had no effect on ventilation.**

Conclusions: **Nonselective NOS inhibitors decreased V_T and $\dot{V}_E$ at rest and at increased carbon dioxide levels but did not alter the slope of the carbon dioxide response. Selective neuronal NOS inhibition had no effect, suggesting that endothelial NOS may be the isoform responsible for altering ventilation. Finally, the cause of the decreased ventilation is not a result of the enhanced anesthetic depth caused by NOS inhibitors.**

(Key words: Anesthesia; hypercapnia, minute ventilation; N^G-L-arginine methylester; 7-nitro-indazole.)

* Medical Student.

† Graduate Student.

‡ Professor of Biomedical Engineering.

§ Associate Professor of Anesthesiology and Biomedical Engineering.

Received from the Departments of Anesthesiology and Biomedical Engineering, University of Virginia Health System, Charlottesville, Virginia. Submitted for publication March 3, 1997. Accepted for publication December 24, 1997.

Address correspondence to Dr. Rich: Department of Anesthesiology, P.O. Box 10010, University of Virginia Health System, Charlottesville, Virginia 22906-0010. Address electronic mail to: gfr2f@virginia.edu

NITRIC oxide (NO) is involved in physiologic roles including neurotransmission and control of vascular smooth muscle tone.[1–3] Various cells constitutively produce NO from L-arginine by the enzyme nitric oxide synthase (NOS).[4] The endothelial or eNOS isoform is primarily localized to the vascular endothelium, whereas the neuronal or nNOS isoform is localized primarily in the central nervous system. Nitric oxide is present in medullary structures and has been shown to modulate respiratory rhythm.[5,6]

Nitric oxide synthase inhibitors have been used to evaluate the role of NO in modulating ventilation. N^G-L-arginine methyl ester (L-NAME), a nonselective NOS inhibitor, injected into the pontine respiratory group prolongs inspiratory time in cats.[5] Systemic administration of L-NAME has been shown to inhibit the ventilatory response to hypoxia in rats and cats.[6] Although these studies suggest that nonselective NOS inhibitors depress ventilation, intraventricular cerebral spinal fluid administration of L-NAME or a selective neuronal NOS inhibitor given to awake dogs has slightly increased ventilation.[7,8] Although it appears from these studies that NOS inhibitors alter ventilation, the ventilatory effects of NOS inhibitors remain controversial. Further, it is not known if NOS inhibitors alter the ventilatory response to carbon dioxide.

This study aimed to determine if NOS inhibitors alter resting ventilation and the ventilatory response to increasing carbon dioxide in rats anesthetized with isoflurane. A selective nNOS inhibitor (7-nitro-indazole, 7-NI) or a nonselective NOS inhibitor (L-NAME and N^G-monomethyl L-arginine [L-NMMA]) was evaluated to determine if the ventilatory effects were a result of endothelial or neuronal NOS inhibition. Because L-NAME and 7-NI decrease the minimum alveolar concentration (MAC), we also compared the ventilatory effects of NOS inhibitors with equivalent increases in isoflurane concentrations to preclude the effect of increased anesthetic depth.[9–11]

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/88/5/1240/392978/0000542-199805000-00015.pdf by guest on 20 April 2024

Materials and Methods

This study was approved by the University of Virginia animal research committee. Male Sprague-Dawley rats were anesthetized with isoflurane and placed in the supine position. A tracheostomy was performed, and a 17-gauge steel cannula was inserted into the trachea for airway control. A small silastic rodent breathing circuit was attached to the tracheal tube, which could be used as an open breathing circuit to allow for fresh gas (oxygen and isoflurane) flow or be changed to a closed system. The rat was allowed to breathe spontaneously on 1.4% isoflurane and oxygen. A femoral artery catheter (PE-50; inner diameter, 0.58 mm) was inserted to determine mean arterial pressure (MAP), heart rate (HR), and arterial blood gases (*p*H, Pa_{O_2}, and Pa_{CO_2}). A femoral venous catheter was inserted for intravenous injection of drugs.

A Silverman screen pneumotachometer, attached to the tracheal tube, was used to measure the respiratory air flow. The pressure decrease was determined using a Validyne MP45 transducer (Northridge, CA) and tidal volume (V_T) was measured by electronic integration (model 8815A; Hewlett-Packard, Beaverton, OR). Respiratory frequency (f) was determined from a strip chart recording of the V_T. Minute ventilation ($\dot{V}_E$) was calculated by f × V_T. The system was calibrated before each experiment using a small rodent ventilator with adjustable f and V_T. The end-tidal carbon dioxide ($ETCO_2$) and isoflurane concentrations were measured from the tracheal tube using a side-stream gas analyzer (Nellcor, Hayward, CA). The aspirate from the gas analyzer was returned to the circle system to prevent loss of carbon dioxide and isoflurane.

The ventilatory response to increasing carbon dioxide was determined by closing the circuit and allowing the rat to rebreathe expired gases. The V_T, f, and $ETCO_2$ were continuously recorded until the $ETCO_2$ increased to 10% (about 3 min). Before determining the ventilatory response to carbon dioxide, a balloon was attached to the circuit and filled with 5% carbon dioxide and 95% oxygen, and the concentration of isoflurane being evaluated. Pilot studies (n = 6) showed that $ETCO_2$underestimated Pa_{CO_2} by 5 ± 4% at baseline and overestimated Pa_{CO_2} by 4 ± 4% and 14 ± 5% at 8% and 10% $ETCO_2$, respectively. Additional pilot studies (n = 6 for each group) showed that this relation was not altered by the presence of isoflurane or L-NAME.

Arterial blood gases were measured using a blood gas analyzer (Ciba-Corning, Medfield, MA) after obtaining 0.25 ml blood. Arterial pressure was measured on the strip chart recorder. L-NAME, L-NMMA, and L-arginine (Sigma Chemical Co., St. Louis, MO) were dissolved in saline, whereas 7-NI (Bio Mol, Aurora, OH) was dissolved in arachis oil. The effects of these drugs were evaluated 40 min after intravenous L-NAME or L-NMMA, or intraperitoneal 7-NI, as was done in previous studies.[9,10] The effects of L-arginine were evaluated 10 min after injection because of its shorter time of onset. The drug doses were based on similar MAC reductions, as previously described.[9,10]

Experimental Groups and Protocol

Fifty-four rats were assigned to one of six experimental groups (n = 9 for each group): time controls (group 1), L-NAME (group 2), L-NMMA (group 3), 7-NI (group 4), increasing isoflurane concentrations (group 5), and L-arginine (group 6). All groups had a baseline response to increasing carbon dioxide determined with the isoflurane concentration of 1.4%. Subsequent carbon dioxide responses were performed using 1.4% isoflurane unless otherwise stated. Group 1 or the time controls evaluated four successive carbon dioxide responses separated by 40 min. Groups 2 and 3 consisted of a baseline carbon dioxide response followed by three carbon dioxide responses at 40-min intervals after intravenous L-NAME or L-NMMA (accumulative doses of 1, 10, and 30 mg/kg). Group 4 consisted of a baseline carbon dioxide response followed by three carbon dioxide responses at 40-min intervals after intraperitoneal 7-NI (accumulative doses of 20, 80, and 400 mg/kg). Group 5 evaluated end-tidal isoflurane concentrations that would be the equivalent to the dose-dependent MAC reductions secondary to the NOS inhibitors. A baseline carbon dioxide response was followed by three carbon dioxide responses at 40-min intervals using isoflurane concentrations of 1.6%, 1.8%, and 2%. These concentrations are equivalent to the percentage decrease in MAC as determined by previous studies (*i.e.*, the largest doses of 7-NI and L-NAME decrease MAC by approximately 40%), thus changing the effective isoflurane concentration from 1.4 to 2%. Group 6 evaluated the effects of intravenous L-arginine alone. This consisted of a baseline carbon dioxide response and a second carbon dioxide response 10 min after L-arginine (300 mg/kg). L-arginine was also administered in two doses of 300 mg/kg separated by 5 min at the end of additional L-NAME experiments (n = 6) to evaluate for reversal of hemodynamic and ventilatory effects.

Arterial blood gas was measured before the first car-

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/88/5/1240/392978/0000542-199805000-00015.pdf by guest on 20 April 2024

bon dioxide response. The resting MAP, HR, f, and V_T were determined at the initiation of each carbon dioxide response. The f and V_T were determined at 8%, 9%, and 10% $ETCO_2$ from the mean of three values around each point. The carbon dioxide response curve was determined from the difference of f, V_T, and $\dot{V}_E$ between 8% and 10% $ETCO_2$. Experimental group comparisons for hemodynamics, resting ventilation, and carbon dioxide responses were made with time controls because f decreased with time. Differences were determined by repeated-measures two-way analysis of variance with Bonferroni's test. All data are presented as x ± SD.

Results

Two rats died of pulmonary edema after injection of the largest dose of L-NAME. The data from these rats were discarded and replaced by that of two additional rats in this group. All other rats survived the experimen tal protocol in good condition. Weight (481 ± 36 g) and temperature (37 ± 1°C) were not different among the six groups. The anesthetized baseline values for *p*H, Pa_{O_2}, and Pa_{CO_2} (7.32 ± 0.02, 392 ± 71 mmHg, and 48 ± 5 mmHg, respectively) were not different among the groups.

Hemodynamics

Baseline MAP and HR were the same in all groups. The MAP was significantly increased by L-NAME (10 and 30 mg/kg) but was not altered by L-NMMA or 7-NI when compared with time controls (fig. 1). The MAP was significantly decreased by isoflurane (1.8% and 2%). The HR was significantly decreased by 7-NI (400 mg/kg), isoflurane (2%), and L-NAME (10 and 30 mg/kg) but not altered by L-NMMA. L-arginine alone did not alter MAP or HR (table 1). L-arginine immediately reversed the effects of L-NAME on MAP (101 ± 17 mmHg) to a level not different than that of the controls.

Resting Ventilation

The baseline f, V_T, and $\dot{V}_E$ values were not different among the groups (table 2). L-NAME, L-NMMA, and 7-NI had no effect on resting f, whereas isoflurane (1.8% and 2%) significantly decreased f compared with time controls. The V_T was decreased by L-NAME and L-NMMA (10 and 30 mg/kg) and isoflurane (1.8% and 2%), whereas 7-NI had no effect. The $\dot{V}_E$ was significantly decreased by L-NAME and L-NMMA (30 mg/kg) and isoflurane (1.8% and 2%) but not altered by 7-NI. L-arginine alone did not alter f, V_T, or $\dot{V}_E$ (table 1). L-arginine reversed the effects of L-NAME (10 and 30 mg/kg) on resting V_T (3.7 ± 0.6 ml) and $\dot{V}_E$ (161 ± 36 ml/

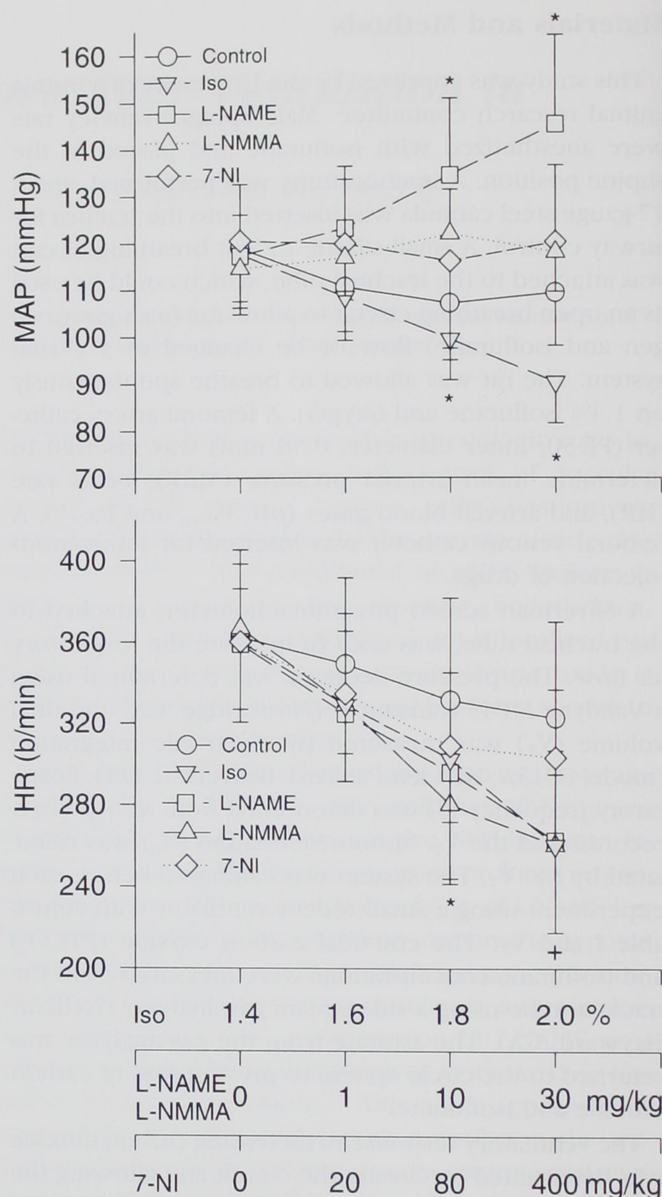

Fig. 1. The effect of 1, 10, and 30 mg/kg N^G-L-arginine methyl ester (L-NAME) and N^G-monomethyl L-arginine (L-NMMA); 20, 80, and 400 mg/kg 7-nitro-indazole (7-NI); or 1.6%, 1.8%, and 2% increasing isoflurane concentrations on resting heart rate (HR) and mean arterial pressure (MAP). For MAP, * denotes that L-NAME (10 and 30 mg/kg) significantly ($P < 0.05$) increased MAP, whereas isoflurane (1.8% and 2%) decreased MAP compared with time controls. For HR, * denotes that L-NAME decreased HR, whereas + denotes that L-NAME (30 mg/kg), 7-NI (400 mg/kg), and isoflurane (2%) decreased HR compared with time controls. Data are means ± SD.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/88/5/1240/392978/0000542-199805000-00015.pdf by guest on 20 April 2024

Table 1. Effects of L-Arginine on Hemodynamic and Ventilation

	Baseline	L-Arginine
MAP (mmHg)	114 ± 18	112 ± 12
HR (beats/min)	360 ± 20	357 ± 21
f (breaths/min)	60 ± 12	58 ± 12
V_T (ml)	3.2 ± 0.6	3.2 ± 0.6
V_E (ml/min)	192 ± 44	186 ± 42
V_E 8%	228 ± 27	234 ± 32
V_E 9%	260 ± 28	270 ± 38
V_E 10%	308 ± 41	308 ± 37
V_E/ET_{CO_2} (ml/min/%)	40 ± 19	37 ± 19

The effects of L-arginine (300 mg/kg) on mean and arterial pressure (MAP), heart rate (HR), respiratory frequency (f), tidal volume (V_T), and minute ventilation (V_E) at rest and 8, 9, and 10% end-tidal carbon dioxide (ET_{CO_2}). L-Arginine did not significantly alter any variable. Data are mean ± SD.

Table 2. Effects of L-NAME, L-NMMA, 7 NI, and Isoflurane on Resting Ventilation

	f (breaths/min)	V_T (ml)	V_E (ml/min)
Controls			
Time 1	60 ± 6	3.2 ± 0.3	190 ± 23
Time 2	53 ± 9	3.4 ± 0.5	182 ± 44
Time 3	50 ± 9	3.7 ± 0.5	186 ± 39
Time 4	44 ± 8	3.8 ± 0.5	170 ± 39
L-NAME			
0 mg/kg	60 ± 7	3.1 ± 0.6	183 ± 35
1 mg/kg	53 ± 11	3.2 ± 0.6	166 ± 44
10 mg/kg	46 ± 14	3.1 ± 0.6*	137 ± 41*
30 mg/kg	40 ± 13	3.1 ± 0.6*	121 ± 32*
L-NMMA			
0 mg/kg	59 ± 6	3.1 ± 0.6	180 ± 30
1 mg/kg	52 ± 9	3.2 ± 0.6	163 ± 41
10 mg/kg	45 ± 14	3.0 ± 0.4*	134 ± 38*
30 mg/kg	40 ± 13	3.0 ± 0.5*	118 ± 31*
7-NI			
0 mg/kg	57 ± 6	3.2 ± 0.3	181 ± 32
20 mg/kg	48 ± 8	3.7 ± 0.3	163 ± 32
80 mg/kg	43 ± 9	3.4 ± 0.3	148 ± 29
400 mg/kg	39 ± 6	3.6 ± 0.3	148 ± 32
Isoflurane			
1.4%	61 ± 6	3.0 ± 0.6	182 ± 25
1.6%	51 ± 9	3.3 ± 0.6	167 ± 21
1.8%	37 ± 6*	3.2 ± 0.6*	120 ± 24*
2.0%	32 ± 8*	3.3 ± 0.6*	105 ± 30*

The effect of L-NAME and L-NMMA (1, 10, and 30 mg/kg), 7-NI (20, 80, and 400 mg/kg), or increasing isoflurane concentrations (1.6, 1.8, and 2.0%) on the resting respiratory frequency (f), tidal volume (V_T), and minute ventilation (V_E). Data are mean ± SD.

* $P < 0.05$ *versus* time controls (1.4% isoflurane).

min) such that they were no different than time controls. The baseline resting $ETCO_2$ (46 ± 4 mmHg or 6.1%) was not different among the six groups. Isoflurane (1.8% and 2%) significantly increased the $ETCO_2$ (53 ± 4 and 54 ± 4 mmHg), whereas the resting $ETCO_2$ was not significantly affected in the other groups.

Ventilatory Response to Increasing Carbon Dioxide

The baseline measurements at 8%, 9%, and 10% $ETCO_2$ for f (59 ± 12, 59 ± 12, and 58 ± 10 breaths/min), V_T (4.1 ± 0.6, 4.8 ± 0.6, and 5.5 ± 0.6 ml), and $\dot{V}_E$ (230 ± 29, 260 ± 27, and 305 ± 32 ml/min) in the time controls were not different than the other groups. L-NAME, L-NMMA, and 7-NI did not alter f, whereas f values were decreased by isoflurane (1.8% and 2%) at 8%, 9%, and 10% $ETCO_2$ compared with time controls (fig. 2). The V_T was decreased by L-NAME and L-NMMA (10 and 30 mg/kg) and isoflurane (1.8% and 2%) at 8%, 9%, and 10% $ETCO_2$ (fig. 3). 7-NI had no effect on V_T. The $\dot{V}_E$ was decreased significantly by L-NAME and L-NMMA (10 and 30 mg/kg) and isoflurane (1.8% and 2%) but was not altered by 7-NI at 8%, 9%, and 10% $ETCO_2$ (fig. 4). L-arginine alone had no effect on ventilation at 8%, 9%, and 10% $ETCO_2$ (table 1, f and V_T not shown). L-arginine reversed the effects of L-NAME (30 mg/kg) on V_T (3.9 ± 0.9 and 4.8 ± 0.9 ml) and $\dot{V}_E$ (160 ± 29 and 218 ± 38 ml/min) at 8% and 10% $ETCO_2$ such that they were no different than time controls.

The change in $\dot{V}_E$ *versus* the change in $ETCO_2$ was significantly decreased by 2% isoflurane compared with values for the time controls (fig. 5). Isoflurane (2%) also decreased the change in V_T *versus* the change in $ETCO_2$ but did not alter f values *versus* $ETCO_2$. L-NAME, L-NMMA, 7-NI, and L-arginine did not significantly alter the change in f, V_T, or $\dot{V}_E$ *versus* with $ETCO_2$.

Discussion

We administered nonselective NOS inhibitors (L-NAME and L-NMMA) or a selective nNOS inhibitor (7-NI) to rats to determine the role of NO in modulating ventilation. The nonselective NOS inhibitors decreased V_T and $\dot{V}_E$ at rest and at increased $ETCO_2$ levels (8–10%). These effects demonstrate a role for NO in the control of spontaneous ventilation in rats anesthetized with isoflurane. In contrast to nonselective NOS inhibitors, a selective neuronal NOS inhibitor did not alter ventilation. Further, the ventilatory changes due to L-

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/88/5/1240/392978/0000542-199805000-00015.pdf by guest on 20 April 2024

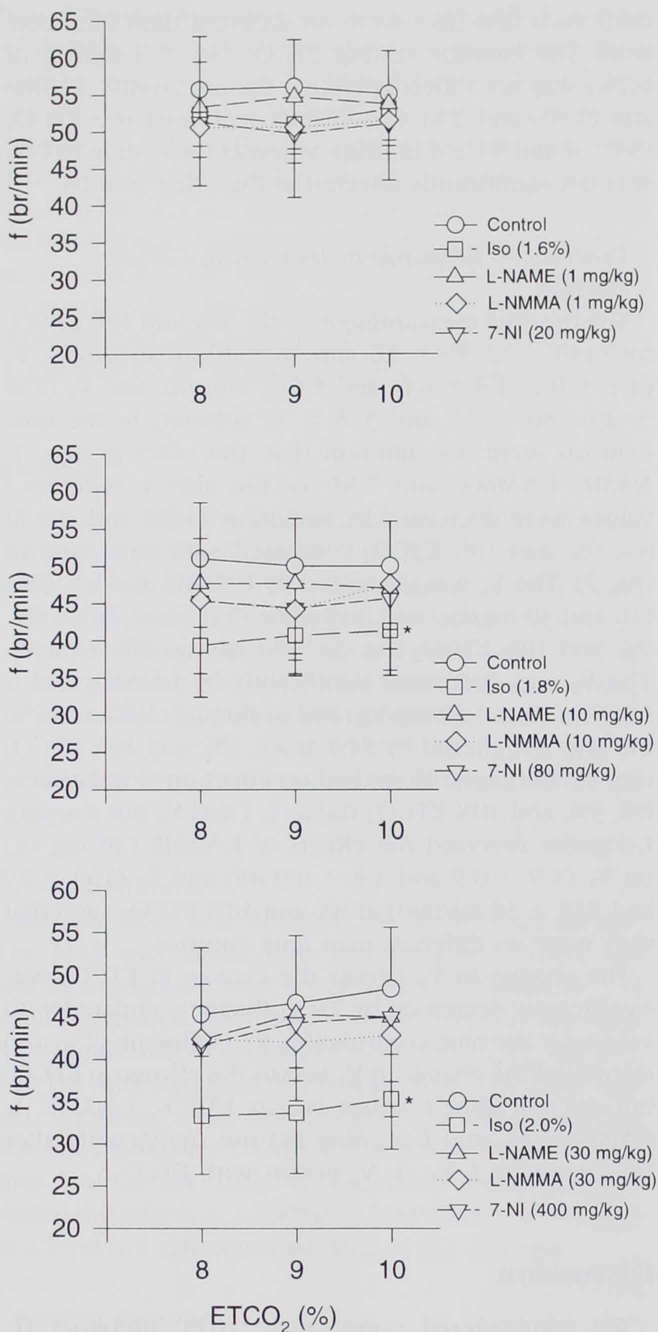

Fig. 2. The effect of 1, 10, and 30 mg/kg N^G-L-arginine methyl ester (L-NAME) and N^G-monomethyl L-arginine (L-NMMA); 20, 80, and 400 mg/kg 7-nitro-indazole (7-NI); or 1.6%, 1.8%, and 2% increasing isoflurane concentrations on respiratory frequency (f) at 8%, 9%, and 10% end-tidal carbon dioxide ($ETCO_2$). * Isoflurane (1.8% and 2%) significantly ($P < 0.05$) decreased f at 8%, 9%, and 10% $ETCO_2$ compared with time controls. Data are means ± SD.

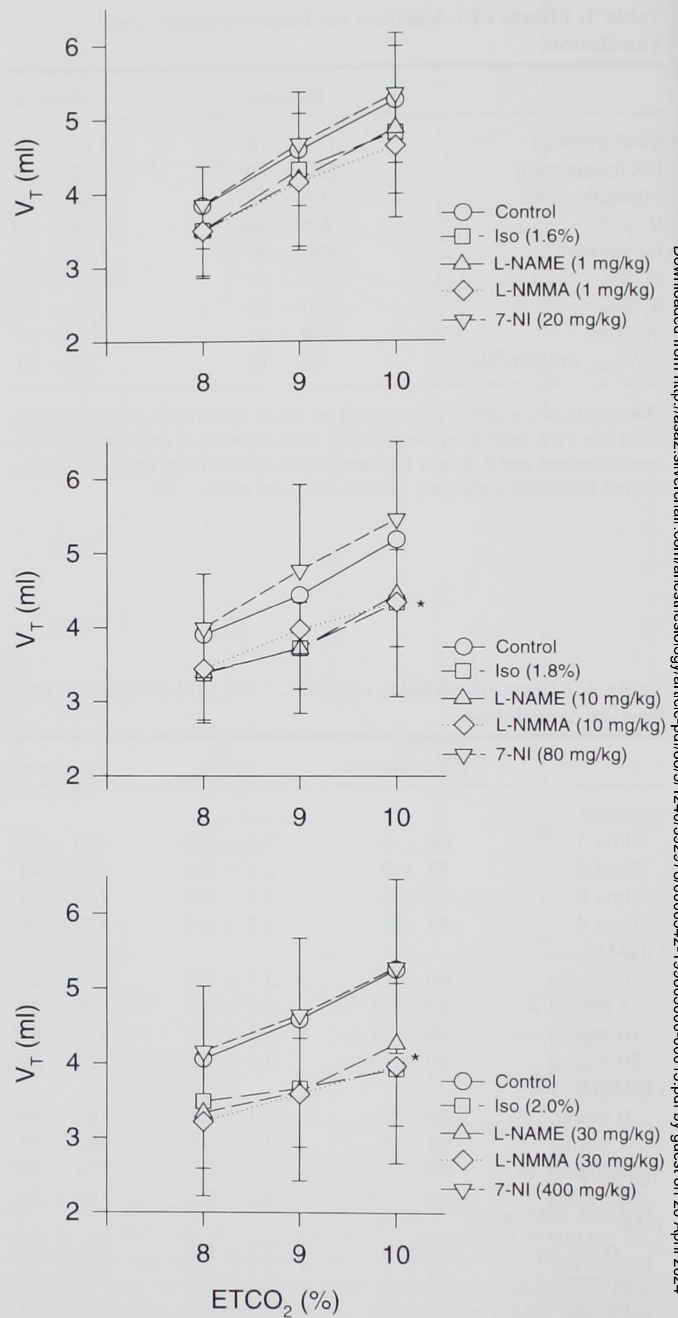

Fig. 3. The effect of 1, 10, and 30 mg/kg N^G-L-arginine methyl ester (L-NAME) and N^G-monomethyl L-arginine (L-NMMA); 20, 80, and 400 mg/kg 7-nitro-indazole (7-NI); or 1.6%, 1.8%, and 2% increasing isoflurane concentrations on tidal volume (V_T) at 8%, 9%, and 10% end-tidal carbon dioxide ($ETCO_2$). *L-NAME and L-NMMA (10 and 30 mg/kg), and isoflurane (1.8% and 2%) significantly ($P < 0.05$) decreased V_T at 8%, 9%, and 10% $ETCO_2$ compared with time controls. Data are means ± SD.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/88/5/1240/392978/0000542-199805000-00015.pdf by guest on 20 April 2024

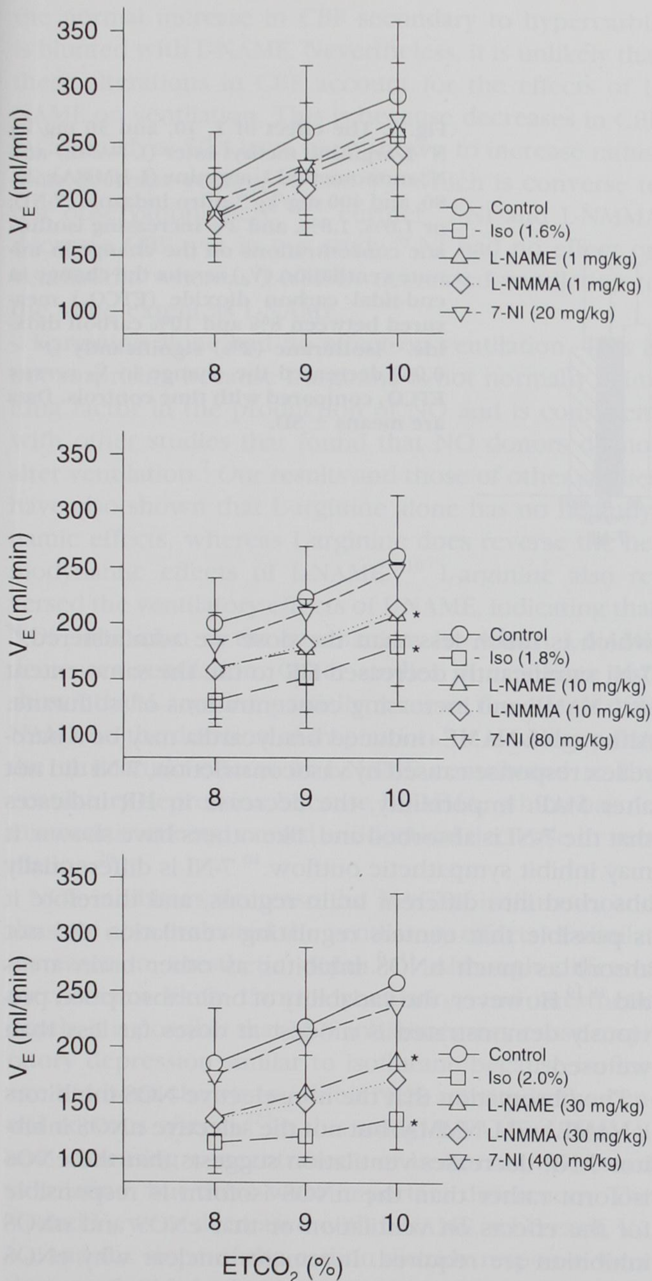

Fig. 4. The effect of 1, 10, and 30 mg/kg N^G-L-arginine methyl ester (L-NAME) and N^G-monomethyl L-arginine (L-NMMA); 20, 80, and 400 mg/kg 7-nitro-indazole (7-NI); or 1.6%, 1.8%, and 2% increasing isoflurane concentrations on minute ventilation ($\dot{V}_E$) at 8%, 9%, and 10% end-tidal carbon dioxide ($ETCO_2$). * L-NAME and L-NMMA (10 and 30 mg/kg) and isoflurane (1.8% and 2%) significantly ($P < 0.05$) decreased $\dot{V}_E$ at 8%, 9%, and 10% $ETCO_2$ compared with time controls. Data are means ± SD.

NAME and L-NMMA were not the same as the ventilatory changes resulting from increasing isoflurane anesthetic depth, which is also caused by NOS inhibitors.

Nitric oxide is involved in various neurotransmitter pathways, although the role of NO in control of ventilation is poorly understood. Nitric oxide synthase has been localized within the central nervous system, including the pontine respiratory group of the brain stem.[5,6] Excitatory and inhibitory pathways involving NO exist within the central nervous system, which may play a role in modulating ventilation. The N-methyl-D-aspartate (NMDA) and muscarinic pathways are excitatory and increase NO-cyclic guanosine monophosphate.[6,12] Activation of brain stem muscarinic receptors has been shown to stimulate ventilation[12] and depress the hypercapnic response,[13] whereas NMDA receptor antagonism has been shown to have minimal effects on inspiratory duration.[14] The γ-aminobutyric acid and α_2-adrenergic pathways, in contrast, are inhibitory and γ-aminobutyric acid receptor activity decreases neuronal cyclic guanosine monophosphate.[12,15] Cerebral intraventricular injection of γ-aminobutyric acid and the α-agonist clonidine have been shown to decrease ventilation in dogs,[15] whereas γ-aminobutyric acid antagonism has minimal effects in cats.[14] It is possible that a combination of blockade of excitatory components, stimulation of inhibitory components of the central nervous system, or both may depress ventilation. This was suggested by Haxhiu *et al.*,[6] who showed that NOS inhibition decreased the ventilatory response to hypoxia while simultaneously decreasing brain stem cyclic guanosine monophosphate.

In our study, L-NAME and L-NMMA caused a significant decrease in V_T and $\dot{V}_E$ at rest and at higher $ETCO_2$ levels, whereas f and the slope of the carbon dioxide response were not altered. Ling *et al.*[5] previously showed that microinjection of L-NAME into the pontine respiratory group prolongs the duration of inspiratory time in cats. Although these authors found that NO is important in respiratory rhythm generation, the effect of NOS inhibitors on f, V_T, $\dot{V}_E$, and the slope of the carbon dioxide response was not determined. Haxhiu *et al.*[6] showed that long-term systemic administration of L-NAME to rats decreased the ventilatory response to hypoxia. This attenuation of ventilation resulting from systemic NOS inhibition is consistent with our results that showed a decrease in ventilation with L-NAME and L-NMMA. In contrast, infusion of L-NAME or a selective nNOS inhibitor into the cerebral spinal fluid of awake dogs was recently shown to slightly decrease resting

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/88/5/1240/392978/0000542-199805000-00015.pdf by guest on 20 April 2024

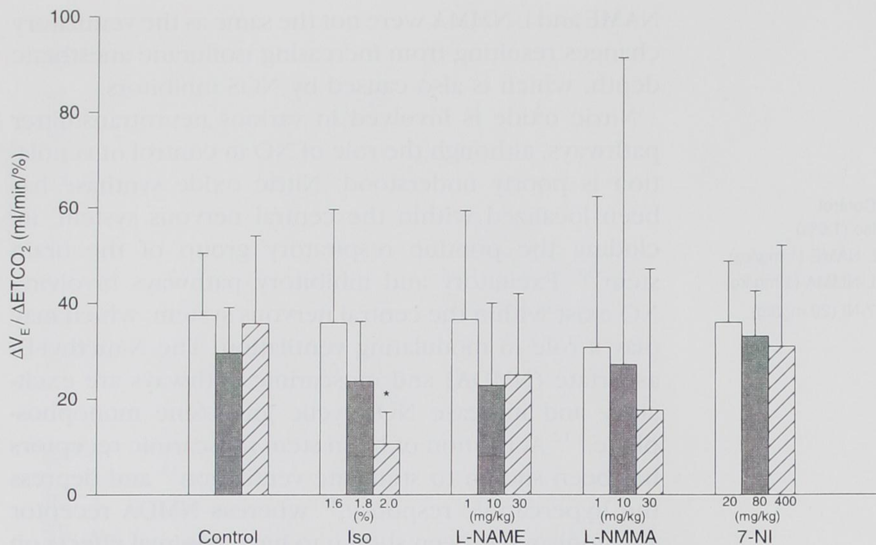

Fig. 5. The effect of 1, 10, and 30 mg/kg N^G-L-arginine methyl ester (L-NAME) and N^G-monomethyl L-arginine (L-NMMA); 20, 80, and 400 mg/kg 7-nitro-indazole (7-NI); or 1.6%, 1.8%, and 2% increasing isoflurane concentrations on the change in minute ventilation ($\dot{V}_E$) *versus* the change in end-tidal carbon dioxide ($ETCO_2$) measured between 8% and 10% carbon dioxide. * Isoflurane (2%) significantly ($P < 0.05$) decreased the change in $\dot{V}_E$ *versus* $ETCO_2$ compared with time controls. Data are means ± SD.

$ETCO_2$ and inhibit morphine-induced respiratory depression.[7,8] This increase in ventilation contrasts with our results, but many methodologic differences between the studies may make comparisons difficult. It is possible that the site of NOS inhibitor administration produces different ventilatory effects, although intraperitoneal and intracerebral ventricular L-NAME produces similar antinociceptive effects in mice.[16] We also cannot preclude that systemic L-NAME and L-NMMA alters neuronal transmission outside the central nervous system, which would be less likely to occur with intraventricular injection. It is also possible that there are species differences or that differences are possibly a result of an anesthetized compared with an awake state.

7-NI had no effect on ventilation, which is surprising because neuronal transmission is thought to be controlled by nNOS.[3] Only one study evaluated the effects of a selective nNOS inhibitor on ventilation. Laurito *et al.*[8] found that cerebral spinal fluid infusion of a nNOS inhibitor slightly increased ventilation to the same extent as L-NAME in awake dogs, implying that nNOS is the responsible isoform. In contrast, our study suggests that nNOS may not play a significant role in control of ventilation. It is unclear if the animal model or route of administration is responsible for the differences in results between the two studies. It is unlikely that insufficient 7-NI was administered in our study because the doses and route of administration of 7-NI that we used were shown to produce a 10–40% reduction in MAC.[10] Intraperitoneal 7-NI also exhibits antinociceptive activity in mice with a median effective dose of 25 mg/kg, which is much less than the dose we administered.[17] 7-NI significantly decreased HR to the the same extent as L-NAME and increasing concentrations of isoflurane. Although L-NAME–induced bradycardia may be a baroreflex response caused by vasoconstriction, 7-NI did not alter MAP. Importantly, the decrease in HR indicates that the 7-NI is absorbed and, like others have shown, it may inhibit sympathetic outflow.[10] 7-NI is differentially absorbed into different brain regions, and therefore it is possible that centers regulating ventilation did not absorb as much nNOS inhibitor as other brain areas did.[18,19] However, the variability of brain absorption previously demonstrated is modest at doses far less than we used.

The observation that the nonselective NOS inhibitors L-NAME and L-NMMA, but not the selective nNOS inhibitor 7-NI, decreases ventilation suggests that the eNOS isoform rather than the nNOS isoform is responsible for the effects on ventilation or that eNOS and nNOS inhibition are required. It remains unclear why eNOS but not nNOS inhibition would alter ventilation, especially because both produce other neuronally mediated changes, such as a decrease in HR and a decrease in MAC.[9–11] The primary role of eNOS is the regulation of vascular tone *via* its effects on the underlying vascular smooth muscle. L-NAME causes vasoconstriction, which has been shown to decrease total or regional cerebral blood flow (CBF).[20] It has also been shown that the vasodilating responsiveness of CBF to carbon dioxide is decreased in the presence of L-NAME.[3,21] Thus it is likely that baseline CBF is decreased and that

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/88/5/1240/392978/0000542-199805000-00015.pdf by guest on 20 April 2024

the normal increase in CBF secondary to hypercarbia is blunted with L-NAME. Nevertheless, it is unlikely that these alterations in CBF account for the effects of L-NAME on ventilation. This is because decreases in CBF by as much as 50% have been shown to increase rather than decrease resting V_T and $\dot{V}_E$, which is converse to our observations here.[22,23] Further, 7-NI and L-NMMA decrease CBF, yet in our study 7-NI had no effect on ventilation, whereas L-NMMA decreased ventilation to the same extent as L-NAME.[3,24,25]

L-arginine alone had no effect on ventilation. This is not surprising because L-arginine is not normally a limiting factor in the production of NO and is consistent with other studies that found that NO donors do not alter ventilation.[7] Our results and those of other studies have also shown that L-arginine alone has no hemodynamic effects, whereas L-arginine does reverse the hemodynamic effects of L-NAME.[9,10] L-arginine also reversed the ventilatory effects of L-NAME, indicating that NOS inhibition is the mechanism by which L-NAME decreases ventilation. Other studies in cats have also shown that L-arginine partially reverses the effects of L-NAME on the duration of inspiration.[6] It is unlikely that the muscarinic effects of L-NAME contributed to the ventilatory depression because L-NMMA, which has no muscarinic effects, caused an identical decrease in ventilation.[26]

NOS inhibitors decrease the MAC of isoflurane, and thus we also evaluated the ventilatory effects of similar increases in anesthetic depth.[9-11] Although different mechanisms likely control ventilation and anesthetic depth, it is possible that NOS inhibitors may cause respiratory depression similar to isoflurane because isoflurane has been shown to inhibit NOS activity in the central nervous system.[27] In this study, L-NAME, L-NMMA, and increasing concentrations of isoflurane decreased ventilation, but there were important differences. The V_T and $\dot{V}_E$ were decreased with L-NAME, whereas f, V_T, $\dot{V}_E$, and the slope of the carbon dioxide response were decreased with isoflurane. L-NAME and L-NMMA do not alter the slope of the carbon dioxide response, which has been well demonstrated with isoflurane.[28] Therefore, the mechanisms by which NOS inhibitors decrease ventilation are probably not entirely the result of increased anesthetic depth.

In conclusion, nonselective NOS inhibitors decreased V_T and $\dot{V}_E$ at rest and at increased carbon dioxide levels, implicating a role for NO in modulating spontaneous ventilation during isoflurane anesthesia. A selective nNOS inhibitor had no effect, which suggests that eNOS may be the isoform responsible for altering ventilation. This study shows that L-NAME and L-NMMA decrease ventilation in rats anesthetized with isoflurane, and that the cause of the decreased ventilation is most likely not secondary to the enhanced anesthetic depth caused by NOS inhibitors.

References

1. Bredt DS, Snyder SH: Nitric oxide, a novel neuronal messenger. Neuron 1992; 8:3-11

2. Furchgott RF: Role of endothelium in responses of vascular smooth muscle. Circ Res 1983; 53:557-73

3. Iadecola C, Pelligrino DA, Moskowitz MA, Lassen NA: Nitric oxide synthase inhibition and cerebrovascular regulation. J Cereb Blood Flow Metab 1994; 14:175-92

4. Moncada S, Palmer RMJ, Higgs EA: Nitric oxide: Physiology, pathophysiology, and pharmacology. Pharmacol Rev 1991; 43:109-42

5. Ling L, Karius DR, Fiscus RR, Speck DF: Endogenous nitric oxide required for an integrative respiratory function in the cat brain. J Neurophysiol 1992; 68:1910-2

6. Haxhiu MA, Chang CH, Dreshaj IA, Erokwu B, Prabhakar NR, Cherniack NS: Nitric oxide and ventilatory response to hypoxia. Resp Physiol 1995; 101:257-66

7. Peligrino DA, Laurito CE, VandeBoncouer TR: Nitric oxide synthase inhibition modulates the ventilatory depressant and antinociceptive actions of fourth ventricular infusions of morphine in the awake dog. Anesthesiology 1996; 85:1367-77

8. Laurito CL, VadeBoncouer TR, Prillaman K, Pelligrino DA: Neuronally-derived nitric oxide (NO) and the ventilatory depression and analgesia accompanying 4th ventricular infusion of morphine in the awake dog [Abstract]. Anesthesiology 1996; 85:A653

9. Johns RA, Moscicki JC, DiFazio CA: Nitric oxide synthase inhibitor dose-dependently and reversibly reduces the threshold for halothane anesthesia: A role for nitric oxide in mediating consciousness? Anesthesiology 1992; 77:779-84

10. Pajewski TN, DiFazio CA, Moscicki JC, Johns RA: Nitric oxide synthase inhibitors, 7-nitro indazole and nitro^G-L-arginine methyl ester, dose dependently reduce the threshold for isoflurane anesthesia. Anesthesiology 1996; 85:1111-9

11. Ichinose F, Huang PL, Zapol WM: Effects of targeted neuronal nitric oxide synthase gene disruption and nitro^G-L-arginine methylester on the threshold for isoflurane anesthesia. Anesthesiology 1995; 83:101-8

12. Burton M, Johnson D, Kazemi H: Adrenergic and cholinergic interaction in central ventilatory control. J Appl Physiol 1990; 68:2092-9

13. Lydic R, Baghdoyan H, Wertz R, White D: Cholinergic reticular mechanisms influence state-dependent ventilatory response to hypercapnia. Am J Physiol (Reg Int Comp Physiol) 1991; 261:738-46

14. Ling L, Darius DR, Speck DF: Pontine-evoked inspiratory inhibitions after antagonism of NMDA, $GABA_A$, or glycine receptor. J Appl Physiol 1993; 74:1265-73

15. Kneussl M, Pappagianopoulos P, Hoop B, Kazemi H: Reversible depression of ventilation and cardiovascular function by ventriculocisternal perfusion with gamma-aminobutyric acid in dogs. Am Rev Respir Dis 1986; 133:1024-8

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/88/5/1240/392978/0000542-199805000-00015.pdf by guest on 20 April 2024

16. Moore PK, Oluyomi AO, Babbedge RC, Wallace P, Hart SL: L-N^G-nitro arginine methyl ester exhibits antinociceptive activity in the mouse. Br J Pharmacol 1991; 102:198-202

17. Moore PK, Babbedge RC, Wallace P, Gaffen ZA, Hart SL: 7-nitro indazole, an inhibitor of nitric oxide synthase, exhibits antinociceptive activity in the mouse without increasing blood pressure. Br J Pharm 1993; 108:296-7

18. Kalisch BE, Connop BP, Jhamandas K, Beninger RJ, Boegman RJ: Differential action of 7-nitro indazole on rat brain nitric oxide synthase. Neurosci Lett 1996; 219:75-8.

19. Babbedge RC, Blank-Ward PA, Hart SL, Moore PK: Inhibition of rat cerebellar nitric oxide synthase by 7-nitro indazole and related substituted indazoles. Br J Pharmacol 1993; 110:225-8

20. Lorente JA, Landin L, De Pablo R, Renes E, Liste D: L-arginine pathway in the sepsis syndrome. Crit Care Med 1993; 21:1287-95

21. Sandor P, Komjati K, Reivich M, Nyary I: Major role of nitric oxide in the mediation of regional CO_2 responsiveness of the cerebral and spinal cord vessels of the cat. J Cereb Blood Flow Metab 1994; 14:49-58

22. Chapman RW, Santiago TV, Edelman NH: Effects of graded reduction of brain blood flow on ventilation in unanesthetized goats. J Appl Physiol: Respir Environ Exercise Physiol 1979; 47:104-11

23. Chapman RW, Santiago TV, Edelman NH: Effects of graded reduction of brain blood flow on chemical control of breathing. J Appl Physiol: Respir Environ Exercise Physiol 1979; 47:1289-94

24. Kovach AGB, Lohinai Z, Marczis J, Balla I, Dawson TM, Syndor SH: The effect of hemorrhagic hypotension and retransfusion and 7-nitro-indazole on rCBF, NOS catalytic activity, and cortical NO content in the cat. Ann N Y Acad Sci 1994; 738:348-68

25. Wang Q, Pelligrino DA, Baughman VL, Koenig HM, Albrecht RF: The role of neuronal nitric oxide synthase in regulation of cerebral blood flow in normocapnia and hypercapnia in rats. J Cereb Blood Flow Metab 1995; 15:774-8

26. Buxton ILO, Cheek DJ, Eckman D, Westfall DP, Sanders KM, Keef KD: N^G-nitro L-arginine methyl ester and other alkyl esters of arginine are muscarinic receptor antagonists. Circ Res 1993; 72:387-95

27. Zuo Z, Johns RA: Halothane, enflurane, and isoflurane do not affect vasal or agonist-stimulated activity of partially isolated soluble and particulate guanylyl cyclases of rat brain. ANESTHESIOLOGY 1995; 83:395-404

28. Fourcade HE, Stevens WC, Larson CP: The ventilatory effects of Forane, a new inhaled anesthetic. ANESTHESIOLOGY 1971; 35:26-31

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/88/5/1240/392978/0000542-199805000-00015.pdf by guest on 20 April 2024