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Nitric Oxide Synthase Inhibitors, 7-Nitro Indazole and Nitro^{G}-L-Arginine Methyl Ester, Dose Dependently Reduce the Threshold for Isoflurane Anesthesia

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Background: **Nitric oxide (NO), a recognized cell messenger for activating soluble guanylate cyclase, is produced by the enzyme NO synthase in a wide variety of tissues, including vascular endothelium and the central nervous system. The authors previously reported the possible involvement of the NO pathway in the anesthetic state by showing that a specific NO synthase inhibitor, nitro^{G}-L-arginine methyl ester (L-NAME), dose dependently and reversibly decreases the minimum alveolar concentration (MAC) for halothane anesthesia. The availability of a structurally distinct inhibitor selective for the neuronal isoform of NO synthase, 7-nitro indazole (7-NI), allowed for the possibility of dissociating the central nervous system effects of neuronal NO synthase inhibition from the cardiovascular effects of endothelial NO synthase inhibition.**

Methods: **The effect of two structurally distinct inhibitors of NO synthase, L-NAME and 7-NI, on the MAC of isoflurane was investigated in Sprague-Dawley rats while concurrently monitoring the animals' arterial blood pressure and heart rate. L-NAME (1 to 30 mg/kg given intravenously, dissolved in 0.9% saline) and 7-NI (20 to 1,000 mg/kg given intraperitoneally, dissolved in arachis oil) were administered after determining control MAC and 30 min before determining MAC in the presence of NO synthase inhibitor.**

Results: **L-NAME and 7-NI caused a dose-dependent decrease from isoflurane control MAC (maximal effect: 35.5 ± 2.5% and 43.0 ± 1.7%, respectively) with a ceiling effect observed for both NO synthase inhibitors (above 10 mg/kg and 120 mg/kg, respectively). L-NAME administration significantly increased systolic and diastolic blood pressures (maximal effect: 39.9 ± 2.2% and 64.3 ± 4.0%, respectively), which were not accompanied by any changes in heart rate. 7-NI administration resulted in no changes in blood pressure and a small but clinically insignificant decrease in heart rate.**

Conclusions: **Inhibition of the NO synthase pathway decreased the MAC for isoflurane, which suggests that inhibition of the NO pathway decreases the level of consciousness and augments sedation, analgesia, and anesthesia. The MAC reduction by two structurally distinct NO synthase inhibitors supports that this is a specific effect on NO synthase. Furthermore, the action of the neuronal NO synthase inhibitor 7-NI supports an effect selective for neuronal NO synthase and also avoids the hypertensive response of generalized NO synthase inhibitors. (Key words: Anesthetics, volatile: Isoflurane. Nitric oxide: nitric oxide synthase inhibitors; Nitro^{G}-L-arginine methyl ester; 7-nitro indazole. Potency: minimum alveolar concentration.)**

NITRIC oxide (NO), first discovered as a potent vasodilator produced by vascular endothelium, is now recognized as the transduction mechanism responsible for activating soluble guanylate cyclase and has been demonstrated in a wide variety of tissue types, including the central nervous system.[1,2] Nitric oxide is synthesized enzymatically by the oxidation of a guanidino nitrogen atom of L-arginine.[3-5] Both constitutive and inducible forms of NO synthase have been described and both are competitively inhibited by analogs of L-arginine, such as nitro^{G}-L-arginine methyl ester (L-NAME) and N^{G}-monomethyl-L-arginine.[4,6] Immunohistochemical localization studies indicate that the constitutive NO synthase is concentrated in endothelial, neuronal, and secretory tissues.[7] Nitric oxide has been shown to mediate the increase in cerebellar cyclic guanosine monophosphate (cGMP) content in response to N-methyl-D-aspartate (NMDA), kainate, and to glutamate.[8-11] Neuronal NO

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synthase activity has been demonstrated in the spinal cord and in several brain regions, including the cerebellum, hypothalamus, midbrain, striatum, and hippocampus.[12,13]

Halothane and other inhalational anesthetics inhibit NO production in vascular endothelium.[14,15] Halothane and enflurane have been shown to decrease cGMP content and to modify synaptic transmission in specific brain regions, including the cerebellum, hippocampus, midbrain, hypothalamus, olfactory bulb, and pituitary gland.[16,17] In addition, halothane depresses synaptic transmission by L-glutamate-stimulated cortical neurons and NMDA-stimulated CA-1 neurons of the hippocampus.[18-20] These observations suggested the hypothesis that inhibition of the L-arginine to NO pathway in the central nervous system may result in a sedative, analgesic, or anesthetic effect. We previously reported the effect of L-NAME, a specific inhibitor of NO synthase on the threshold for halothane anesthesia as assessed by the ability of L-NAME to reduce the MAC for halothane in Sprague-Dawley rats.[21] The MAC reduction by L-NAME was accompanied by an increase in systolic and diastolic blood pressure and a small decrease in heart rate. The hemodynamic effects of L-NAME administration that we observed here are similar to those observed by other investigators in rats and in dogs and are probably a result of the inhibition of endothelial cell NO production.[22-25]

Recently a neuronally selective NO synthase inhibitor, 7-nitro indazole (7-NI), was developed that demonstrates selectivity for cerebellar NO synthase while being devoid of effects on the cardiovascular system.[26] Although it has minimal pressor effects *in vivo*, 7-NI is a very potent inhibitor of NO synthase activity in homogenates derived from endothelial cells.[27] The selectivity of 7-NI appears to be due to a preferential uptake of the inhibitor into neuronal cells rather than endothelial cells.[28] We tried to use this neuronally selective NO synthase inhibitor, which is structurally distinct from L-arginine analogs, to confirm the specificity of the MAC reducing effect and to more directly implicate neuronal NO synthase in MAC reduction. Because the hemodynamic manifestations of NO synthase inhibition, presumably from their effect on endothelial NO synthase activity, appeared to limit the utility of L-NAME in the *in vivo* setting, we compared the efficacy of L-NAME to the efficacy of the neuronally selective 7-NI in reducing the MAC for isoflurane anesthesia.

Materials and Methods

After obtaining institutional animal care committee approval, we examined the reduction of isoflurane MAC in response to increasing concentrations of 7-NI and L-NAME. Isoflurane was obtained from Ohmeda (Liberty Corner, NJ). Nitro[G]-L-arginine methyl ester, L-arginine, and arachis oil were obtained from Sigma Chemical Company (St. Louis, MO). 7-Nitro indazole was obtained from Bio Mol (Aurora, OH).

Sixty-six male Sprague-Dawley rats (296 ± 3 g) were each placed in a clear plastic cone and anesthetized with 5% isoflurane and oxygen for 3 to 5 min. The inspired isoflurane concentration was reduced to 2% and the animal was allowed to breathe spontaneously until cannulation of a femoral artery and vein with PE50 tubing had been accomplished. The trachea was then intubated with a 16-gauge polyethylene catheter. The isoflurane concentration was decreased further to 1.5% and ventilation was controlled with a Harvard animal respirator using measurement of arterial blood gases to maintain normal p_{O_2}, p_{CO_2}, and pH. Electrocardiogram and systolic and diastolic blood pressures were monitored using a Grass polygraph and Gould pressure transducer. Temperature was measured using a Yellow Springs (Yellow Springs Instrument Company, Inc., Yellow Springs, OH) thermistor and maintained at normothermia with a heating blanket and warning lights.

A fine polyethylene catheter, PE10, was introduced through and beyond the endotracheal tube until obstruction to passage was met and then withdrawn 1 to 2 mm. Gas samples for measuring alveolar anesthetic concentrations were obtained by withdrawing 10 ml gas through the catheter into gas-tight glass syringes over 3 to 5 min at the time of tail clamp and then assayed using gas chromatography with a Varian model 3700 chromatograph with a flame ionization detector. Constant alveolar concentration of isoflurane was verified by analyzing triplicate samples. Control MAC was established according to the methods described by Eger and colleagues[29] using a long hemostat (8-inch Rochester Dean Hemostatic Forceps) clamped to the first ratchet lock on the tail for 1 min. The tail was always stimulated proximal to a previous test site. Gross movement of the head, extremities, and/or body was taken as a positive test, whereas grimacing, swallowing, chewing, or tail flick were considered negative. The isoflurane concentration was reduced in decrements of 0.12% to 0.15% until the negative response became positive,

allowing 12- to 15-min equilibration after changes in concentration.[30] The MAC was considered to be the concentration midway between the highest concentration that permitted movement in response to the stimulus and the lowest concentration that prevented movement.

Due to the different solubilities of the two NO synthase inhibitors investigated in this article, the 7-NI was administered *via* intraperitoneal injection after sonication in arachis oil and the L-NAME, being soluble in aqueous solutions, was administered *via* intravenous bolus.[25] The volume in which the drugs were administered was held constant. 7-NI and L-NAME were administered in 3 ml arachis oil and 1 ml H_2O, respectively. After initial baseline MAC determination, 7-NI at a dose of 20, 60, 80, 120, 500, or 1,000 mg/kg was administered *via* intraperitoneal injection after sonication in arachis oil. L-NAME at 1, 5, 10, 20, or 30 mg/kg was administered as an intravenous bolus. The order of administration of the various doses of NO synthase inhibitors was performed in an unblinded, random manner. Four to six animals were studied at each of the inhibitor concentrations. An isoflurane concentration was chosen at which movement did not occur in the last negative response before the positive test. At this isoflurane concentration, 30 min after the administration of 7-NI or L-NAME, MAC was again determined with the concentration of isoflurane reduced and response to tail clamp checked every 12 to 15 min thereafter until a positive response was achieved.

L-arginine was infused as a 300-mg/kg bolus in two animals after MAC determination in the presence of L-NAME (30 mg/kg). Within 1 min after administering L-arginine, both animals appeared to awaken as assessed by spontaneous movement. Thus they were returned to a concentration of isoflurane above their control MAC level. The isoflurane concentration was then reduced in decrements of 0.12 to 0.15% until the negative response became positive, allowing 12 to 15 min equilibration after changes in concentration, permitting determination of the MAC after L-arginine reversal.

To control for the possibility that the vehicle (arachis oil) used to administer 7-NI may have had an effect on MAC determinations, five animals had their MACs determined under isoflurane anesthesia as described above. Forty-five and 120 min after administering 3 ml arachis oil, the MAC was again determined. The stability of the MAC determinations over time, in the presence of 7-NI, was assessed in the following manner. Three animals had their MAC determined as described above. The MAC was again determined 75 and 140 min after administering 7-NI (20 mg/kg) in 3 ml arachis oil.

All data are reported as means ± SEM. Statistical analysis was performed using analysis of variance with multiple-range testing (Neuman-Keul's test) where needed. $P < 0.05$ was accepted as significant.

Results

The control value for isoflurane MAC in the rat was 1.23 ± 0.01 vol%, which correlates with previous determinations.[31] As seen in figure 1, 20, 60, 80, 120, 500, and 1,000 mg/kg 7-NI caused a dose-dependent decrease from isoflurane control MAC of 15.0 ± 0.9%, 18.7 ± 1.0%, 31.5 ± 2.2%, 39.0 ± 2.0%, 43.0 ± 1.7%, and 41.8 ± 2.2%, respectively. Each concentration was significantly decreased ($P < 0.001$) from the isoflurane control MAC. A ceiling effect for the 7-NI was observed when the dose was greater than 120 mg/kg dose. L-NAME 1, 5, 10, 20, and 30 mg/kg also caused a dose-dependent decrease from isoflurane control MAC of 3.9 ± 1.0%, 3.1 ± 0.2%, 27.3 ± 2.9%, 34.1 ± 1.8%, and 35.3 ± 2.5%, respectively. Each concentration was significantly decreased ($P < 0.001$, except for the lowest concentration, 1 mg/kg, where $P < 0.01$) from the isoflurane control MAC. A ceiling effect was observed when the dose was greater than 10 mg/kg. No untoward effects were observed for either NO synthase inhibitor, even at the highest doses.

As seen in figure 2, the administration of 20, 60, 80, 120, 500, and 1,000 7-NI mg/kg resulted in no changes in either systolic or diastolic blood pressure. Absolute control blood pressure was 135 ± 6 mmHg systolic and 100 ± 7 mmHg diastolic. As seen in figure 3, 20, 60, 80, 120, 500, and 1,000 mg/kg 7-NI administration resulted in a small decrease in heart rate (7.2 ± 2.6%, 10.1 ± 1.8%, 2.8 ± 1.8%, 4.9 ± 0.7%, 3.7 ± 1.4%, and 6.0 ± 1.2%, respectively; $P < 0.05$ for all doses except the 80 mg/kg dose). Absolute control heart rate was 404 ± 2 bpm. The administration of 1, 5, 10, 20, and 30 mg/kg L-NAME resulted in increases in both systolic blood pressure (4.4 ± 1.9%, 27.2 ± 2.5%, 36.3 ± 2.5%, 39.9 ± 2.2%, and 37.8 ± 2.9%, respectively; $P < 0.01$ for all doses except the 1-mg/kg dose) and diastolic blood pressure (2.4 ± 3.1%, 45.6 ± 7.9%, 57.4 ± 4.2%, 64.3 ± 4.0%, and 43.3 ± 5.7%, respectively; $P < 0.01$ for all doses except the 1-mg/kg dose). Absolute control

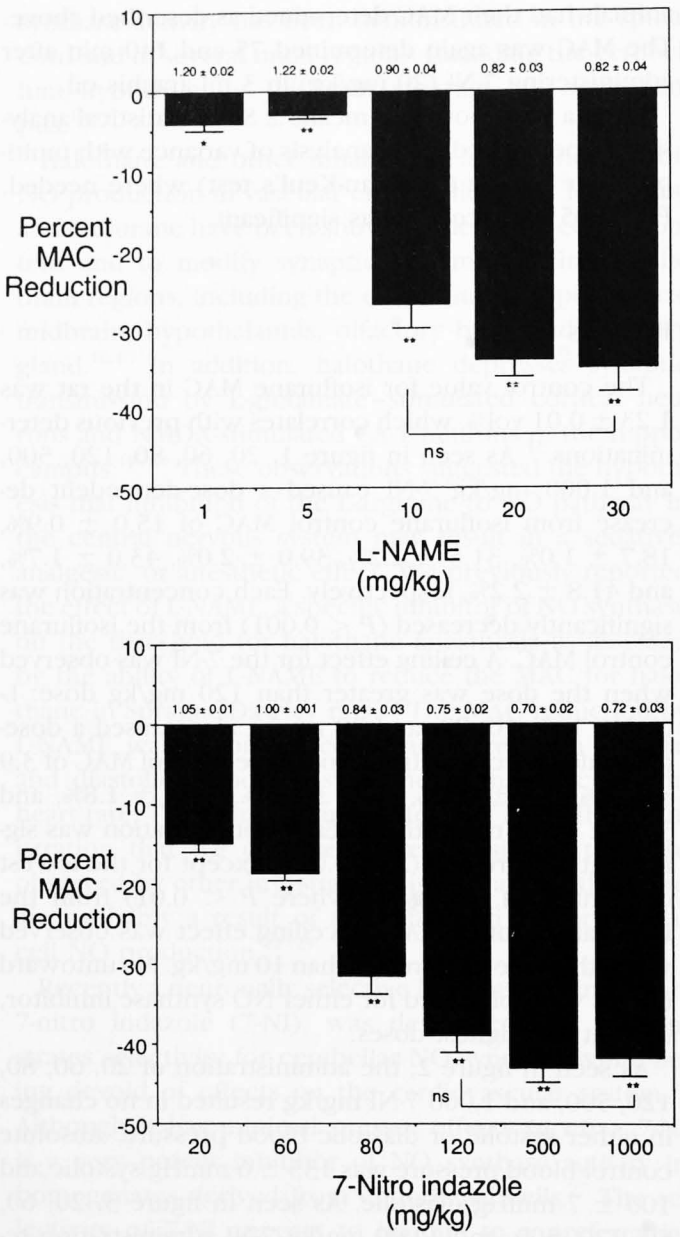

Fig. 1. Isoflurane minimum alveolar concentration (MAC) reduction by increasing concentrations of the NO synthase inhibitors 7-nitro indazole (7-NI) and nitro^G-L-arginine methyl ester (L-NAME). Data are presented as the mean ± SEM; n = 6 animals for each data point, except n = 4 for the 1,000 7-NI mg/kg dose. *$P < 0.01$ *versus* control and **$P < 0.001$ *versus* control. ns = not significantly different.

blood pressure was 119 ± 1 mmHg systolic and 79 ± 1 mmHg diastolic. The L-NAME–induced changes in blood pressure were not accompanied by any changes in heart rate. Absolute control heart rate was 395 ± 3 bpm.

L-arginine at a dose of 300 mg/kg caused an immediate and complete reversal of MAC reduction induced by L-NAME 30 mg/kg ($P < 0.05$) along with significant increases in systolic and diastolic blood pressure (6.5 ± 2.2% and 16.7 ± 3.3%, respectively; $P < 0.001$).

The effect of the vehicle (arachis oil) used to administer 7-NI on MAC was assessed over time. The control MAC for these five animals was 1.22 ± 0.02 vol%. The MAC after 45 and 120 min was 1.20 ± 0.02 vol% and 1.25 ± 0.01 vol%, respectively. Neither the 45- nor the

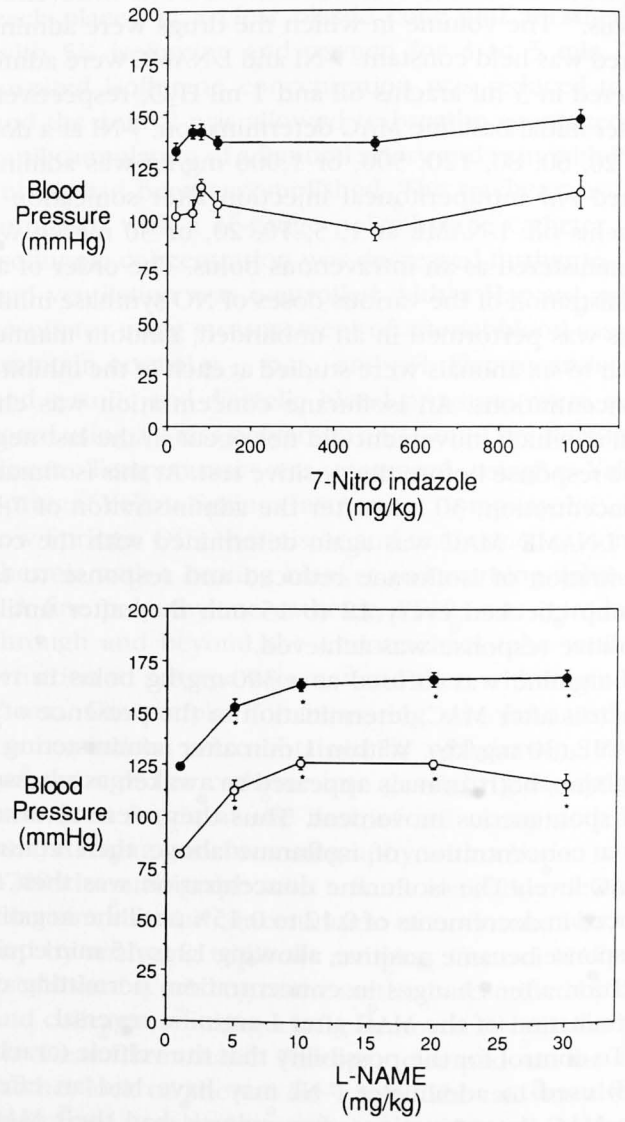

Fig. 2. The effect of the NO synthase inhibitors 7-nitro indazole (7-NI) and nitro^G-L-arginine methyl ester (L-NAME) on systolic and diastolic blood pressure under isoflurane anesthesia. Data are presented as the mean ± SEM; n = 6 animals for each data point, except n = 4 for the 1,000 mg/kg 7-NI dose. *$P < 0.01$ *versus* control. Systolic blood pressure (●); diastolic blood pressure (○).

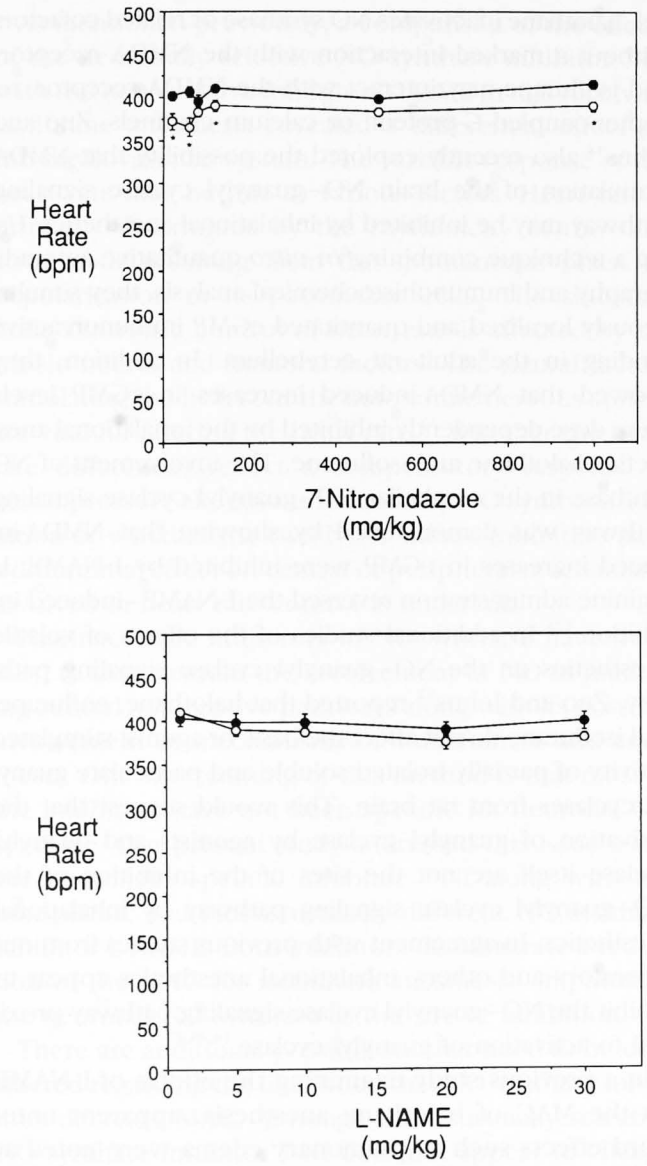

Fig. 3. The effect of the NO synthase inhibitors 7-nitro indazole (7-NI) and nitro^{G}-L-arginine methyl ester (L-NAME) on heart rate under isoflurane anesthesia. Data are presented as the mean ± SEM; n = 6 animals for each data point, except n = 4 for the 1,000 mg/kg 7-NI dose. *$P < 0.05$ *versus* control. Control heart rate (●); NOS inhibitor heart rate (○).

120-min time points were significantly different from the control value. The stability of the MAC determinations, in the presence of 7-NI, was assessed 75 and 140 min after administering the NO synthase inhibitor. The control MAC for these five animals was 1.21 ± 0.01 vol%. The MAC after 75 and 140 min was 1.01 ± 0.03 vol% and 1.05 ± 0.03 vol%, respectively. The 75- and 140-minute time points were not significantly different from each other, indicating a relative stability of the MAC determinations, in the presence of 7-NI, during the time course of our experiments.

Discussion

Previously we suggested that halothane may inhibit the production of NO in the central nervous system, and that this inhibition may have a sedative, analgesic, or anesthetic effect.[21] Intravenous administration of L-NAME, but not nitro^{G}-D-arginine methyl ester, dose dependently decreased the MAC for halothane. This MAC reduction persisted for 2 h after L-NAME injection and was immediately and completely reversed by bolus infusion of a competitive dose of L-arginine, but not by D-arginine, suggesting a specific site of action.

In our experiments, the intraperitoneal administration of 7-NI and intravenous administration of L-NAME caused a dose-dependent decrease from isoflurane control MAC. With both inhibitors, a ceiling effect was observed. No untoward effects were observed for either NO synthase inhibitor, even at the largest doses. The two inhibitors differed, however, in their hemodynamic effects. The administration of 7-NI resulted in no changes in either systolic or diastolic blood pressure, but resulted in a small but clinically insignificant decrease in heart rate. The administration of L-NAME resulted in significant increases in both systolic and diastolic blood pressure, which were not accompanied by any changes in heart rate.

Using methods similar to ours, Adachi and colleagues,[32] while demonstrating the cardiovascular changes induced by L-NAME under halothane anesthesia, failed to confirm the reduction in MAC. Because the experimental design used by Johns and associates[21] and Adachi and colleagues were very similar, an explanation for the discrepancy between the observations from these two laboratories is uncertain. Our findings, replicated now with a different volatile anesthetic and an additional NO synthase inhibitor, are consistent with our previous findings and with other observed effects of NO synthase inhibition on the central nervous system and cardiovascular system.[4,20,33,34]

The implication of the NO pathway in states of wakefulness and anesthesia is further supported in a recent study using a neuronal NO synthase knockout mouse model. Ichinose and colleagues[35] described the use of homologous recombination to generate knockout mice

showing no evidence of neuronal NO synthase gene expression. They compared the effects of L-NAME on the MAC of isoflurane and on the righting reflex response between wild-type and neuronal NO synthase knockout mice. Administration of L-NAME significantly decreased the MAC of isoflurane anesthesia in the normal mice but did not affect the MAC of isoflurane in the knockout mice. This showed that the reduction of MAC and inhibition of the righting reflex by the NO synthase inhibitor were clearly and specifically linked to the inhibition of neuronal NO synthase. Also noteworthy was the fact that the neuronal knockout mice exhibited a normal MAC for isoflurane and demonstrated no obvious change in baseline consciousness. This would imply the existence of compensatory mechanisms to retain consciousness and pain responses, a finding consistent with the presumed importance of these vital functions.

No apparent side effects were noted in rats receiving either L-NAME or 7-NI. In a previous study, rats receiving the largest dose of L-NAME (30 mg/kg) appeared to have pulmonary edema while being anesthetized with halothane. In our present study and in those by Adachi and colleagues[32] and Triner and coworkers,[38] this phenomena was not observed. Because the rats in the study by Adachi and colleagues also received halothane and those in our study received isoflurane, it is difficult to speculate on this difference.

Researchers in our laboratory and others have shown that various inhalational anesthetics can inhibit NO production in vascular endothelium.[13,14,36] There is significant evidence for an interaction of inhalational anesthetics with the neuronal nitric oxide–guanylyl cyclase signaling pathway. Inhalational anesthetics are generally accepted to inhibit NO synthase activity and decrease cGMP levels *in vivo* in the cerebellum and other regions of the brain.[15,37,38] Controversy remains in that some studies fail to demonstrate an inhibition of *in vitro* NO synthase activity.[39,40] The mechanism of inhibition of the NO–guanylyl cyclase signaling pathway by inhalational anesthetics remains unresolved. Terasako and associates[41] reported that halothane (2%) and isoflurane (2%) suppressed NMDA-stimulated formation of cGMP in rat cerebellum and that the sites of action of these two volatile anesthetics were different. They speculated that, at the concentrations studied, halothane inactivates NO synthase or related cofactors without a marked interaction with the NMDA receptor, and isoflurane may interact with the NMDA receptor, receptor-coupled G-protein, or calcium channels. Zuo and Johns[42] also recently explored the possibility that NMDA stimulation of the brain NO–guanylyl cyclase signaling pathway may be inhibited by inhalational anesthetics. Using a technique combining *in vitro* quantitative autoradiography and immunohistochemical analysis, they simultaneously localized and quantitated cGMP immunoreactive binding in the adult rat cerebellum. In addition, they showed that NMDA-induced increases in cGMP levels were dose dependently inhibited by the inhalational anesthetics halothane and isoflurane. The involvement of NO synthase in the cerebellar NO–guanylyl cyclase signaling pathway was demonstrated by showing that NMDA-induced increases in cGMP were inhibited by L-NAME. L-arginine administration reversed the L-NAME–induced inhibition.[42a] In additional studies of the effects of volatile anesthetics on the NO–guanylyl cyclase signaling pathway, Zuo and Johns[42] reported that halothane, enflurane, and isoflurane do not affect the basal or agonist-stimulated activity of partially isolated soluble and particulate guanylyl cyclases from rat brain. This would suggest that the activation of guanylyl cyclase by agonists and guanylyl cyclase itself are not the sites of the inhibition of the NO–guanylyl cyclase signaling pathway by inhalational anesthetics. In agreement with previous studies from our laboratory and others, inhalational anesthetics appear to inhibit the NO–guanylyl cyclase signaling pathway proximal to activation of guanylyl cyclase.[14,38,43]

In a previous study examining the effects of L-NAME on the MAC of halothane anesthesia, apparent untoward effects such as pulmonary edema were noted at the highest dose.[20] It is therefore noteworthy that in our present study using isoflurane as the inhalational anesthetic, neither 7-NI nor L-NAME exhibited any apparent untoward effects, even at the highest doses.

Consistent with our previously reported data, L-NAME resulted in significant increases in both systolic and diastolic blood pressures in anesthetized rats.[20] In the presence of isoflurane, the L-NAME–induced changes in blood pressure were not accompanied by a change in heart rate. In the previous experiment, the use of halothane was accompanied by a decrease in heart rate. The two experiments were identical in design, except for the volatile anesthetic. Inhalational anesthetics are known to produce dose-dependent and drug-specific circulatory effects, including a differential effect on heart rate.§

§ Eger EI II. Isoflurane [Forane], A compendium and reference, Second edition. Madison, Wisconsin, Ohio Medical Products, 1985, pp 1–110.

As mentioned previously, a comparison of the effects of the neuronally selective NO synthase inhibitor 7-NI with the nonselective inhibitor L-NAME in rats receiving isoflurane anesthesia revealed a differential effect on the cardiovascular system. The variable response of the heart rate may be due to various factors. Heart rate is primarily determined by the rhythmicity of the sinoatrial node resulting from the spontaneous phase IV depolarization of its pacemaker cells. The sinoatrial node's intrinsic control of heart rate is affected by extrinsic neural and humoral factors. The neural factors stimulating the heart *via* the autonomic nervous system are vital for altering the heart rate.[44] Whether the heart rate differences reflect a compensatory response to blood pressure alterations induced by NO synthase inhibition or whether the two NO synthase inhibitors have a differential effect on central or peripheral mechanisms involved in heart rate control is uncertain.

Pharmacologic inhibitors of NO synthase have been used to demonstrate the involvement of NO in mediating central nociceptive pathways along with its possible involvement in mechanisms of wakefulness and anesthesia. One shortcoming of this method is that most of the inhibitors have not been specific for neuronal NO synthase. Our present study addressed this issue using the neuronally specific inhibitor 7-NI along with the nonspecific and mechanistically different NO synthase inhibitor L-NAME. Both inhibitors demonstrate a reduction of the MAC for isoflurane anesthesia, implicating the neuronal NO synthase as the site of inhibition.

There are additional possibilities that need to be considered regarding the likelihood that NO synthase inhibitors decrease MAC. Although the neuronally selective NO synthase inhibitor 7-NI does not appear to change blood pressure and only minimally alters heart rate, neuronally derived NO, in addition to endothelially derived NO, is involved in maintaining and regulating regional cerebral blood flow under normal and disease states.[45,46] Kovach and associates[47] noted that inhibition of NO synthase activity after administering a nonselective NO synthase inhibitor, as well as the neuronally selective NO synthase inhibitor 7-NI, decreased cerebral and spinal cord blood flow in a heterogeneous manner. The possibility that alterations in cerebral blood flow by NO synthase inhibitors may in fact account for some or all of the decrease in MAC is beyond the scope of this study.

Although blockade of movement is a central feature of the general anesthetic state, the sites and mechanisms by which volatile anesthetics achieve a state of unresponsiveness remain unknown. Recent data have suggested that motor responses may be inhibited by anesthetics at the level of the spinal cord.[48,49] This line of reasoning was further supported by the findings that preferentially anesthetizing the brain, with minimal delivery of anesthetic to the rest of the body, markedly increased cerebral anesthetic requirements, suggesting that the spinal cord is an important site of anesthetic inhibition.[50] Although this would imply that the brain does not influence somatic responses to anesthetics, ablation of discrete brain stem neurons or electric stimulation of the periaqueductal gray matter has been shown to alter MAC.[51,52]

Inhibition of the NO synthase pathway, using both nonselective and neuronally selective NO synthase inhibitors, decreases the MAC for isoflurane. Although uncertainly remains regarding the sites and mechanisms by which anesthetics reduce the somatic responses to noxious stimuli, the reduction in the MAC by two structurally distinct NO synthase inhibitors suggests the specificity of the effect on NO synthase. Furthermore, the action of the structurally distinct and neuronally selective NO synthase inhibitor 7-NI supports a selective effect on neuronal NO synthase resulting in a dose-dependent reduction in the MAC for isoflurane anesthesia in rats while avoiding the hypertensive response of generalized NO synthase inhibitors. These findings further support a role for the NO pathway in mechanisms of anesthesia and analgesia.

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