

Nitric Oxide Synthase Inhibitor Dose-dependently and Reversibly Reduces the Threshold for Halothane Anesthesia

A Role for Nitric Oxide in Mediating Consciousness?

Roger A. Johns, M.D.,* Jeffrey C. Moscicki, M.S.,† Cosmo A. DiFazio, M.D., Ph.D.‡

Nitric oxide is a newly recognized cell messenger for the activation of soluble guanylate cyclase and is produced from L-arginine by the enzyme nitric oxide synthase in a wide variety of tissues, including vascular endothelium and brain. Inhalational anesthetics inhibit nitric oxide production from vascular endothelium and also decrease resting cyclic guanosine monophosphate content in multiple brain regions. Halothane has been shown to depress neurotransmission by L-glutamate and N-methyl-D-aspartate. These amino acid neurotransmitters are known to increase neuronal cyclic guanosine monophosphate content by stimulation of nitric oxide production. To investigate the possible involvement of the L-arginine-to-nitric oxide pathway in the anesthetic state, the effect of a specific nitric oxide synthase inhibitor, nitro^G-L-arginine methyl ester, on the minimum alveolar concentration (MAC) for halothane anesthesia was determined in Sprague-Dawley rats. Bolus injection of nitro^G-L-arginine methyl ester at 0, 1, 5, 10, 20, and 30 mg/kg resulted in a dose-dependent reduction in MAC for halothane of 0 ± 0 , 2.3 ± 0.4 , 21.5 ± 3.9 , 30.5 ± 2.4 , 51.0 ± 7.8 , and $26.0 \pm 2.8\%$, respectively. Nitro^G-L-arginine methyl ester had no effect on MAC for halothane. Bolus infusion of L-arginine 300 mg/kg after MAC reduction by nitro^G-L-arginine methyl ester 10 mg/kg resulted in an immediate and complete reversal of the MAC reduction. No reversal was observed after infusion of D-arginine 300 mg/kg. MAC reduction by nitro^G-L-arginine methyl ester 1, 5, 10, 20 and 30 mg/kg was accompanied by an increase in systolic blood pressure (1 ± 3 , 29 ± 6 , 27 ± 3 , 45 ± 3 , and $27 \pm 5\%$ respectively) and diastolic blood pressure (1 ± 6 , 30 ± 6 , 29 ± 7 , 53 ± 2 , and $29 \pm 7\%$ respectively), and a small decrease in heart rate (-2 ± 3 , -8 ± 4 , -11 ± 2 , -5 ± 8 , and $-15 \pm 3\%$, respectively). This dose-dependent and reversible decrease in MAC by nitro^G-L-arginine methyl ester suggests that inhibition of the nitric oxide pathway decreases the level of consciousness and augments anesthesia, analgesia, or sedation. (Key words: Anesthetics, volatile: halothane. Endothelium: endothelium-derived relaxing factor; nitric oxide. Enzymes: guanylate cyclase; nitric oxide synthase. Potency: MAC.)

ENDOTHELIUM-DERIVED relaxing factor (EDRF)/nitric oxide (NO), first discovered as a potent vasodilator produced by vascular endothelium,¹ is now recognized as the transduction mechanism for the activation of soluble guanylate cyclase and has been demonstrated in a wide variety of tissue types, including brain.² Extensive recent

literature has suggested that EDRF is NO or a chemically related species.² NO is synthesized enzymatically by the oxidation of a guanidino nitrogen atom of L-arginine (fig. 1).³⁻⁵ Both constitutive and inducible forms of NO synthase have been described, and both are specifically inhibited by analogues of L-arginine such as nitro^G-L-arginine methyl ester (LNAME) and N^G-monomethyl-L-arginine.^{2,6} Immunohistochemical localization studies indicate that the constitutive NO synthase is concentrated in endothelial, neuronal, and secretory tissues.⁷ NO has been shown to mediate the increase in cerebellum cyclic guanosine monophosphate (GMP) content in response to N-methyl-D-aspartate (NMDA)⁸⁻¹⁰ to kainate¹¹ and to glutamate.⁹ Brain NO synthase activity has been demonstrated in several brain regions, including cerebellum, hypothalamus, midbrain, striatum, and hippocampus.¹²

Halothane and other inhalational anesthetics inhibit NO production in vascular endothelium.¹³⁻¹⁵ Halothane¹⁶ and enflurane¹⁷ have been shown to decrease cyclic GMP content and to modify synaptic transmission in specific brain regions, including cerebellum, hippocampus, midbrain, hypothalamus, olfactory bulb, and pituitary. In addition, halothane depresses synaptic transmission by L-glutamate-stimulated cortical neurons¹⁸ and NMDA-stimulated CA1 neurons of the hippocampus.^{19,20}

These observations suggested the hypothesis that inhibition of the L-arginine-to-NO pathway in the central nervous system may result in an anesthetic, analgesic, or sedative effect. To test this hypothesis, we studied the effect of LNAME, a specific inhibitor of NO synthase, on the threshold for halothane anesthesia as assessed by the ability of LNAME to reduce the minimum alveolar concentration (MAC) for halothane in the Sprague-Dawley rat.

Materials and Methods

After institutional animal care committee approval had been obtained, we examined the reduction of halothane MAC in response to increasing concentrations of LNAME. Sprague-Dawley rats (308 ± 10 g) each were placed in a clear plastic cone and anesthetized with 3.5% halothane and oxygen for 3-5 min. Halothane concentration was reduced to 1.5%, and the animal breathed spontaneously until cannulation of a femoral artery and vein with PE50 tubing had been accomplished. The tra-

* Associate Professor of Anesthesiology.

† Laboratory Specialist.

‡ Professor of Anesthesiology.

Received from the Department of Anesthesiology, University of Virginia Health Sciences Center, Charlottesville, Virginia. Accepted for publication June 12, 1992.

Address reprint requests to Dr. Johns: Department of Anesthesiology, Box 238, University of Virginia Health Sciences Center, Charlottesville, Virginia 22908.

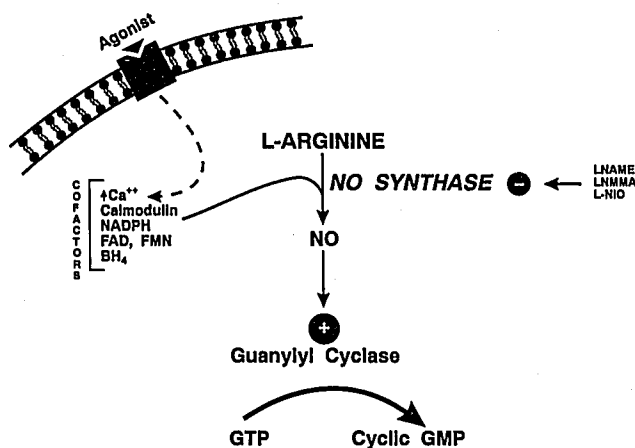


FIG. 1. The nitric oxide (NO) signaling pathway. In the brain and endothelium, NO is produced from L-arginine by similar constitutive enzymes called NO synthase(s). These enzymes are activated by the binding of calcium (Ca^{2+}) and calmodulin, often in response to agonist-receptor interaction leading to increased cytosolic calcium. They are homologous to P450 reductase enzymes, having recognition sites for reduced nicotinamide adenine dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD), and flavin mononucleotide (FMN). Tetrahydrobiopterin (BH_4) is another cofactor. Following its production, NO binds to the heme moiety of guanylyl cyclase, which catalyzes the production of cyclic 3',5'-guanosine monophosphate (cyclic GMP) from guanosine triphosphate (GTP). Specific analogs of L-arginine, including nitro-L-arginine methyl ester (LNAME), N^G -monomethyl-L-arginine (LNMMA), and N-imino ornithine (L-NIO), are competitive inhibitors of NO synthase.

chea was intubated with a 16-G polyethylene catheter. Halothane concentration was then decreased further to 1%, 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 . The electrocardiogram and systolic and diastolic blood pressures were monitored using a Grass polygraph and Gould pressure transducer. Temperature was measured by a Yellow Springs thermistor and maintained at normothermia with a heating blanket and warming lights.

A fine polyethylene catheter, PE10, was introduced through and beyond the endotracheal tube until obstruction to passage was met and then was withdrawn 1–2 mm. Gas samples for measurement of alveolar anesthetic concentrations were obtained by withdrawing 10 ml gas through the catheter into gas-tight glass syringes over 3–5 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 halothane was verified by analyzing triplicate samples. Control MAC was established according to the methods described by Eger *et al.*²¹ using a long hemostat (8-inch Rochester Dean Hemostatic Forceps) clamped to the first ratchet lock on the tail for 1 min. In every case the tail was stimulated proximal to a previous

test site. Gross movement of the head, extremities, and/or body was taken as a positive test result, whereas grimming, swallowing, chewing, or tail flick were considered negative. The halothane concentration was reduced in decrements of 0.12–0.15% until the negative response became positive, with 12–15 min equilibration allowed after changes in concentration.²² 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.

After initial baseline MAC determination, LNAME at 1, 5, 10, 20, or 30 mg/kg was administered as an intravenous bolus. Six to twelve animals were studied at each of the LNAME concentrations. A halothane concentration was chosen at which movement did not occur in the last negative response before the positive test. At this halothane concentration, 30 min after the bolus dose of LNAME, the animal was tested again for reactivity to tail clamp. Halothane concentrations were reduced and response to tail clamp checked every 12–15 min thereafter until a positive response was achieved. In some experiments after MAC determination in the presence of 10 mg/kg LNAME, L-arginine or D-arginine was infused as a 300-mg/kg bolus. Within 1 min after administration of L-arginine, all animals appeared to awaken as assessed by spontaneous movement. They therefore were returned to a pre-LNAME concentration of 0.82% (1 MAC) halothane, at which point they still exhibited a positive response to the tail clamp stimulus. The effect of the stereoisomer of LNAME, nitro^G-D-arginine methyl ester, on MAC for halothane was determined in additional experiments.

DATA ANALYSIS

All data are reported as mean \pm standard error of the mean. Statistical analysis was performed using analysis of variance with multiple-range testing (the Neuman-Keuls test) where needed. $P < 0.05$ was accepted as significant.

CHEMICALS AND DRUGS

Halothane was obtained from Halocarbon Labs (Hackensack, NJ). LNAME, L-arginine, and D-arginine were obtained from Sigma Chemical Company (St. Louis, MO). Nitro^G-D-arginine methyl ester was obtained from Peninsula Laboratories (Belmont, CA).

Results

The control value for halothane MAC was 0.82 ± 0.02 vol%, which is similar to previous determinations in rats.^{23,24} LNAME 1, 5, 10, 20, and 30 mg/kg caused a dose-related decrease from halothane control MAC of 2.3 ± 0.4 , 21.5 ± 3.9 ($P < 0.01$), 30.5 ± 2.4 ($P < 0.01$),

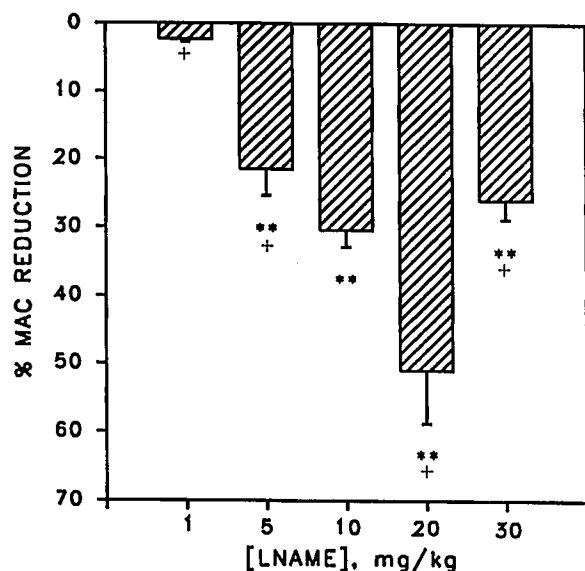


FIG. 2. Halothane minimum alveolar concentration (MAC) reduction by increasing concentrations of LNAME (nitro^G-L-arginine-methyl-ester). Data are presented as mean ± SEM; N = 9 (1 mg/kg), 6 (5 mg/kg), 18 (10 mg/kg), 7 (20 mg/kg), and 3 (30 mg/kg). **Significantly different from control ($P < 0.01$); †Significantly different from previous concentration ($P < 0.01$).

51.0 ± 7.8 ($P < 0.01$), and 26.0 ± 2.8 ($P < 0.01$)%, respectively (fig. 2). Each concentration, except 10 mg/kg, was significantly different ($P < 0.01$) from the preceding concentration. The 30-mg/kg dose resulted in pulmonary edema in three rats. No other untoward effects were observed. Nitro^G-D-arginine methyl ester 10 mg/kg had no effect on halothane MAC.

The administration of LNAME 1, 5, 10, 20, and 30 mg/kg resulted in increases in both systolic blood pressure (1 ± 3, 29 ± 6 [$P < 0.01$], 27 ± 3 [$P < 0.01$], 45 ± 3 [$P < 0.01$], and 27 ± 5% [$P < 0.01$], respectively) and diastolic blood pressure (1 ± 6, 30 ± 6 [$P < 0.01$], 29 ± 7 [$P < 0.01$], 53 ± 2 [$P < 0.01$], and 29 ± 7% [$P < 0.01$], respectively). Absolute control blood pressure was 120 ± 2 mmHg systolic and 98 ± 3 mmHg diastolic. These LNAME-induced changes in blood pressure were accompanied by a slight decrease in heart rate (-2 ± 3, -8 ± 4,

-11 ± 2 [$P < 0.05$], -5 ± 8, -15 ± 3% [$P < 0.05$], respectively). Absolute control heart rate was 382 ± 7 beats/min.

L-arginine 300 mg/kg caused an immediate and complete reversal of MAC reduction induced by LNAME 10 mg/kg ($P < 0.01$). In contrast, D-arginine 300 mg/kg had no effect on LNAME-induced MAC reduction (table 1).

The increase of systolic and diastolic blood pressure caused by LNAME 10 mg/kg was partially but not significantly reversed by L-arginine, whereas D-arginine had no significant effect (table 1). Heart rate returned to baseline after L-arginine and was not altered by D-arginine (table 1).

Discussion

Our laboratory^{14,15} and others¹³ have demonstrated the ability of inhalational anesthetics to inhibit NO production in vascular endothelium. The current data suggest the novel possibility that halothane may inhibit the production of NO in the central nervous system and that this inhibition may have a sedative, anesthetic, or analgesic effect. Bolus injection of LNAME but not nitro^G-D-arginine methyl ester decreased the MAC for halothane in a dose-dependent manner. This MAC reduction persisted for 2 h after LNAME 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.

Although the mechanism of general anesthesia remains largely unknown, a component of many theories is that general anesthesia is primarily the result of altered synaptic transmission.^{18,25-27} Studies of the synaptic actions of intravenous and inhalational anesthetics have been reported to fall into two general but not mutually exclusive categories: depression of excitatory transmission^{18,28-30} and enhancement of inhibitory transmission.^{25,27,31} Both excitatory and inhibitory amino acid-mediated neurotransmitter systems are widespread in the central nervous system and have been reported to be influenced by general anesthetics.^{18,25,32-35}

TABLE 1. Effect of L-Arginine and D-Arginine on LNAME-induced Changes in Halothane MAC, Blood Pressure, and Heart Rate

	Control	LNAME, 10 mg/kg	L-ARG Reversal	D-ARG Reversal
Halothane MAC volume (%)	0.82 ± 0.02	0.57 ± 0.01*	> 0.82	0.59 ± 0.2*
Systolic BP (mmHg)	119 ± 4	153 ± 3*	145 ± 5*	155 ± 6*
Diastolic BP (mmHg)	99 ± 4	127 ± 4*	119 ± 4*	96 ± 5*
Heart rate (beats/min)	387 ± 8	342 ± 6*	398 ± 7	386 ± 10*
N (no. of rats)	47	28	6	4

Values are mean ± SEM.

MAC = minimum alveolar concentration; LNAME = nitro-L-arginine methyl ester; BP = blood pressure; L-ARG = L-arginine, 300

mg/kg; D-ARG = D-arginine, 300 mg/kg.

* Significantly different from control ($P < 0.01$).

Neurotransmission in response to acetylcholine, glutamate, and glycine has long been associated with increased brain cyclic GMP concentrations.³⁶ Garthwaite *et al.*⁸ first demonstrated that the increase in cyclic GMP concentrations in rat cerebellum in response to NMDA stimulation was the result of NO release. Bredt and Snyder¹⁰ subsequently demonstrated that NO mediates glutamate-linked increase of cyclic GMP concentrations in cerebellum and isolated and characterized NO synthase from rat brain.³⁷ Immunohistochemical localization of NO synthase⁷ demonstrated that the synthase was localized in discrete neuronal populations. Immunostaining appeared in the molecular and granule cell layers of the cerebellum, the superior and inferior colliculi, and the granule cell layer of the olfactory bulb as well as the posterior lobe of the pituitary. Lesser staining occurred in the superficial layers of the cerebral cortex, the dentate gyrus of the hippocampus, and the bed nucleus of the stria terminalis. Localized staining also occurred in the islands of Calleja, the diagonal band of Broca, and the mamillary nuclei. The largest concentrations of NO synthase in the brain, as assessed by the activity of cytosolic preparations of the enzyme, were in the cerebellum, followed by the hypothalamus and midbrain, the striatum, and the hippocampus, with the least activity found in the medulla oblongata.¹²

Halothane has been demonstrated to have a marked inhibitory effect on resting cyclic GMP concentrations in specific rat brain regions.¹⁶ Effects were prominent in the cerebellum, the midbrain, the hippocampus, substantia nigra, thalamus, hypothalamus, olfactory bulb, cortex, frontal cortex, pineal gland, and pituitary gland. After exposure to 2% halothane, the decrease in cyclic GMP in these regions ranged from 10–60% of control. This halothane-induced depression of brain cyclic GMP content may be a result of the inhibitory action of halothane on brain NO production, similar to its effect in endothelium. The ability of LNAME, a specific inhibitor of NO synthesis, to decrease halothane MAC suggests an important role of the L-arginine-to-NO pathway in regulating consciousness.

After the reversal of LNAME-induced MAC reduction with L-arginine, animals were immediately returned to the baseline halothane concentration that existed before LNAME administration, at which point they continued to respond to tail clamp. This elevation of MAC during L-arginine reversal suggests that L-arginine itself may increase anesthetic requirement or have a general analeptic effect. The NO pathway may play a role in maintaining wakefulness.

The recently reported involvement of NO in peripheral nociceptive pathways³⁸ is unlikely to explain the current data. Opiates, in addition to their central and spinal sites of action, have been shown to cause analgesia through a

peripheral mechanism.³⁹ The stimulation of cyclic GMP through NO release has been demonstrated to be responsible for the peripheral analgesia induced by both morphine and acetylcholine.^{38,40} This effect would not account for the observed reduction in MAC, however, because the inhibition of peripheral analgesia pathways by LNAME and other L-arginine analogues should decrease analgesia and would likely increase rather than reduce MAC for halothane. A report by Moore *et al.*⁴¹ demonstrated that intraperitoneal (1–75 mg/kg) and intracerebroventricular (0.1–100 µg per mouse) injection of L-NAME in the mouse elicited a dose-related antinociception, as assessed by the formalin-induced paw-licking procedure, that was reversed by a 600-mg/kg intraperitoneal dose of L-arginine. These data are consistent with a central analgesic action of L-NAME and with our current report of the effect of LNAME on MAC.

The hemodynamic effects of LNAME administration observed in this study are similar to those observed by other investigators in rats and in dogs and are likely a result of the inhibition of endothelial cell EDRF production.^{42–45} The increase in blood pressure in response to LNAME may have been exaggerated in the current study because the halothane concentration was reduced simultaneously and progressively as LNAME concentration increased, because of the MAC reduction. Halothane alone produces a dose-dependent decrease in blood pressure, primarily through a decrease in myocardial contractility and secondarily through a decrease in systemic vascular resistance. Thus, any decrease in halothane concentration would have tended to potentiate the increase in blood pressure caused by LNAME. The failure of blood pressure effects to reverse significantly provides evidence that the observed changes in MAC are not accounted for in any way by the elevated blood pressure.

The largest dose of LNAME, 30 mg/kg, was observed to cause fulminant pulmonary edema in three of six rats studied at this dose. The other three, which form the basis for the data presented, also exhibited respiratory distress but to a lesser degree. This manifested as airway noises consistent with pulmonary congestion. This pulmonary congestion may account for the decreased MAC reduction and decreased elevation of blood pressure at this concentration, because the uptake of anesthetic is likely to have been altered by ventilation-perfusion mismatching. We cannot rule out a ceiling effect occurring at higher doses of L-NAME because these complications resulted in a necessarily incomplete dose-response curve. No untoward effects of the L-arginine analogues or L-arginine itself were observed at lower concentrations.

NMDA-mediated excitatory neurotransmission is a very likely site of our observed effect of LNAME on MAC for halothane. The anesthetic action of ketamine is thought to be mediated through NMDA receptor

inhibition⁴⁶; ketamine and the noncompetitive NMDA receptor antagonists MK-801 and phencyclidine reduce the volatile anesthetic requirement.^{47,48} Another recent report has demonstrated that specific NMDA receptor inhibitors⁴⁹ and riluzole,⁵⁰ a non-receptor-mediated inhibitor of glutamate (NMDA receptor)-stimulated neurotransmission, administered intravenously to rats caused a dose-dependent decrease in MAC for halothane. Because NMDA receptor activation stimulates NO synthase and subsequent NO and cyclic GMP production in neurons, this may well be the mechanism by which LNAME infusion decreases halothane MAC in our studies and further suggests a role for inhibition of the NMDA pathway in mechanisms of sedation, analgesia, or anesthesia.

These studies of the effects of intravenous inhibitors of NO synthase on anesthetic threshold, while provocative and strongly suggestive, do not provide specific direct evidence of an interaction between the NO signaling pathway and inhalational anesthetics in the central nervous system. In addition to an inhibitory action on NO synthase, it is possible that LNAME is acting at other sites such as specific arginine transporters on the cell membrane or on other metabolic pathways involved in arginine metabolism. Further studies in isolated tissue, cell, and subcellular preparations will be necessary to determine definitively the specific site of LNAME action. The effect of NO synthase inhibitors on the MAC for other volatile anesthetics as well as anesthetics that do not inhibit EDRF needs to be examined.

References

1. Furchgott RF, Zawadzki JV: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288:373-376, 1980
2. Moncada S, Palmer RMJ, Higgs EA: Nitric oxide: Physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 43:109-142, 1991
3. Palmer RMJ, Ashton DS, Moncada S: Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* 333:664-666, 1988a
4. Schmidt HHHW, Nauh H, Wittfoht W, Gerlach J, Prescher KE, Klein MM, Niroomand F, Bohme E: Arginine is a physiological precursor of endothelium-derived nitric oxide. *Eur J Pharmacol* 154:213-216, 1988
5. Kelm M, Feelisch M, Spahr R, Piper HM, Noack E, Schrader J: Quantitative and kinetic characterization of nitric oxide and EDRF-released from cultured endothelial cells. *Biochem Biophys Res Commun* 154:236-244, 1988
6. Johns RA, Peach MJ, Linder J, Tichotsky A: N^G-monomethyl L-arginine inhibits endothelium-derived relaxing factor stimulated cyclic GMP accumulation in cocultures of endothelial and vascular smooth muscle cells by an action specific to the endothelial cell. *Circ Res* 67:979-985, 1990
7. Bredt DS, Hwang PM, Snyder SH: Localization of nitric oxide synthase indicating a neural role for nitric oxide. *Nature* 347:768-770, 1990
8. Garthwaite J, Charles SL, Chess-Williams R: Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. *Nature* 336:385-388, 1988
9. Garthwaite J, Garthwaite G, Palmer RMJ, Moncada S: NMDA receptor activation induces nitric oxide synthesis from arginine in rat brain slices. *Eur J Pharmacol* 172:413-416, 1989
10. Bredt DS, Snyder SH: Nitric oxide mediates glutamate linked enhancement of cGMP levels in the cerebellum. *Proc Natl Acad Sci USA* 86:9030-9033, 1989
11. Garthwaite J, Southam E, Anderton M: A kainate receptor linked to nitric oxide synthesis from arginine. *J Neurochem* 53:1952-1954, 1989
12. Forstermann U, Gorsky LD, Pollock JS, Schmidt HHHW, Heller M, Murad F: Regional distribution of nitric oxide synthesizing enzyme(s) in rat brain. *Biochem Biophys Res Commun* 168:727-732, 1990
13. Muldoon SM, Hart JL, Bowen KA, Freas W: Attenuation of endothelium-mediated vasodilation by halothane. *ANESTHESIOLOGY* 68:31-37, 1988
14. Uggeri MJ, Johns RA: Halothane attenuates both receptor and non-receptor-mediated EDRF production in rat thoracic aorta (abstract). *ANESTHESIOLOGY* 73:A604, 1990
15. Uggeri MJ, Proctor CJ, Johns RA: Halothane, enflurane, and isoflurane attenuate both receptor and non-receptor-mediated EDRF production in rat thoracic aorta. *ANESTHESIOLOGY* 76:1012-1017, 1992
16. Kant GJ, Muller TW, Lenox RH, Meyerhoff JL: In vivo effects of pentobarbital and halothane anesthesia on levels of adenosine 3', 5'-monophosphate and guanosine 3', 5'-monophosphate in rat brain regions and pituitary. *Biochem Pharmacol* 29:1891-1896, 1980
17. Vulliamoz Y, Verosky M, Alpert M, Triner L: Effect of enflurane on cerebellar cGMP and on motor activity in the mouse. *Br J Anaesth* 55:79-84, 1983
18. Richards CD, Smaje JC: Anaesthetics depress the sensitivity of cortical neurones to L-glutamate. *Br J Pharmacol* 58:347-357, 1976
19. Pearce RA, Stringer JL, Lothman EW: Effect of volatile anesthetics on synaptic transmission in the rat hippocampus. *ANESTHESIOLOGY* 71:591-598, 1989
20. MacIver MB, Roth SH: Inhalation anaesthetics exhibit pathway-specific and differential actions on hippocampal synaptic responses in vitro. *Br J Anaesth* 60:680-691, 1988
21. Eger EI II, Saidman LJ, Brandstater B: Minimum alveolar anesthetic concentration: A standard of anesthetic potency. *ANESTHESIOLOGY* 26:756-763, 1965
22. Eger EI II: Effect of inspired anesthetic concentration on the rate of rise of alveolar concentration. *ANESTHESIOLOGY* 24:153-157, 1963
23. DiFazio CA, Brown RE, Ball CG, Heckel CG, Kennedy SS: Additive effects of anesthetics and theories of anesthesia. *ANESTHESIOLOGY* 36:57-63, 1972
24. Hecker BR, Lake CL, DiFazio CA, Moscicki JC, Engle JS: The decrease of the minimum alveolar anesthetic concentration produced by sufentanil in rats. *Anesth Analg* 62:987-990, 1983
25. Nicoll RA: The effects of anaesthetics on synaptic excitation and inhibition in the olfactory bulb. *J Physiol (Lond)* 223:803-814, 1972
26. Gage PW, McKinnon D, Robertson B: The influence of anesthetics on postsynaptic ion channels, Molecular and Cellular Mechanisms of Anesthetics. Edited by Rothe SH, Miller KW. New York, Plenum Press, 1986, pp 139-153
27. Keane PE, Biziere K: The effects of general anesthetics on GABAergic synaptic transmission. *Life Sci* 41:1437-1448, 1987

28. Crawford JM: Anaesthetic agents and the chemical sensitivity of cortical neurones. *Neuropharmacology* 9:31-46, 1970
29. Barker JL, Gainer H: Pentobarbital: Selective depression of excitatory postsynaptic potentials. *Science* 182:720-722, 1973
30. Teichberg VI, Tal N, Goldberg O, Luini A: Barbiturates, alcohols and the CNS excitatory neurotransmission: Specific effects on the kainate and quisqualate receptors. *Brain Res* 291:285-292, 1984
31. Collingridge GL, Gage PW, Robertson B: Inhibitory post-synaptic currents in rat hippocampal CA1 neurones. *J Physiol (Lond)* 356:551-564, 1984
32. Richards CD: On the mechanism of halothane anaesthesia. *J Physiol (Lond)* 233:439-456, 1973
33. Richards CD, White AE: The actions of volatile anaesthetics on synaptic transmission in the dentate gyrus. *J Physiol (Lond)* 252: 241-257, 1975
34. Galindo A: Effects of procaine, pentobarbital and halothane on synaptic transmission in the central nervous system. *J Pharmacol Exp Ther* 169:185-195, 1969
35. Schofield CN: Potentiation of inhibition of general anaesthetics in neurones of the olfactory cortex in vitro. *Pflugers Arch* 383: 249-255, 1980
36. Garthwaite J: Nitric oxide synthesis linked to activation of excitatory neurotransmitter receptors in the brain, Nitric Oxide from L-Arginine: A Bioregulatory System. Edited by Moncada S, Higgs EA. Amsterdam, Elsevier, 1990, pp 115-137
37. Bredt DS, Snyder SH: Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme. *Proc Natl Acad Sci USA* 87:682-685, 1990
38. Ferreira SH, Duarte IDG, Lorenzetti BB: The molecular mechanism of action of peripheral morphine analgesia: Stimulation of the cGMP system via nitric oxide release. *Eur J Pharmacol* 201:121-122, 1990
39. Ferreira SH: Prostaglandins: Peripheral and central analgesia, *Advances in Pain Research and Therapy*. Volume 5. Edited by Bonica JJ, Lindblom U, Iggo A. New York, Raven Press, 1983, p 627
40. Duarte IDG, Lorenzetti BB, Ferreira SH: Peripheral analgesia and activation of the nitric oxide-cyclic GMP pathway. *Eur J Pharmacol* 186:289, 1990
41. 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* 102:198-202, 1991
42. Gardiner SM, Compton AM, Kemp PA, Bennett T: Regional and cardiac haemodynamic effects of NG-nitro-L-arginine methyl ester in conscious, Long Evans rats. *Br J Pharmacol* 101:625-631, 1990
43. Gardiner SM, Compton AM, Kemp PA, Bennett T: Regional and cardiac haemodynamic responses to glyceryl trinitrate, acetylcholine, bradykinin and endothelin-1 in conscious rats: Effects of NG-nitro-L-arginine methyl ester. *Br J Pharmacol* 101:632-639, 1990
44. Klabunde RE, Ritger RC, Helgren MC: Cardiovascular actions of inhibitors of endothelium-derived relaxing factor (nitric oxide) formation/release in anesthetized dogs. *Eur J Pharmacol* 199: 51-59, 1991
45. van Gelderen EM, Heiligers JPC, Saxena PR: Haemodynamic changes and acetylcholine-induced hypotensive responses after N^G-nitro-L-arginine methyl ester in rats and cats. *Br J Pharmacol* 103:1899-1904, 1991
46. Yamamura T, Harada K, Okamura A, Kemmotsu O: Is the site of ketamine anesthesia the N-methyl D-aspartate receptor? *ANESTHESIOLOGY* 72:704-710, 1990
47. Scheller MS, Zornow MH, Fleischer JE, Shearman GT, Greber TF: The noncompetitive N-methyl D-aspartate receptor antagonist MK-801 profoundly reduces volatile anesthetic requirements in rabbits. *Neuropharmacology* 28:677-681, 1989
48. Daniell LC: The noncompetitive N-methyl D-aspartate antagonists, MK-801, phencyclidine and ketamine, increase the potency of general anaesthetics. *Pharmacol Biochem Behav* 36:111-115, 1990
49. Perkins WJ, Morrow DR: A dose dependent reduction in halothane M.A.C. in rats with a competitive N-methyl-D-aspartate (NMDA) receptor antagonist. *Anesth Analg* 74:S233, 1992
50. Mantz J, Cheramy A, Thierry A-M, Glowinski J, Desmoulets J-M: Anesthetic properties of riluzole (54274 RP), a new inhibitor of glutamate neurotransmission. *ANESTHESIOLOGY* 76:844-848, 1992