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# *Nitric Oxide*

## *Involvement in the Effects of Anesthetic Agents*

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**There has been an explosive increase in the amount of interesting information about the physiologic and pathophysiologic roles of nitric oxide in cardiovascular, nervous, and immune systems. The possible involvement of the nitric oxide–cyclic guanosine monophosphate pathway in the effects of anesthetic agents has been the focus of many investigators. Relaxations of cerebral and peripheral arterial smooth muscle as well as increases in cerebral and other regional blood flows induced by anesthetic agents are mediated mainly *via* nitric oxide released from the endothelium and/or the nitrergic nerve and also *via* prostaglandin $I_2$ or endothelium-derived hyperpolarizing factor. Preconditioning with volatile anesthetics protects against ischemia-reperfusion–induced myocardial dysfunction and cell death or neurotoxicity, possibly through nitric oxide release. Inhibition of nitric oxide synthase decreases the anesthetic requirement. Involvement of nitric oxide in the effects of volatile, intravenous, and local anesthetics differs. This review article includes a summary of information about the sites and mechanisms by which various anesthetic agents interact with the nitric oxide–cyclic guanosine monophosphate system.**

THIS review article covers the involvement of nitric oxide, and also endothelium-derived hyperpolarizing factor (EDHF) or prostaglandin $I_2$ ($PGI_2$), in the effects of anesthetic agents on regional and systemic circulation, including the myocardium, and the central and peripheral nervous systems; and we will discuss the different effectiveness of volatile, intravenous, and local anesthetic agents in experimental mammals. Some information regarding human materials is also included; however, the available information is still insufficient to construct a clinically applicable hypothesis by extrapolating the data from experimental mammals to humans.

This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 5A.

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A detailed discussion about nitric oxide–morphine interactions is beyond the scope of the current review and must remain for a future review article.

Endothelium-derived relaxing factor (EDRF), discovered by Furchgott and Zawadzki,[1] was determined to be biochemically and functionally identical to nitric oxide.[2–4] Introduction of nitric oxide synthase (NOS) inhibitors[5] accelerated the progress of investigations to clarify the important roles of nitric oxide in the regulation of not only cardiovascular functions but also central and peripheral nerve functions and immune reactions. Nitric oxide has beneficial–destructive duality; nitric oxide formed *via* constitutive NOS mainly has physiologically pivotal functions as an endothelial messenger, neurotransmitter, or neuromodulator, whereas nitric oxide formed in excess through inducible NOS is detrimental to cell viability.[6] $PGI_2$ (prostacyclin) synthesized from arachidonic acid in the endothelial cells possesses a vasodilator action and an antiaggregatory property as nitric oxide does.[7] There are evidences supporting the hypothesis that vascular endothelial cells can liberate one or more active substances, other than nitric oxide and $PGI_2$, that result in hyperpolarization of vascular smooth muscle cell membranes associated with muscular relaxation; therefore, it is called EDHF.[8]

Besides the major effects on the central nervous system in eliciting unconsciousness, analgesia, and decreased skeletal muscle tone, anesthetic agents exert a variety of actions on the whole body, mainly on the cardiovascular and nervous systems. There is evidence that nitric oxide and other endothelium-derived vasodilating factors are involved in mechanisms underlying the action of anesthetic agents; *i.e.*, contribution to vasodilator and hypotensive responses to anesthetics, beneficial effects of anesthetic preconditioning against ischemic damage in the heart and brain, and involvement in alterations of the minimum alveolar concentration for volatile anesthesia (MAC).

## Syntheses and Actions of Nitric Oxide and Other Endothelium-derived Relaxing Factors

### *Nitric Oxide*

Nitric oxide is produced when L-arginine is transformed to L-citrulline through catalysis by NOS in the

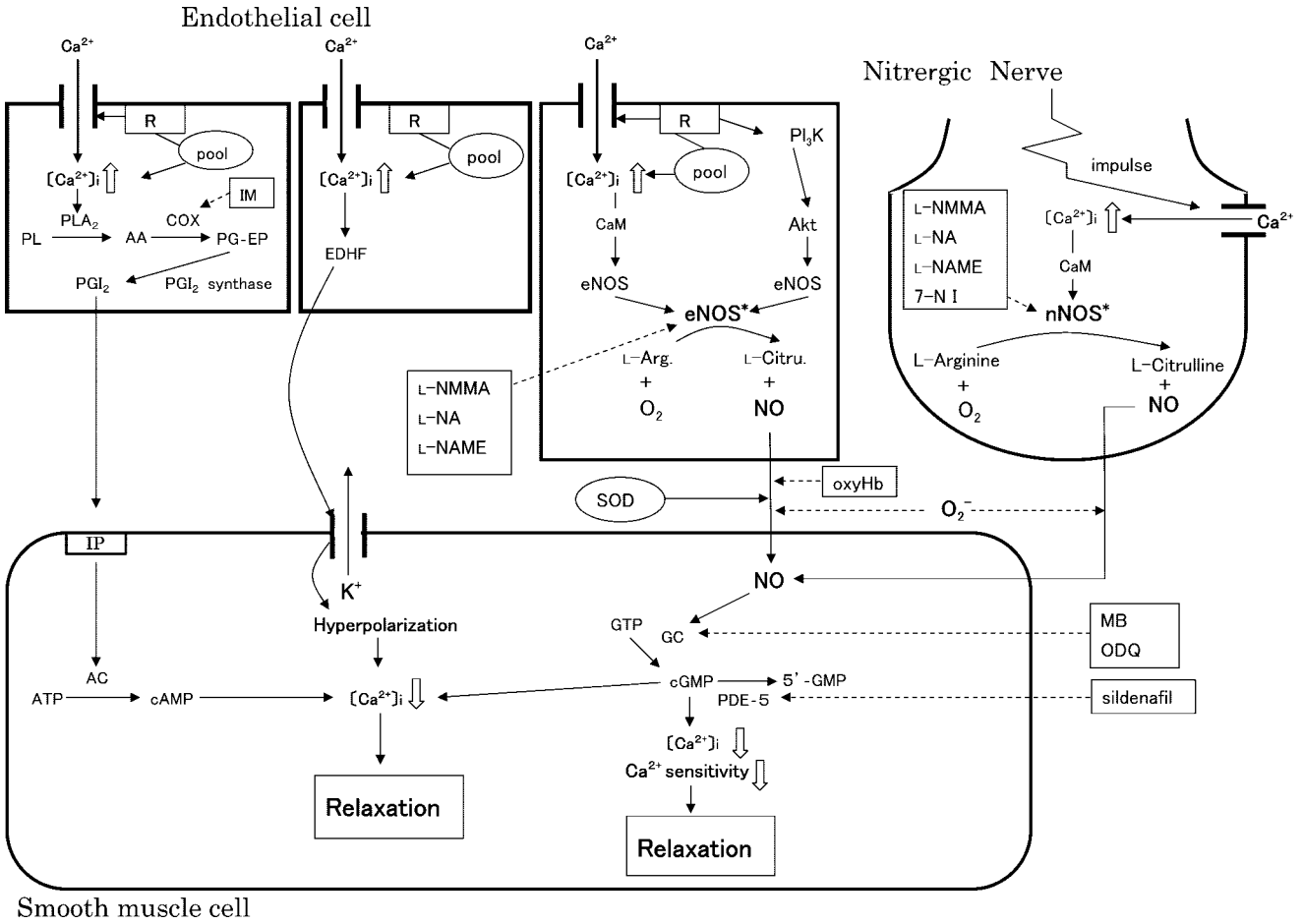


**Fig. 1. Schematic presentation of information pathways *via* nitric oxide (NO), prostaglandin $I_2$ ($PGI_2$), and endothelium-derived hyperpolarizing factor (EDHF) from endothelial cells or NO from nitrergic nerves to vascular smooth muscle cells. In the *third square from the left* (for endothelial nitric oxide synthase [eNOS]), the transmembrane influx of $Ca^{2+}$ and its mobilization from intracellular storage sites are elicited by activation of drug receptors (R), such as muscarinic, peptidergic (bradykinin and substance P), and $\alpha_2$-adrenergic receptors, located on the endothelial cell membrane or by mechanical stimuli such as shear stress. Shear stress, bradykinin, or insulin induces the phosphorylation of $Ser^{1177/1179}$ of eNOS through phosphatidylinositol-3 kinase ($PI_3K$) and the downstream serine/threonine protein kinase Akt (protein kinase B), resulting in increased NO formation. This mechanism does not require the increase in intracellular $Ca^{2+}$ for NO production. At the *top right*, nitrergic nerves (postganglionic parasympathetic) innervating the vascular wall participate in maintaining vasodilatation in cerebral arteries that are scarce in adrenergic vasoconstrictor innervation and also contribute to functionally counteract the adrenergic vasoconstrictor nerves in peripheral blood vessels to maintain blood flow homeostasis. In the *top left square*, activation of receptors by agonists or mechanical stress applied to the endothelial cell membrane leads to transmembrane $Ca^{2+}$ influx; the cations activate phospholipase $A_2$ ($PLA_2$) to form arachidonic acid (AA), thus increasing the $PGI_2$ synthesis. $PGI_2$ liberated from the endothelial cells binds to $PGI_2$ (IP) receptors located in smooth muscle cell membranes, activates adenylyl cyclase (AC), and stimulates cyclic adenosine monophosphate (cAMP) production, resulting in vascular smooth muscle relaxation. *Solid line* denotes stimulation; *dotted line* denotes inhibition; *R* denotes receptive site for chemical or mechanical stimuli; *pool* denotes $Ca^{2+}$ storage site. 7-NI = 7-nitroindazol; ATP = adenosine triphosphate; $[Ca^{2+}]_i$ = intracellular $Ca^{2+}$ concentration; CaM = calmodulin; cGMP = cyclic guanosine monophosphate; COX = cyclooxygenase; GC = guanylyl cyclase; GTP = guanosine triphosphate; IM = indomethacin; L-Arg. = L-arginine; L-Citru. = L-citrulline; L-NA = $N^G$-nitro-L-arginine; L-NAME = $N^G$-nitro-L-arginine methylester; L-NMMA = $N^G$-monomethyl-L-arginine; MB = methylene blue; nNOS = neuronal nitric oxide synthase; NOS* = activated nitric oxide synthase; $O_2$ = oxygen; $O_2^-$ = superoxide anion; oxyHb = oxyhemoglobin; PDE-5 = phosphodiesterase-5; PG-EP = prostaglandin endoperoxide; PL = phospholipids; SOD = superoxide dismutase.**

presence of oxygen and a number of cofactors: reduced nicotinamide adenine dinucleotide phosphate, tetrahydrobiopterin, calmodulin, heme, flavin adenine dinucleotide, and flavin mononucleotide. $Ca^{2+}$ is required for the activation of neuronal NOS (nNOS, NOS I) and endothelial NOS (eNOS, NOS III) but not inducible or immunologic NOS (iNOS, NOS II). The nNOS, mostly a soluble enzyme, is constitutively expressed in the brain[9] and peripheral nerves. eNOS is also constitutively expressed mostly in particulate fractions of the endothelial cell.[10] iNOS is not constitutively expressed but is induced mainly in macrophages with bacterial lipopolysaccharide and cytokines.

**Nitric Oxide Derived from the Endothelium.** Endothelial NOS binds to caveolin 1 in the caveolae, microdomains of the plasma membrane. Caveolin 1 inhibits eNOS activity, and this interaction is regulated by $Ca^{2+}$/calmodulin.[11] eNOS intracellularly migrates in response to increased cytosolic $Ca^{2+}$ in the presence of calmodulin and is activated for nitric oxide synthesis (fig. 1). The synthesis of nitric oxide by NOS isoforms is inhib-

ited by L-arginine analogs, including *N* $^{G}$-monomethyl-L-arginine (L-NMMA),[5] *N* $^{G}$-nitro-L-arginine (L-NA), L-NA methylester (L-NAME), and asymmetric dimethylarginine.[12] 7-Nitroindazol (7-NI) is one of the most promising nNOS inhibitors so far introduced.[13]

Endothelial nitric oxide causes vasodilatation, decreased vascular resistance, decreased blood pressure, inhibition of platelet aggregation and adhesion, inhibition of leukocyte adhesion and transmigration, and reduced vascular smooth muscle proliferation, and acts to prevent atherosclerosis. Nitric oxide or nitrovasodilators activate soluble guanylyl cyclase and produce cyclic guanosine monophosphate (cGMP) from guanosine triphosphate in smooth muscle cells. Methylene blue, oxyhemoglobin, and 1H[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one[14] inhibit the activity of soluble guanylyl cyclase. Accumulation of cGMP causes activation of cGMP-dependent protein kinase, which involves a reduction of intracellular $Ca^{2+}$ and a decrease in the sensitivity of contractile elements to $Ca^{2+}$, resulting in smooth muscle relaxation (fig. 1). cGMP is degraded by phosphodiesterase type 5 to 5′-GMP.

**Nitric Oxide Synthesized by Neuronal NOS.**

*Autonomic Nerves.* Nonadrenergic noncholinergic inhibitory responses to autonomic nerve stimulation are mainly mediated through nitric oxide synthesized by nNOS; nitric oxide plays a crucial role as a neurotransmitter from the peripheral efferent nerves in blood vessels[15] (fig. 1) and gastrointestinal tracts.[16,17] On the other hand, afferent nitrergic nerves control some sensory information processing, such as pain[18,19] and reflex.[20]

*Central Nervous System.* In the brain, nitric oxide functions mainly as a neuromodulator. Nitric oxide signaling seems to be essential for two forms of neural plasticity: long-term potentiation in the hippocampus and long-term depression in the cerebellum. These forms of neural plasticity underlie aspects of both learning and information storage in the brain.[21] Glutamate participates mainly in synaptic interactions, but with the help of nitric oxide, the strength of excitatory input might be nonsynaptically signaled to the surrounding monoaminergic neurons in the brain. Nitric oxide formed by *N*-methyl-D-aspartate (NMDA) receptor activation diffuses to adjacent nerve terminals to modulate neurotransmitter release.[22] Nitric oxide can also regulate secretion of hormones and neuropeptides. The nitric oxide–cGMP pathway may also be involved in sleep[23] and the circadian clock.[24]

**Nitric Oxide Synthesized by Inducible NOS.** Under pathologic conditions (*e.g.*, during inflammation), high levels of nitric oxide are produced after induction of the expression of iNOS, mainly in macrophages.[25] Nitric oxide possesses the protective–destructive duality inherent in every other major component of the immune response. On the one hand, it exerts beneficial effects by acting as an antibacterial, antiparasitic, antiviral agent, or tumoricidal agent; on the other hand, high levels of nitric oxide, if uncontrolled, elicits detrimental effects that are produced because nitric oxide reacts with concomitantly produced superoxide anions, thereby generating highly toxic compounds such as peroxynitrite and hydroxyl radicals.

*Prostaglandin $I_2$*

The prostaglandin family, including $PGI_2$ (prostacyclin), is synthesized from arachidonic acid formed from phospholipids through phospholipase $A_2$. Cyclooxygenase synthesizes prostaglandin endoperoxides from arachidonic acid (fig. 1), and its activity is inhibited by aspirin, indomethacin, ibuprofen, and other nonsteroidal antiinflammatory drugs. An enzyme that transforms prostaglandin endoperoxides to $PGI_2$ was found in microsomes prepared from the aorta[7] and in cultured endothelial cells.[26]

*Endothelium-derived Hyperpolarizing Factor*

Endothelium-dependent vasodilatation is not always blocked by inhibitors of NOS and cyclooxygenase. Acetylcholine elicited relaxation and hyperpolarization of muscle cell membranes in the rat aorta and pulmonary artery with intact endothelium, the mechanical response being abolished by hemoglobin and methylene blue without any effect on hyperpolarization, suggesting that acetylcholine releases two different substances, nitric oxide and EDHF, from endothelial cells (fig. 1).[8] Like its cousins, nitric oxide and $PGI_2$, EDHF is an important regulator of blood flow.[27] The electrical and mechanical responses mediated by EDHF are blocked by treatment with $K^+$ channel inhibitors or exposure to high $K^+$ media. $Ca^{2+}$-activated and adenosine 5′-triphosphate (ATP)–sensitive $K^+$ channels seem to play a major role in hyperpolarization that is responsible for muscle relaxation. Although different mechanisms of action of EDHF are reported in a variety of blood vessels, there is still considerable debate regarding the chemical nature of EDHF.[28,29]

## Actions of Anesthetics in Relation to Nitric Oxide

*Cardiovascular System*

**Isolated Blood Vessels and Platelets.** Endothelium-derived relaxing factor is released from vascular endothelial cells both under basal conditions and after stimulation with various agonists or mechanical stress *in vitro* and *in vivo*. Anesthetic agents modulate vascular tone and platelet aggregation by changing the basal and stimulated release of vasodilator factors from the endothelium.

*Volatile Anesthetics.*

**Effects on basal release of nitric oxide, EDHF, and $PGI_2$.** In intraparenchymal arterioles in the rat brain slice, halothane, as well as sodium nitroprusside (SNP),

**Table 1. Cerebral Vasodilatation and Blood Flow Increase Induced by Volatile Anesthetics in Various Mammals**

| Reference, Year | Animal | Anesthetic Dose | Response | Mediator |
|---|---|---|---|---|
| Eskinder *et al.*,[35] 1992 | Dog | 2% and 3% halothane | Cerebral artery relaxation | Cyclic GMP but not NO |
| Harkin *et al.*,[30] 1997 | Rat | 0.5–2.5% halothane | Brain slice arteriole relaxation | Possibly NO (not determined) |
| Staunton *et al.*,[33] 2000 | Rat | 0.6–2.6% halothane | Cerebral microvascular dilation | NO derived from nNOS but not from eNOS |
| Koenig *et al.*,[113] 1993 | Rat | 1–3% halothane | Pial vasodilatation | NO |
| McPherson *et al.*,[114] 1993 | Dog | 1 MAC halothane<br>1 MAC isoflurane<br>70% $N_2O$ | CBF increase | NO |
| Hudetz *et al.*,[115] 1994 | Rat | 1–2 MAC halothane | CBF increase | NO and prostaglandin |
| Smith *et al.*,[32] 1995 | Rat | 1.7 MAC halothane | CBF increase | NO and prostaglandin |
| Moore *et al.*,[116] 1994 | Pig | 1 MAC isoflurane | CBF increase | NO and prostaglandin |
| Okamoto *et al.*,[117] 1997 | Mouse | 1.2–2.4% isoflurane | CBF increase | NO from eNOS at 1.2 and 1.8% isoflurane<br>NO from nNOS at 2.4% isoflurane |

CBF = cerebral blood flow; eNOS = endothelial nitric oxide synthase; GMP = guanosine monophosphate; MAC = minimum alveolar concentration; $N_2O$ = nitrous oxide; nNOS = neuronal nitric oxide synthase; NO = nitric oxide.

elicited vasodilatation.[30] Although whether nitric oxide was responsible for vasodilator response to halothane was not actually determined, these authors postulated the involvement of nitric oxide on the basis of findings obtained from studies on rat pial vessels *in vivo* by Keonig *et al.*[31] and Smith *et al.*[32] In hippocampal arterioles of rat brain slices, halothane-induced vasodilatation was attenuated by treatment with 7-NI or L-NAME to a similar extent, whereas acetylcholine-induced vasodilatation was not inhibited by 7-NI but was converted to constriction by L-NAME, suggesting that halothane-induced dilatation is mediated, in part, by neurally derived nitric oxide and that eNOS does not play a major role in the dilatation of hippocampal microvessels[33] (table 1). The halothane-induced relaxation in isolated rabbit basilar arteries was endothelium independent.[34] On the other hand, halothane increased tissue cGMP levels in isolated canine cerebral arteries; halothane, unlike SNP, did not modulate the activity of soluble guanylyl cyclase, whereas halothane, like atrial natriuretic peptide, stimulated the particulate guanylyl cyclase activity.[35] Their conclusion was that although cGMP is involved in the halothane-induced relaxation of canine cerebral arteries, nitric oxide does not seem to participate in the increase in cGMP levels.

Halothane and enflurane had no significant effect on NOS activity in cultured bovine aortic endothelial cells.[36] Halothane caused decreases in tension in the canine carotid and rabbit aortic preparations but increased tension in the femoral artery; these effects were not altered by removal of the endothelium.[37] The rat aortic endothelium attenuated the vasodilator effect of isoflurane by a mechanism that was abolished by inhibition of NOS activity.[38]

There were findings suggesting that halothane and desflurane induce the release of nitric oxide and vasodilating prostaglandins in coronary arteries of blood-perfused isolated rabbit hearts, whereas in contrast, these mediators are not involved in the coronary vasodilating effect of isoflurane.[39] In superfused small mesenteric arteries and veins, isoflurane elicited hyperpolarization that was abolished by inhibitions of $Ca^{2+}$-activated and ATP-sensitive $K^+$ channels, cyclic adenosine monophosphate, and protein kinase A, but not by inhibitions of nitric oxide, cGMP, and protein kinase G, equally in normotensive and spontaneously hypertensive rats.[40]

**Effects on stimulated release of nitric oxide, EDHF, and $PGI_2$.** Both receptor-mediated and non–receptor-mediated endothelium-dependent relaxation of rat aortic rings in response to methacholine and $Ca^{2+}$ ionophore A23187, respectively, were attenuated by halothane and enflurane at 2 MAC and by isoflurane at 1 MAC.[41] Halothane and isoflurane attenuated acetylcholine-induced, endothelium-dependent relaxation and decreased the acetylcholine-stimulated levels of cGMP in the rat aorta.[42] Sevoflurane impaired relaxations induced by acetylcholine, bradykinin, and $Ca^{2+}$ ionophore A23187 of canine and rabbit mesenteric artery rings but did not affect the relaxation to nitroglycerin.[43,44] In the rabbit perfused lung, isoflurane attenuated the L-NAME–sensitive relaxation to acetylcholine but did not change the L-NAME–insensitive nitroglycerin-induced relaxation.[45] It seems that isoflurane inhibits nitric oxide–dependent relaxation by acting at a site distal to the endothelial cell receptor–mediated responses but proximal to guanylyl cyclase activation of vascular smooth muscle.

Halothane attenuated endothelium-dependent relaxations of the isolated rabbit aorta and canine femoral and carotid arteries in response to acetylcholine and bradykinin; however, halothane did not affect relaxations caused by nitroglycerin.[37] In endothelial cell–vascular smooth muscle cocultures, halothane and isoflurane inhibited bradykinin–, ATP–, and $Ca^{2+}$ ionophore–stimulated, nitric oxide–dependent cGMP accumulation but did not depress nitrovasodilator-induced cGMP formation, suggesting that the anesthetics seem to inhibit

nitric oxide–guanylyl cyclase signaling distal to receptor activation and proximal to nitric oxide activation of guanylyl cyclase in the endothelial cells.[46] In rat aortic rings, halothane and isoflurane inhibited methacholine-stimulated, nitric oxide–mediated vasorelaxation but did not alter the cGMP increase caused by iNOS in the lipopolysaccharide-treated rings, suggesting that these anesthetics inhibit only receptor/$Ca^{2+}$-activated NOS action and that direct inhibition of NOS, soluble guanylyl cyclase, or an interaction with nitric oxide is not responsible for anesthetic inhibition of endothelium-dependent relaxation.[47]

In cultured porcine aortic endothelial cells, sevoflurane diminished bradykinin-induced transient increase in intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) and reduced the amount of nitric oxide released by bradykinin from endothelial cells.[48] In rat aortic strips, halothane and L-NAME inhibited carbachol-induced, endothelium-dependent relaxation in a similar manner, and halothane inhibited carbachol-induced increases in $[Ca^{2+}]_i$.[49] Using fura-2–loaded rat pulmonary arterial valve leaflets, sevoflurane was found to inhibit the increase in endothelial $[Ca^{2+}]_i$ induced by bradykinin.[50]

Nakamura *et al.*[51] have demonstrated that mechanisms underlying the inhibition of endothelium-dependent relaxation differed among anesthetics; isoflurane inhibits the response mainly by the formation of endothelial nitric oxide, sevoflurane may inactivate nitric oxide or inhibit the action of nitric oxide, and the effect of halothane may be due to the inhibition of nitric oxide actions on vascular smooth muscle. Halothane and enflurane, but not isoflurane, inhibited bradykinin- and ATP-stimulated $Ca^{2+}$ transients in bovine endothelial cells; limitations of $Ca^{2+}$ availability to activate eNOS could account for part of the inhibition of endothelium-dependent, nitric oxide–mediated vasodilatation by volatile anesthetics.[52] Using a bioassay method for detection of EDRF/nitric oxide with bovine endothelial cells as a donor tissue and the endothelium-denuded rabbit aortic ring as an assay tissue, Blaise *et al.*[53] noted that enflurane added to the perfusate either upstream or downstream to the assay tissue attenuated the aortic relaxation induced under stimulation by bradykinin of endothelial cells, whereas isoflurane added either upstream or downstream to endothelial cells potentiated the relaxation induced by the basal release of EDRF but attenuated the relaxation to bradykinin-stimulated release of EDRF. It seems that enflurane decreases the stability of EDRF/nitric oxide released after bradykinin stimulation, whereas isoflurane increases the stability or action of the basal EDRF and decreases the stability of the bradykinin-stimulated EDRF. The reason for different actions of these anesthetics on the stability of EDRF/nitric oxide under basal and stimulated conditions remains unanswered.

There was evidence suggesting the idea that the effects of halothane and isoflurane on $Ca^{2+}$ homeostasis in bovine aortic endothelial cells reflect a reduction of the thapsigargin- or bradykinin-evoked $Ca^{2+}$ influx, which would be consequent to a cellular depolarization caused by an inhibition of the $Ca^{2+}$-activated $K^+$ channel activity initiated after cell stimulation.[54] Halothane inhibited endothelium-dependent relaxation caused by acetylcholine in rat aortic rings to a greater extent than in mesenteric arterial rings; this anesthetic also inhibited nitric oxide–independent EDHF-mediated relaxation in the mesenteric artery.[55] Halothane seems to have an ability to inhibit endothelium-dependent relaxation in the aorta (mainly nitric oxide dependent) more than in the mesenteric artery (nitric oxide and EDHF dependent). Desflurane, enflurane, and sevoflurane selectively inhibited the acetylcholine-induced release of EDHF, and halothane and isoflurane inhibited the nitric oxide–mediated relaxant response to acetylcholine as well as the response mediated by EDHF in isolated rabbit carotid arteries; relaxations induced by SNP were not influenced by any of the anesthetics tested.[56] In addition, cytochrome P-450 inhibitors abolished the EDHF-mediated relaxation elicited by acetylcholine. Therefore, attenuation by volatile anesthetics of the EDHF-mediated, acetylcholine-induced relaxation seems to be attributable to inhibition of the cytochrome P-450–dependent synthesis of EDHF by the endothelium. The same authors[57] also observed that isoflurane, etomidate, and thiopental, but not phenobarbital, attenuated the EDHF-mediated vasodilator response to bradykinin in the coronary microcirculation of isolated perfused rat hearts after inhibition of nitric oxide and $PGI_2$ formation.

In rabbit small mesenteric arteries, acetylcholine caused endothelium-dependent relaxation and hyperpolarization; the relaxation in response to low concentrations of acetylcholine was abolished by L-NA, oxyhemoglobin, and methylene blue, and the L-NA–resistant relaxation and hyperpolarization elicited by higher concentrations of acetylcholine were both blocked by tetraethylammonium.[58] Isoflurane, enflurane, and sevoflurane inhibited both of these L-NA–sensitive and L-NA–resistant, tetraethylammonium-sensitive responses but did not affect the SNP-induced relaxation. In the rat cremaster muscle microcirculation, L-NMMA inhibited acetylcholine- and bradykinin-induced vasodilatation during isoflurane but not halothane or ketamine anesthesia; exposure to superfusion fluids with high $K^+$, an inhibitor of EDHF action, unmasked acetylcholine-stimulated, nitric oxide–dependent relaxation during halothane or ketamine anesthesia, suggesting that anesthetics can alter the balance between nitric oxide and EDHF vasodilatation in the microcirculation and that nitric oxide–dependent mechanisms are enhanced and EDHF action is inhibited during isoflurane anesthesia.[59] Gambone *et al.*[60] and Seki *et al.*[61] obtained evidence suggesting that isoflurane and halothane attenuated endothelium-dependent vasorelaxation of

isolated canine pulmonary arteries by inhibiting ATP-sensitive $K^+$ channel activity.

There are evidences casting some doubt about the selective inhibition of volatile anesthetics on the actions of endothelial nitric oxide and EDHF. In rat aortic rings, relaxations induced by acetylcholine, exogenous nitric oxide, and nitroglycerin were attenuated by halothane, which also inhibited the nitric oxide–stimulated cGMP content, suggesting that the site of action of halothane is within the vascular smooth muscle, rather than on the synthesis or release of EDRF from the endothelium, and that its action may involve an interference with guanylyl cyclase activation.[62,63] The inhibition by halothane, enflurane, and isoflurane of acetylcholine– and nitric oxide–induced relaxations in vascular smooth muscle was suggested to be the result of competition between nitric oxide and anesthetics for the heme moiety on the soluble guanylyl cyclase.[64] The same group[65] provided evidence indicating that halothane and inhibitors of soluble guanylyl cyclase, methylene blue and 6-anilino-5,8-quinolinedione (LY 83583), may act through competitive antagonism at a common site of action on soluble guanylyl cyclase in the EDRF–nitric oxide relaxation pathway. Halothane attenuated nitroglycerin-induced, endothelium-independent relaxation of the rat aorta by suppressing $Ca^{2+}$ dynamics in the smooth muscle.[66] Diminished coronary vasodilation induced by bradykinin and serotonin after NOS inhibition in isolated guinea pig hearts was largely restored or enhanced by halothane, sevoflurane, or isoflurane.[67] In isolated, perfused rat mesenteric arteries, halothane and isoflurane do not seem to affect endothelium-dependent vasodilatations induced by acetylcholine; however, after the NOS activity is inhibited, high concentrations of halothane, but neither isoflurane nor the lower concentration of halothane, seem to impair endothelium-dependent vasodilatation, possibly mediated by tetraethylammonium-sensitive $K^+$ channels.[68]

Ogawa *et al.*[69] provided evidence suggesting that the endothelium and vascular smooth muscle of the canine basilar artery are more susceptible to oxidative stress than those of the mesenteric artery and that halothane at clinically relevant concentrations exerts no significant influence on this vascular injury. Sevoflurane, when administered in combination with nitroglycerin, enhanced the development of nitroglycerin tolerance under hyperoxic conditions, possibly by generation of superoxide anions or hydroxyl radicals within vascular smooth muscle.[70]

*Intravenous Anesthetics.*

**Effect on basal release of nitric oxide, EDHF, and $PGI_2$.** Propofol was suggested to induce endothelium-dependent relaxation of the isolated rat aorta, possibly *via* release of vasodilator prostaglandins.[71] High concentrations of propofol relaxed bovine coronary artery rings, the response being suppressed by endothelium denudation or treatment with methylene blue.[72] In cocultures of porcine aortic endothelial and smooth muscle cells, propofol increased cGMP formation, and this increase was inhibited after treatment with either L-NA or hemoglobin; when applied to smooth muscle cells alone, propofol did not result in an increase in cGMP levels, suggesting that propofol stimulates the production and release of nitric oxide from cultured endothelial cells.[73] In isolated rat distal coronary arteries, propofol produced vasodilatation that was attenuated by endothelial denudation and treatment with L-NA or indomethacin but was not affected by glibenclamide, an ATP-sensitive $K^+$ channel inhibitor, indicating a possible involvement of nitric oxide and vasodilator prostaglandins in the endothelium-dependent vasodilatation.[74] Because the inhibitory effect of propofol on contraction induced by norepinephrine and vasopressin in the endothelium-intact spontaneously hypertensive rat aorta was observed in the presence of NOS inhibitors but not cyclooxygenase inhibitors, Boillot *et al.*[75] suggested that propofol either induces the release of vasodilating cyclooxygenase metabolites from the endothelium or inhibits vasoconstrictor cyclooxygenase metabolites. Propofol decreased perfusion pressure elevated by increasing $K^+$ concentrations in the perfused rat lung; indomethacin and L-NAME did not affect the response to propofol, but glibenclamide inhibited it.[76] In superfused small mesenteric arteries and veins of the rat, propofol resulted in hyperpolarization and relaxation of smooth muscle, the responses being abolished by inhibition of $Ca^{2+}$-activated and ATP-sensitive $K^+$ channels and by inhibition of nitric oxide and cGMP.[77] It seems that propofol-induced hyperpolarization and relaxation is due to activation of both of the $K^+$ channels that are mediated by the nitric oxide– cGMP pathway. The toxic effects on endothelial cells incubated with the peroxynitrite donor 3-morpholino sydnonimine were decreased by treatment with propofol, which reacted with peroxynitrite more rapidly than did tyrosine, resulting in an inhibition of nitrotyrosine formation.[78] The antioxidant property of propofol may be partly attributed to its scavenging effect on peroxynitrite.

On the other hand, there was evidence indicating that propofol-induced vasodilatation in the rat pulmonary vascular bed is not mediated or modulated by the release of nitric oxide, opening of ATP-sensitive $K^+$ channels, or the release of vasodilator prostaglandins.[79] Ketamine caused pulmonary vasodilatation, possibly mediated by an L-type $Ca^{2+}$ channel–sensitive pathway.[80] Vasorelaxation of rat aortic rings induced by *S*(+)- and *R*(−)-ketamine was not affected by removal of the endothelium and treatment with L-NA or glybenclamide.[81]

UK14,304, an $\alpha_2$-adrenoceptor agonist used as an adjunct to anesthetics, produced relaxation of isolated rat middle cerebral arteries that were blocked by removal of the endothelium or addition of L-NAME or pertussis toxin, suggesting that endothelial nitric oxide-dependent

rat cerebral relaxation induced by $\alpha_2$-adrenoceptor stimulation is mediated by a pertussis toxin–sensitive G protein.[82]

**Effect on stimulated release of nitric oxide, EDHF, and $PGI_2$.** From studies comparing the effect of nonbarbiturate intravenous anesthetics on relaxations induced by acetylcholine and SNP in isolated rat aortae, propofol and ketamine were found to suppress endothelium-dependent relaxation, but midazolam had no influence on it,[83] leading us to postulate that the inhibitory effect of ketamine is mediated by suppression of nitric oxide formation, whereas that of propofol may be mediated at least partly by suppression of nitric oxide function. Etomidate at clinically relevant concentrations attenuated endothelium-dependent relaxation induced by acetylcholine of rat aortic rings, possibly by acting at a site distal to the endothelial muscarinic receptor but proximal to guanylyl cyclase activation of vascular smooth muscle.[84]

Acetylcholine-induced vasorelaxation seemed to be mediated by two components, nitric oxide and a cytochrome P-450 metabolite, likely to be an EDHF, in canine pulmonary arterial rings, and propofol was suggested to selectively attenuate the acetylcholine-induced relaxation by inhibiting both of these endothelium-derived mediators.[85] Etomidate and ketamine attenuated vasorelaxation of canine pulmonary arterial rings in response to acetylcholine and bradykinin; relaxations induced by these agonists were inhibited by L-NAME or tetrabuthylammonium alone and were abolished by combined treatment.[86] Inhibitory effects of these anesthetics may be associated with both nitric oxide– and EDHF–mediated components. There were findings indicating that etomidate, but not ketamine, attenuated the endothelium-dependent component of levcromakalim (an ATP-sensitive $K^+$ channel activator)–induced canine pulmonary arterial relaxation *via* an inhibition of the cyclooxygenase pathway.[87] In canine pulmonary vein rings with the intact endothelium, propofol and thiopental attenuated relaxation induced by levcromakalim, and the anesthetic-induced inhibition of levcromakalim relaxation was decreased after pretreatment with L-NAME but not with indomethacin.[88] These anesthetics seem to attenuate the endothelium-dependent component of ATP-sensitive $K^+$ channel–induced vasorelaxation *via* an inhibitory effect on the nitric oxide pathway.

*Local Anesthetics.* The procaine-induced relaxation in rat aortic rings may be mediated through multiple mechanisms: A substantial portion of the relaxation is caused by endothelial nitric oxide; the activation of tetraethylammonium-sensitive $K^+$ channels contributes in part to the procaine-induced, endothelium-independent relaxation; and procaine may directly inhibit external $Ca^{2+}$ entry and internal $Ca^{2+}$ release in smooth muscle cells.[89]

Local anesthetics, lidocaine, bupivacaine, and mepivacaine, reduced endothelium-dependent relaxation to bradykinin in isolated porcine ciliary arteries, whereas the endothelium-independent relaxation to the nitric oxide donor 3-norpholino sydnonimine was unaffected; L-arginine reduced the inhibitory effect of bupivacaine, suggesting that local anesthetics impair endothelial formation of nitric oxide from L-arginine.[90] Relaxations induced by acetylcholine and SNP of the rat aorta were attenuated by lidocaine, tetracaine, bupivacaine, and ropivacaine, whereas papaverine-induced relaxations were inhibited by the former three but were augmented by ropivacaine; cGMP levels in acetylcholine-stimulated aortae were reduced by the former three but were not affected by ropivacaine.[91]

Lidocaine reduced relaxation elicited by ATP-sensitive $K^+$ channel openers cromakalim and pinacidil in the isolated rat aorta without the endothelium[92]; alkalinization to pH 7.6 augmented the inhibitory effect of lidocaine, whereas acidification to pH 7.2 abolished this effect.[93] In the isolated endothelium-denuded rat carotid artery, lidocaine inhibited relaxations induced by ATP-sensitive $K^+$ channel openers but did not affect the response to hypoxia that was mediated by ATP-sensitive $K^+$ channel opening.[94] These authors[95] obtained evidence suggesting that lidocaine reduces vasodilatation possibly mediated by ATP-sensitive $K^+$ channels in rat cerebral microvessels but not vasodilatation by inward rectifier $K^+$ channels. Lidocaine also inhibited vasorelaxation and hyperpolarization in response to levcromakalim in porcine coronary artery.[96] These findings may indicate that lidocaine interferes with the vasodilatation mediated *via* EDHF that is liberated from endothelial cells in response to chemical or physical stimuli and activates ATP-sensitive $K^+$ channels in vascular smooth muscle cells. In the rat aorta with intact endothelium, *R*(+)-bupivacaine and *S*(−)-bupivacaine inhibited vasorelaxation in response to levcromakalim, whereas ropivacaine did not affect this relaxation; in the aorta without endothelium, *R*(+)-bupivacaine inhibited vasorelaxation to the ATP-sensitive $K^+$ channel opener, whereas *S*(−)-bupivacaine reduced the relaxation only in the highest concentration used.[97]

*Studies in Human Materials.*

**Effects on basal release of nitric oxide, EDHF, and $PGI_2$.** In human mesenteric artery rings, thiopental elicited endothelium-dependent relaxation that was inhibited by L-NAME and indomethacin, whereas propofol-induced relaxation was endothelium independent.[98] It seems that relaxations induced by thiopental, but not propofol, are mediated by nitric oxide and vasodilator prostaglandins. Bodelsson *et al.*[99] provided evidence suggesting that propofol at clinically relevant concentrations promotes relaxations *via* EDHF in isolated human omental arteries and *via* both nitric oxide and EDHF in human omental veins. Sevoflurane promoted endothelium-dependent relaxation in human omental arteries and veins,

probably *via* an enhancement of the response of smooth muscle to relaxing mediators such as cGMP.[100]

In cultured human endothelial cells, isoflurane inhibited the capacitative $Ca^{2+}$ entry, suggesting that isoflurane apparently depresses nitric oxide–mediated vasodilatation when the observed inhibition is not compensated for downstream of the eNOS activation.[101] Bupivacaine-induced relaxation of isolated human umbilical arteries was not mediated by nitric oxide and prostaglandins.[102] Ketamine in therapeutic concentrations decreased the level of nitrite and eNOS protein production in human umbilical vein endothelial cells and inhibited the levels of eNOS messenger RNA and bradykinin-enhanced $[Ca^{2+}]_i$, suggesting that the inhibitory effect of ketamine on nitric oxide biosynthesis is associated with a pretranslational inhibition of eNOS expression and a posttranslational decrease in eNOS activity due to a reduction of $[Ca^{2+}]_i$ levels.[103]

**Effects on stimulated release of nitric oxide, EDRF, and $PGI_2$.** In isolated human pulmonary arteries, halothane inhibited the endothelium-dependent, nitric oxide–mediated relaxation by acetylcholine but did not affect the response to SNP, the adenylyl cyclase activator forskolin, and the ATP-sensitive $K^+$ channel opener cromakalim, suggesting that the inhibitory effect of halothane on acetylcholine-induced relaxations is associated with interference with the nitric oxide pathway at a site before activation of soluble guanylyl cyclase in smooth muscle.[104] On the other hand, the relaxant response to acetylcholine, which was resistant to both NOS and cyclooxygenase blockade, was depressed by a $Ca^{2+}$-activated $K^+$ channel blocker, and a cytochrome P-450 inhibitor in isolated human renal arterial segments; both etomidate and thiopental attenuated the relaxation induced by acetylcholine, but not the response by SNP, suggesting that these anesthetics inhibit the EDHF-mediated relaxant response to acetylcholine in human renal arteries.[105] Propofol at clinically relevant concentrations attenuated tumor necrosis factor $\alpha$–induced apoptosis and decreased the Bcl-2/Bax ratio in human umbilical vein endothelial cells; this was accompanied by increased nitric oxide production.[106]

**Effects on platelets and leukocytes.** Sevoflurane was suggested to inhibit secondary platelet aggregation induced by adenosine 5′-diphosphate or epinephrine in human platelets, possibly reducing thromboxane $A_2$ formation by suppressing cyclooxygenase activity; halothane seemed to suppress both thromboxane formation and binding to its receptors; and isoflurane did not affect platelet aggregation.[107] Propofol (40 $\mu$M) enhanced, whereas 100 $\mu$M suppressed, adenosine- and epinephrine-induced secondary aggregation of human platelets without affecting primary aggregation.[108] Sevoflurane and propofol had an inhibitory effect on intraoperative and early postoperative platelet aggregation in patients, whereas isoflurane had no effect.[109] However, the authors did not determine whether nitric oxide or cyclooxygenase products were involved in the inhibitory effect. Propofol-induced inhibition of human platelet aggregation was greater in whole blood than in platelet-rich plasma, inhibition of platelet aggregation correlated with inhibition of thromboxane $A_2$ synthesis, and the anesthetic potentiated the nitric oxide–cGMP pathway, mainly by increasing the synthesis of nitric oxide by leukocytes.[110] In surgical patients who received a bolus injection of propofol, platelet aggregation was reduced in whole blood and in platelet-rich plasma plus leukocytes, platelet thromboxane $B_2$ was reduced, and plasma levels of nitrites plus nitrates increased, indicating that the propofol-induced inhibition of platelet aggregation results from the decrease in thromboxane synthesis and increase in nitric oxide production.[111] However, halothane, ketamine, etomidate, and thiopentone were found to reduce NOS activity in human polymorphonuclear leukocytes; this effect was specific because other enzymes were unaffected.[112]

In summary, on the basis of studies using blood vessels isolated from experimental animals, volatile and intravenous anesthetics in clinically relevant concentrations interfere with the synthesis and release of EDRF, mainly nitric oxide, from endothelial cells in response to chemical stimuli or with its actions on vascular smooth muscle. These anesthetics stimulate the basal release of EDRF to cause vasorelaxation sometimes only at concentrations higher than clinically relevant ones. Local anesthetics also inhibit the stimulated release of nitric oxide from the endothelium. As expected, there is heterogeneity in the responsiveness to anesthetics between drugs with different chemical structures even in the same category, regions of blood vessels used, or animal species. Information about the effects of anesthetic agents on human blood vessels in relation to the basal and stimulated release of EDRF (nitric oxide, EDHF, and/or $PGI_2$) is still insufficient to raise a validated hypothesis.

**Blood Flow and Blood Pressure Responses.**

*Cerebral Blood Flow.* In rats anesthetized with nitrous oxide–fentanyl, suffusions of 1–3% halothane produced pial arteriolar and venular dilatations that were suppressed by NOS inhibition, whereas vasodilatation induced by SNP was not affected; L-NA and L-NAME constricted pial arterioles and venules, suggesting that nitric oxide production contributes to halothane-induced dilatation of cerebral microvessels.[113] In dogs anesthetized with pentobarbital, inhaled halothane, isoflurane, and nitrous oxide increased cerebral blood flow, and L-NAME prevented increased cerebral blood flow by the volatile anesthetics.[114] In rats in which the mean blood pressure and laser Doppler flow under steady state conditions were achieved at 0.5 or 1 MAC of halothane, raising the level of inspired halothane increased cerebrocortical blood flow and decreased cerebrovascular resistance; these responses were attenuated by intravenous L-NAME,

further attenuation being attained by treatment with indomethacin, suggesting that nitric oxide is not an obligatory mediator, but may have a permissive role, in halothane-induced cerebral vasodilation.[32,115] In pentobarbital-anesthetized pigs, isoflurane increased cerebral blood flow, and both L-NAME and indomethacin attenuated the response to isoflurane.[116] Okamoto *et al.*[117] provided evidence that isoflurane-induced increase in regional blood flow of the cerebral cortex is preserved in nNOS gene–deficient mice; in wild-type mice, eNOS and nNOS contribute to isoflurane-induced cerebral hyperemia (table 1). Contrarily, in rats under urethane-chloralose anesthesia, 7-NI induced an increase in mean arterial pressure, and halothane eliminated the 7-NI–induced pressor effect; on the other hand, cerebral blood flow decreased after 7-NI injection regardless of the type of anesthesia.[118] nNOS seems to be more important in the cerebral vasculature, and eNOS seems to be more important in the peripheral vasculature. The authors suggested that halothane interferes with eNOS-mediated vascular tone but not with nNOS-mediated control of cerebral blood flow. Todd *et al.*[119] suggested that nitric oxide may not be the primary mediator responsible for the effects of isoflurane and pentobarbital on rabbit cerebral blood flow, but rather acts to influence background vascular tone in animals anesthetized with these drugs. Ketamine reduced isoflurane-induced cerebral vasodilatation in pentobarbital-anesthetized rabbits with a closed cranial window, apparently independent of nitric oxide formation, whereas sevoflurane-induced cerebral vasodilatation was not affected by ketamine.[120]

As presented so far, cerebral blood flow increase in response to anesthetics may be mediated by nitric oxide formed by nNOS and eNOS. Cerebral vascular tone and blood flow under resting conditions are regulated by the basal release of nitric oxide from perivascular nitrergic nerves and endothelial cells in various mammals.[121] Postganglionic neurons from the pterygopalatine ganglion innervate cerebral arteries, and preganglionic neurons innervating the pterygopalatine ganglion originate through the greater petrosal nerve, possibly from the superior salivatory nucleus in the brainstem.[122–124] There are glutamatergic, GABAergic, and glycinergic inputs to superior salivatory neurons in the rat.[125,126] Excitatory inputs to the superior salivatory nucleus are expected to evoke activation of nitrergic nerves innervating cerebral vasculature and activation of cholinergic nerves innervating lacrimal, nasal, and salivary glands.[123,127,128] Whether the parasympathetic nucleus in the brainstem is stimulated by anesthetics remains to be elucidated.

Oxotremorine, a muscarinic agonist, increased blood flow to forebrain regions in isoflurane-anesthetized dogs but did not change cerebral blood flow in pentobarbital-anesthetized dogs; L-NAME decreased baseline blood flow and prevented oxotremorine-induced hyperemia.[129] Stimulation of NOS may be involved in the muscarinic agonist–induced hyperemia; however, the reason for different susceptibility of the response to oxotremorine during isoflurane and pentobarbital anesthesia was not determined. Oxotremorine-induced cerebral hyperemia was preserved in rats anesthetized with nitrous oxide–fentanyl.[130] Sevoflurane dose-dependently increased brain tissue nitrite and impaired the autoregulation.[131] The volatile anesthetic may impair cerebrovascular autoregulation by mechanisms secondary to the increase in perivascular nitric oxide availability. In anesthetized pigs, inhaled nitric oxide increased cerebral blood volume and cerebral transit time, whereas cerebral blood flow remained unchanged, indicating a vasodilator action of inhaled nitric oxide in the cerebral vasculature, which may occur preferentially in the venous compartment.[132] In spontaneously hypertensive rats that had undergone middle cerebral artery occlusion, the volume of injured brain in the thiopental group was smaller than that in the halothane control group, and the volumes of injured brain in the etomidate and isoflurane groups were larger than those in the control and thiopental groups; there was evidence to support the speculation that the detrimental effect of etomidate may result from nitric oxide of cerebral endothelial origin being bound by the iron component of free hemoglobin associated with etomidate-induced hemolysis, and the adverse effect of isoflurane may be due to cerebral perfusion pressure associated with vasodilatation.[133]

*Coronary Blood Flow.* In conscious rats, L-NAME decreased blood flow to the heart; during either barbiturate or halothane anesthesia, L-NAME did not alter coronary blood flow, indicating that both barbiturate and halothane seem to inhibit endothelial nitric oxide–mediated regulation of coronary hemodynamics.[134] In conscious dogs, intravenous L-NMMA increased coronary blood flow and did not affect coronary vascular resistance, whereas in halothane-anesthetized dogs, L-NMMA induced a coronary vasoconstriction.[135] The nitric oxide system seems to be involved in the control of coronary vascular tone in the presence of halothane. In rat epicardial arteries, flow-induced vasodilatation was endothelium dependent and mediated by both nitric oxide and prostanoids; isoflurane attenuated flow-induced vasodilatation, possibly by decreasing synthesis, the actions of nitric oxide and prostanoids, or both, whereas halothane enhanced it, possibly by increasing synthesis, the action of nitric oxide, or both.[136] In goats anesthetized with pentobarbital, indices of myocardial metabolism were lower than those anesthetized with ketamine; the duration of the reactive hyperemia was shorter in the ketamine group than in the pentobarbital group, suggesting that pentobarbital decreases metabolic activity, whereas ketamine reduces the hyperemic response, in which impaired endothelial function seems to be involved.[137]

In barbiturate-anesthetized dogs, sevoflurane increased retrograde coronary collateral blood flow, the effect being attenuated by the $Ca^{2+}$-activated $K^+$ channel inhibitor iberiotoxin but not L-NAME.[138] In perfused guinea pig hearts, isoflurane slightly increased coronary flow but not effluent nitric oxide concentrations; halothane, isoflurane, and sevoflurane did not alter the bradykinin-induced increase in coronary flow and effluent nitric oxide and L-citrulline concentrations.[139]

*Pulmonary Blood Flow.* Nitric oxide was reduced in the exhalate of horses anesthetized with halothane compared with intravenous anesthetics, ketamine, guaiphenesin, and romifidine, and mean pulmonary artery pressure was higher during halothane anesthesia compared with intravenous anesthesia.[140] Propofol had no effect on the baseline pulmonary vascular pressure–flow relation in dogs compared with the conscious state; pulmonary vasodilator responses to bradykinin and proline–nitric oxide were similar in the conscious and propofol-anesthetized states, whereas in contrast, acetylcholine-induced vasodilatation was attenuated during propofol anesthesia.[141] Propofol may cause a specific defect in the signal transduction pathway for acetylcholine-induced pulmonary vasodilatation, and this defect seems to involve the endothelial component of the response.

There was evidence indicating that the lidocaine-induced increase of pulmonary vascular resistance was enhanced when a capacity for compensatory vasodilatation including the EDRF–nitric oxide pathway was exhausted in halothane-anesthetized dogs.[142] In a canine cross-circulation model treated with ibuprofen, pulmonary vasoconstriction induced by lidocaine infusion after treatment with L-NA was greater than that before the NOS inhibitor, and it was reversed to the level present without L-NA by additional administration of L-arginine, leading to the conclusion that lidocaine-induced pulmonary vasoconstriction is modulated by the EDRF–nitric oxide pathway in dog lung.[143]

*Renal Blood Flow.* In conscious and barbiturate-anesthetized rats, L-NAME decreased renal blood flow, but during halothane anesthesia, L-NAME did not alter renal blood flow.[134] Halothane anesthesia may eliminate the synthesis of nitric oxide or its action. Halothane caused renal vasoconstriction and inhibited the nitric oxide–guanylyl cyclase signaling pathway in the rabbit kidney.[144] Renal hemodynamic responses to halothane may be induced, in part, through an inhibition of this pathway. Neither halothane nor sevoflurane at 0.8 MAC altered renal blood flow and renal interstitial cGMP and nitrite/nitrate levels in dogs, but both anesthetics decreased these values at 2.4 MAC; changes in cGMP and nitrite/nitrate concentrations were correlated with renal blood flow changes during anesthesia, suggesting that halothane- and sevoflurane-induced decreases in intrarenal nitric oxide levels result from reduction in blood flow.[145]

*Blood Flow in Other Materials.* Carotid, mesenteric, and renal vasoconstriction induced by L-NMMA was blunted in dogs during halothane-anesthesia compared with awake dogs.[146] In sevoflurane-anesthetized rats, the elevation of sevoflurane concentration evoked adhesive responses of leukocytes, concurrent with platelet margination and rolling in mesenteric venules, such changes in microvessels being presented by pretreatment with hemin, a heme oxygenase-1 inducer; nitric oxide suppression by L-NAME deteriorated microvascular flows irrespective of the presence or absence of hemin.[147] Endogenous carbon monoxide seems to attenuate sevoflurane-induced microvascular endothelial interactions with leukocytes and platelets, although local nitric oxide levels may dominate microvascular flow *in situ*. It was demonstrated that administration of ketamine led to increased plasma nitric oxide levels, induction of metabolic acidosis, and oxidative damage, although without reaching hepatic damage in rats, and that when experimental hypothermia was induced, ketamine affected hepatic blood flow.[148] The authors suggested that researchers doing studies on physiologic processes involving nitric oxide should exercise caution if anesthesia is induced by ketamine

In rats anesthetized with thiopental, halothane elicited arteriolar dilatation in the rat diaphragm, which was abolished by indomethacin and mefenamic acid but was not modified by L-NA.[149] Halothane-induced vasodilatation may be mediated by vasodilator prostaglandins but not by nitric oxide. Bazin *et al.*[150] found that diaphragmatic arteriolar diameters in rats, regulated by vasodilator prostaglandins, were greater during etomidate than during thiopental or propofol anesthesia.

*Blood Pressure.* Increases in mean arterial pressure induced in rats that received intravenous L-NA, possibly attained by depression of basal release of nitric oxide, differed under the influence of anesthetic agents, as follows: Althesin (mixture of alphaxalone and alphadolone) > conscious = pentobarbital = chloralose = ketamine = urethane > enflurane ≫ halothane.[151] Whether the attenuation by volatile anesthetics of the pressor response to L-NA is associated with the anesthetic-induced interference with the baseline nitric oxide synthesis or nitric oxide availability remained unanswered. On the other hand, L-NMMA elicited a greater increase in blood pressure in urethane/α-chloralose– and pentobarbital–anesthetized rats than in conscious rats, suggesting that the experimental conditions (anesthetized or conscious) modify the contribution of spontaneously released nitric oxide to blood pressure regulation *in vivo*.[152] Halothane blunted or blocked systemic hemodynamic responses to NOS inhibition seen in conscious and barbiturate-anesthetized rats.[134] Propofol induced increases in heart rate, coronary blood flow, and

carotid blood flow and a decrease in systemic vascular resistance, whereas intralipid, the solvent for propofol, increased carotid and mesenteric vascular resistance; in the presence of intralipid, the L-NMMA–induced pressor response and systemic and regional vasoconstriction were more pronounced than in control dogs.[153]

*Studies in Humans.* Hypotension induced by propofol, but not etomidate, in angiotensin-converting enzyme inhibitor–treated hypertensive patients was suggested to be the result of the additive effect of the similar endothelium-dependent mechanisms of action of propofol and angiotensin-converting enzyme inhibitor, *i.e.*, increase in production and release of nitric oxide.[154]

Lidocaine and other local anesthetic agents stimulated nitric oxide generation in human peripheral neutrophils under resting conditions, this effect being attenuated by NOS inhibition; these anesthetics also enhanced formyl-methionyl-leucyl-phenylalanine– or phorbol myristate acetate–induced nitric oxide generation.[155] Therefore, nitric oxide is suggested to mediate various pharmacologic effects of the local anesthetics on the host defense mechanism and the control of blood pressure. In forearm skin of healthy, male subjects, after the traumatic effects of injection had subsided, L-NAME reduced the vasodilator response to intradermally injected prilocaine, whereas aspirin had no effect, leading to the conclusion that vasoactive effects of the local anesthetic are mediated partly through the release of endothelial nitric oxide and, although other mechanisms may also be involved, the cyclooxygenase pathway does not seem to play a role.[156]

**Heart.**

*Volatile Anesthetics.* Cardiac functions and metabolism are regulated by anesthetic agents as well as nitric oxide released from the vascular endothelium and endocardium. Beneficial ways to protect the myocardium against coronary circulatory insufficiency are the major concern of many clinicians. Anesthetic preconditioning or postconditioning would be one of the useful strategies performed by anesthesiologists.

**Preconditioning.** In isolated, perfused guinea pig hearts, left ventricular pressure and coronary flow recovered to a greater extent after ischemic preconditioning and anesthetic preconditioning (APC) with sevoflurane than in hearts given no treatment before ischemia (the control), treated with ischemic preconditioning plus glibenclamide, or treated with APC plus glibenclamide; effluent nitric oxide concentrations increased in APC and ischemic preconditioning groups after ischemia, compared with the control group and each of the glibenclamide groups; coronary flow increases to bradykinin and SNP were greater after APC and ischemic preconditioning. The protective effects of APC and ischemic preconditioning, possibly mediated by nitric oxide, were reversed by ATP-sensitive $K^+$ channel blockade.[157] The authors of the same group[158] noted that effluent dityrosine, a marker of peroxynitrite, was decreased, left ventricular pressure was increased, and infarct size was reduced in the APC group, compared with the nontreated ischemic control group, APC plus reactive oxygen species (ROS) scavengers group, and APC plus L-NAME group in isolated guinea pig hearts. APC seems to be initiated by ROS, and the protective and ROS/reactive nitrogen species–reducing effects of APC are attenuated when bracketed by ROS scavengers or nitric oxide inhibition. Similar findings were also obtained with isoflurane that appears to confer delayed cardioprotection in the rat, triggered by ROS and reactive nitrogen species.[159] It was suggested that ROS and nitric oxide, or reaction products including peroxynitrite, mediate attenuation of sevoflurane-induced mitochondrial electron transport in the guinea pig heart; this may lead to a positive feedback mechanism with augmented ROS generation to trigger APC secondary to altered mitochondrial function.[160]

In rabbits with myocardial ischemia–reperfusion injury, the infarct size was reduced, and plasma lactate dehydrogenase and creatine kinase levels and duration of ventricular arrhythmia were decreased in the desflurane preconditioning group, as compared with those in the control and L-NAME plus desflurane–treated groups.[161] Delayed APC by isoflurane (1 day before experimentation) reduced infarct size in male rabbits subjected to ischemia–reperfusion, and L-NAME, but not aminoguanidine or 7-NI, abolished the isoflurane-induced protection. In addition, infarct size was reduced, and eNOS protein expression was greater, in female *versus* male rabbits; infarct size was unchanged in female rabbits with and without isoflurane pretreatment, and L-NAME, but not iNOS and nNOS inhibitors, increased infarct size.[162] Female sex–induced reductions in infarct size may be mediated by eNOS, but remote isoflurane exposure before ischemia and reperfusion does not seem to produce additional cardioprotection *in vivo*. Postischemic left ventricular function in the rat heart was improved 48 h after inhalation of 1.5 MAC isoflurane, and iNOS expression and activity in the heart were increased 24–72 h after isoflurane; a selective iNOS inhibitor, 1400W, abolished iNOS activation and cardioprotection.[163]

Nuclear factor-κB (NF-κB)–DNA binding activity was increased at the end of reperfusion in the ischemic rat heart, and cytosolic NF-κB inhibitor was decreased; APC with sevoflurane attenuated NF-κB activation and reduced the expression of tumor necrosis factor α, interleukin 1, and iNOS, together with decreases in infarct size and creatine kinase release and improvement of myocardial function.[164] Attenuation of NF-κB activation and subsequent down-regulation of NF-κB–dependent inflammatory gene expression seems to play an important role in the protective mechanism of APC against acute myocardial injury. Flavoprotein fluorescence, an

index for mitochondrial ATP-sensitive $K^+$ channel activity, was increased and infarct size after ischemia was reduced by APC with isoflurane in isolated perfused rat hearts; coadministration of adenosine and *S*-nitroso-*N*-acetyl-penicillamine, an nitric oxide donor, with isoflurane conferred a highly significant reduction of infarct size and improvement of left ventricular function without increasing flavoprotein oxidation over isoflurane alone.[165] Therefore, it was concluded that mitochondrial ATP-sensitive $K^+$ channel activation seems to be a crucial mediator of cardioprotection afforded by APC with isoflurane and that enhanced cardioprotection conferred by combined preconditioning may be mediated through both mitochondrial ATP-sensitive $K^+$ channel–dependent and –independent mechanisms.

**Postconditioning.** In pentobarbital-anesthetized rabbits, postconditioning (four cycles of coronary artery reperfusion-coronary artery occlusion after 30 min of coronary artery occlusion) reduced myocardial infarct size after ischemia–reperfusion *versus* the control; isoflurane inhaled throughout the experiment reduced infarct size in control and enhanced the protective effect of postconditioning. When isoflurane was administered only during reperfusion, infarct size was not changed, but its combination with postconditioning reduced infarct size; L-NA abolished the effect of postconditioning alone or in combination with isoflurane during reperfusion.[166] Anesthetic postconditioning with isoflurane improved functional recovery in the rat heart and decreased acute infarct size and lactate dehydrogenase release; this protection was abolished by LY294002, which inhibited phosphorylation of protein kinase B/Akt and its downstream targets glycogen synthase kinase 3$\beta$, eNOS, and p70S6 kinase.[167] Infarct-remodeled myocardium seems to be receptive to protection by isoflurane postconditioning *via* protein kinase B/Akt signaling. Brief exposure to isoflurane during early reperfusion after prolonged coronary occlusion in barbiturate-anesthetized rabbits reduced infarct size of the left ventricle; the Erk1/2 inhibitor PD 098059, the p70s6K inhibitor rapamycin, and L-NAME, but neither the iNOS inhibitor aminoguanidine nor the nNOS inhibitor 7-NI, abolished the protection produced by isoflurane.[168]

**Miscellaneous.** Nitric oxide synthase activity in the guinea pig heart was higher and the nitric oxide pool (nitric oxide plus nitrite) was lower in the group of animals that had been given isoflurane and oxygen mixture *via* a facemask, compared with those of the control and oxygen groups; in the oxygen group, malondialdehyde was higher compared with the other groups, suggesting that isoflurane prevents peroxidation reactions in heart tissues, possibly by scavenging toxic oxygen radicals produced under hyperoxygenation conditions as occurs with general anesthesia.[169] In isolated rat papillary muscles, the administration of sevoflurane caused a reduction in contractility, and the negative effect was not altered by treatment with L-NA; during continuous administration of sevoflurane, the positive inotropic effect of isoproterenol was not influenced by L-NA.[170]

*Intravenous Anesthetics.* Thiopental decreased myocardial function in isolated cat papillary muscles with intact endocardial endothelium, and the negative inotropic effect of the anesthetic at low doses was abolished when the endothelium was removed or by L-NAME.[171] Propofol caused negative chronotropy and the enhancement of nitrite production in cultured rat ventricular myocytes, and these effects were depressed by atropine, methoctramine, or L-NMMA; propofol displaced [$^3$H]quinuclidinyl benzilate binding to the cell membrane of myocytes, suggesting that the negative chronotropy induced by propofol is mediated in part by $M_2$ muscarinic receptor activation, which involves the enhancement of nitric oxide production in cultured ventricular myocytes.[172] Propofol decreased peak shortening of ventricular myocytes from diabetic rats, reduced actomyosin ATPase activity, and increased troponin I phosphorylation in myofibrils compared with values in normal rats; protein kinase C (PKC) inhibition prevented the propofol-induced increase in troponin phosphorylation and decrease in shortening; expression of PKC-$\alpha$, PKC-$\delta$, PKC-$\varepsilon$, and constitutive NOS were up-regulated and iNOS was expressed in diabetic cardiomyocytes.[173] Increases in PKC and NOS expression in combination with troponin phosphorylation seem to contribute to the decrease in $[Ca^{2+}]_i$ and myofilament $Ca^{2+}$ sensitivity; propofol is suggested to decrease $[Ca^{2+}]_i$ and shortening *via* a PKC- and NOS-dependent pathway.

The duration of ventricular tachycardia was less in urethane-anesthetized rats during the occlusion and reperfusion periods when compared with that in pentobarbital-anesthetized rats, but the incidence of ventricular fibrillation during reperfusion was higher; L-NAME had no significant effect on the difference observed between the two anesthetic groups.[174] In isolated, perfused guinea pig hearts, ischemia–reperfusion without anesthetics increased coronary neutrophil adherence; *S*(+)-ketamine reduced postischemic adherence, as did the racemate, and although *R*(−)-ketamine had no effect on adhesion, it increased vascular leakage in the presence of L-NA.[175]

*Local Anesthetics.* In anesthetized (halothane plus nitrous oxide) and paralyzed rats, the average doses of intravenous bupivacaine producing arrhythmias and asystole were markedly lower with L-NAME treatment than with saline treatment, and plasma concentrations of bupivacaine were higher with L-NAME treatment; however, electroencephalographic epileptiform activity was less intense in the L-NAME–treated animals.[176] Studies from the same research group[177] demonstrated that L-NAME decreased the tetracaine and lidocaine doses that produced arrhythmias and asystole, with a greater dose-reducing effect on tetracaine than lidocaine, *versus* sa-

line treatment; plasma concentrations of lidocaine, but not tetracaine, were higher in L-NAME–treated rats than in saline-treated ones. These data implied that inhibition of nitric oxide production enhances the cardiotoxicity of lidocaine and tetracaine; however, altered drug clearance by NOS inhibition is insufficient to explain these findings.

*Nervous System*

**Brain.** The synthesis and release of nitric oxide and constitutive NOS activity in the brain are modulated by anesthetic agents, resulting in alterations in the central nervous function and cerebral blood flow; nitric oxide is involved in anesthetic preconditioning-induced neuroprotection against ischemic injury or excitatory amino acids; and MAC for volatile anesthetics is reduced by treatment with NOS inhibitors, nitric oxide scavengers, and guanylyl cyclase inhibitors.

*Volatile Anesthetics.*

**Basal release of nitric oxide and NOS activity.** Cyclic guanosine monophosphate, but not cyclic adenosine monophosphate, was increased in the whole brain of halothane-exposed rats.[178] In rats, unstimulated cerebellar nitric oxide concentrations were greater during anesthesia with isoflurane than anesthesia with halothane, and L-NAME pretreatment reduced nitric oxide concentrations during isoflurane, but not halothane, anesthesia, indicating that increased nitric oxide production during isoflurane anesthesia is expected to impact central neuronal function and cerebral blood flow and vascular resistance.[179] In rats anesthetized with isoflurane, there was loss of the righting reflex coincident with an elevation in hippocampal nitrite/nitrate levels, whereas rats exposed to nitrous oxide showed loss of the righting reflex but no change in hippocampal nitrite/nitrate; when rats were pretreated with L-NAME, the isoflurane-induced increases in nitrite/nitrate were suppressed.[180] Nitric oxide contents in the cortex and cerebellum were increased in rats anesthetized with halothane compared with those in waking rats; the changes of nitrite/nitrate contents were more drastic in the cortex than in the cerebellum.[181] In rats anesthetized with halothane, isoflurane, and sevoflurane, nitric oxide contents in the brain cortex were greater as compared with the nonanesthetized animals; L-NA abolished the increase in nitric oxide content produced by volatile anesthetics.[182] The behavioral effects of nitrous oxide in the light–dark exploration test in mice were attenuated after treatment with the nitric oxide scavenger hemoglobin and the nNOS inhibitor *S*-methyl-L-thiocitrulline but were unaltered by either an eNOS inhibitor or an iNOS inhibitor; exposure to nitrous oxide increased NOS activity in the cerebellum and corpus striatum.[183] Nitric oxide produced by nNOS may be involved in nitrous oxide–linked anxiolytic-like behavior. Sevoflurane and isoflurane anesthesia increased the nitric oxide concentration in the rat brain cortex and decreased that in the cerebellum. The nitric oxide increase was abolished by pretreatment with an iNOS inhibitor, and anesthesia enhanced the increase in nitric oxide concentration in the brain cortex after intraventricular lipopolysaccharide administration, suggesting that a putative role for iNOS in the increase in nitric oxide levels produced by volatile anesthetics, whereas nNOS activity is probably inhibited during anesthesia.[184]

Halothane and isoflurane decreased NOS activity in rat brain extracts.[185] NOS activity and cGMP levels were similar in all brain regions in rats, and during halothane anesthesia, cGMP contents were decreased.[186] Crude rat and bovine brain NOS activity was not affected by halothane and enflurane.[35]

**Stimulated release of nitric oxide.** In rat cerebellar slices, halothane suppressed formation of cGMP after stimulation by NMDA and D-aspartate that increases intracellular $Ca^{2+}$ but not after stimulation by SNP; and isoflurane suppressed NMDA-stimulated, but not D-aspartate– and SNP–stimulated, formation of cGMP, whereas thiopental suppressed NMDA–, D-aspartate–, and SNP–stimulated formation of cGMP.[187] Increase in cGMP production in cultured rat cerebral neurons in response to NMDA, quisqualate, and kainate was inhibited by halothane or isoflurane at clinically relevant concentrations, whereas the increase in cGMP production stimulated by SNP was not influenced by these anesthetics, suggesting that halothane or isoflurane inhibited the nitric oxide–cGMP signaling pathway stimulated by excitatory amino acids and the site of this inhibition is proximal to the activation of nNOS.[188]

In contrast, isoflurane at clinically relevant concentrations enhanced the stimulated effect of glutamate, NMDA, or kainate on cGMP production in cultured rat cortical neurons, whereas halothane or enflurane had no effect.[189] In rat cerebellar slices, isoflurane enhanced the NMDA-stimulated NOS activity, whereas halothane produced no effect; however, the NMDA-stimulated cGMP production was inhibited by both anesthetic agents, this effect being unaltered by a mixture of superoxide dismutase and catalase or by glycine, a coagonist of NMDA receptors.[190] The inhibitory effect of these anesthetics on cGMP accumulation may not be due to either their interaction with the glycine binding site of the NMDA receptor or the action of superoxide anions.

**Preconditioning.** Anesthesia with isoflurane or halothane before permanent middle cerebral artery occlusion in rats reduced infarct volumes compared with the control; Western blot analysis from cortical extracts of rats with APC revealed an increase in the iNOS protein, and aminoguanidine eliminated the infarct-sparing effect of the preconditioning.[191] In 7-day-old rats subjected to left common carotid arterial ligation followed by hypoxia, isoflurane preconditioning did not alter the mortality but did increase the weight ratio of left/right cerebral

**Table 2. Modulation by Nitric Oxide of Volatile Anesthetic Minimum Alveolar Concentration in Experimental Animals**

| Reference, Year | Animal | Anesthetic | Treatment (Dose) | MAC Change | Mediator |
|---|---|---|---|---|---|
| Johns *et al.*,[195] 1992 | Rat | Halothane | L-NAME (20 mg/kg IV) | Decrease (51%) | NO |
| Ichinose *et al.*,[201] 1995 | Mouse (nNOS-KO) | Isoflurane | | None | |
| | Mouse (wild) | Isoflurane | L-NAME (acute) (25 mg/kg IP) | Decrease (28%) | NO |
| | Mouse (wild) | Isoflurane | L-NAME (chronic) | None | |
| Pajewski *et al.*,[199] 1996 | Rat | Isoflurane | L-NAME (30 mg/kg IV) | Decrease (35%) | NO |
| | | | 7-NI (500 mg/kg IP) | Decrease (43%) | NO *via* nNOS |
| Chen *et al.*,[196] 1997 | Rabbit | Isoflurane | L-NAME (30 mg/kg IV) | Decrease (11%) | NO |
| Chen *et al.*,[197] 1998 | Rat | Isoflurane | L-NAME (30 mg/kg IV) | Decrease (37%) | NO |
| Chen *et al.*,[198] 1999 | Rat | Isoflurane | Carboxy-PTIO (0.6 mg/kg IV) | Decrease (19%) | NO |
| Fukuda *et al.*,[200] 1999 | Rat | Halothane | 7-NI (500 mg/kg IP) | Decrease (87%) | NO *via* nNOS |
| Masaki and Kondo,[204] 1999 | Rat | Sevoflurane | MB (5 mg ICV) | Decrease (28%) | Cyclic GMP |
| Tao *et al.*,[205] 2000 | Rat | Isoflurane | G-kinase inhibitor (100 μg/10 μl IT) | Decrease (30%) | NO/cyclic GMP/G-kinase |
| | | Isoflurane | NO donor | Increase | NO |
| Cechova and Pajewski,[206] 2004 | Rat | Isoflurane | ODQ (500 mg/kg IP) | Decrease (52%) | NO/cyclic GMP |
| Engelhardt *et al.*,[202] 2006 | Mouse | Isoflurane | 7-NI (120 mg/kg IP) | Decrease (25%) | NO *via* nNOS |
| | Mouse (nNOS-KO) | Isoflurane | 7-NI (120 mg/kg IP) | Decrease (38%) | NO *via* nNOS* |

* The authors suggested that only minimal neuronal nitric oxide synthase (nNOS) activity is required to maintain cellular homeostasis or that alternative compensatory pathways (up-regulation of nNOS splice variants such as nNOSg and nNOSb) exist.

7-NI = 7-nitroindazol; G-kinase = cyclic GMP–dependent protein kinase; GMP = guanosine monophosphate; ICV = intracerebroventricular; IP = intraperitoneal; IT = intrathecal; IV = intravenous; KO = knockout; L-NAME = $N^G$-nitro-L-arginine methylester; MB = methylene blue; NO = nitric oxide.

hemispheres in the survivors; isoflurane induced a time-dependent increase in iNOS proteins, and the APC-induced neuroprotection was abolished by aminoguanidine.[192] Isoflurane preconditioning reduced the neurotoxicity induced by glutamate, NMDA, and α-amino-3-hydroxy-5-methyl-4-isoxazol propionic acid in rat cerebellar slices; this neuroprotection was abolished by protein kinase inhibitors or L-NAME.[193] In contrast, hypoxic injury in rat cerebrocortical slices was attenuated by $Na^+$ channel blockers, such as lidocaine and dibucaine, and $Ca^{2+}$ channel blockers, such as verapamil and ω-conotoxin, and halothane abolished the protective effects of these channel blockers; all channel blockers tested attenuated hypoxia-evoked nitric oxide synthesis, estimated from the extracellular cGMP formation, and halothane blocked these actions of channel blockers.[194] Therefore, halothane was suggested to reverse the $Na^+$ and $Ca^{2+}$ channel blockade, leading to the attenuation of its cerebroprotective actions possibly *via* a restoration of nitric oxide synthesis.

**MAC for volatile anesthetics.** Bolus injection of L-NAME to rats resulted in a dose-dependent reduction in MAC for halothane anesthesia, and infusion of L-arginine reversed the MAC reduction by L-NAME, suggesting that inhibition of the nitric oxide pathway decreases the level of consciousness and augments anesthesia, analgesia, or sedation[195] (table 2). The MAC for isoflurane was reduced in the presence of L-NAME in rabbits[196] and rats[197]; the NOS inhibitor also inhibited the activity of constitutive NOS in the cerebellum.[197] Bolus injection of carboxy-PTIO, a nitric oxide scavenger, reduced the MAC value of isoflurane and increased cerebellar NOS activity during isoflurane anesthesia, suggesting that the level of nitric oxide may set a baseline from which isoflurane then acts.[198] Inhibition of the NOS pathway by L-NAME and 7-NI decreased the MAC for isoflurane in rats.[199] The effectiveness of 7-NI is consistent with the effect being selective for nNOS. 7-NI also decreased halothane MAC in rats, which was accompanied by suppression of the nNOS activity and reduction of the number of nicotinamide adenine dinucleotide phosphate-diaphorase–positive cells and the staining intensity of the axons in the locus ceruleus and spinal cord, supporting the hypothesis that the nitric oxide signaling pathway is related to MAC.[200]

Targeted disruption of the nNOS gene did not modify the MAC for isoflurane and the righting reflex $ED_{50}$ in knockout mice; however, acute administration of L-NAME decreased the isoflurane MAC and righting reflex $ED_{50}$ in wild-type mice but did not alter those values in knockout mice (table 2). In addition, the wild-type mice, when given L-NAME for a week, showed values identical to those of the untreated wild-type mice.[201] Therefore, the authors suggested that although acute inhibition of NOS reduces the anesthetic requirements of wild-type mice, a chronic deficiency of nNOS or a week-long administration of L-NAME does not decrease the MAC for isoflurane. However, 7-NI reduced isoflurane MAC, the righting reflex, and spontaneous motor activity in both wild-type and nNOS knockout mice, indicating that the NMDA receptor–nitric oxide–cGMP pathway remain a credible target for modulating the effects of isoflurane.[202]

In mice, acute administration of 7-NI decreased sevoflurane MAC and cerebellar cGMP; 4-day-long gavage feeding with 7-NI decreased cGMP, but sevoflurane MAC was reduced only for the first 2 days, indicating

dissociation between the two parameters during long-term nNOS inhibition.[203] There may be cGMP–independent compensatory mechanisms that mediate nociception when NOS is chronically inhibited. In contrast, soluble guanylyl cyclase inhibition by methylene blue decreased sevoflurane MAC and brain cGMP content in rats, and sevoflurane itself also decreased cGMP contents in the brain *in vivo* and inhibited the nitric oxide–stimulated guanylyl cyclase activity *in vitro*.[204] It was suggested that the inhibition of the nitric oxide– cGMP pathway at the soluble guanylyl cyclase level could be involved in anesthetic or analgesic effects, and the inhibitory effect of sevoflurane on guanylyl cyclase would be one of the sites of action of this anesthetic. Rp-8-p-CPT-cGMPS, a selective cGMP-dependent protein kinase I$\alpha$ inhibitor, decreased isoflurane MAC in rats, whereas in contrast, the nitric oxide donor NOC-12 increased it; Rp-8-p-CPT-cGMPS produced a reversal of the isoflurane MAC increase induced by NOC-12.[205] cGMP-dependent protein kinase I$\alpha$ seems to mediate the action on the nitric oxide– cGMP pathway in anesthetic mechanisms at the spinal cord level. Soluble guanylyl cyclase inhibition by 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one also reduced the MAC for isoflurane without any significant hemodynamic change in rats.[206]

*Intravenous Anesthetics.* Thiopental, ketamine, midazolam, and etomidate caused a decrease in NOS activity in the rat brain.[207] Riluzole, L-NAME, and 7-NI inhibited nNOS activity in the rat hippocampus, and riluzole competed with 7-NI for inhibition of nNOS activity.[208] The $\alpha_2$-adrenoceptor agonist dexmedetomidine decreased the nitric oxide–mediated synthesis of cGMP in *Xenopus laevis* larvae, similar to the effects of volatile and intravenous anesthetics, suggesting that the nitric oxide– cGMP pathway is an important mediator of the anesthetic action of these compounds.[209] However, ketamine, pentobarbital, fentanyl, and midazolam did not affect the NOS activity in the rat brain.[185,210]

Hypothermic and hypnotic responses to ketamine and pentobarbital were augmented in NOS-inhibited mice.[211] Treatment of *Xenopus laevis* tadpoles with L-NAME reduced anesthetic requirements of thiopental, propofol, and ketamine; and the effect of L-NAME was reversed by L-arginine.[212] 7-NI prolonged the duration of methohexital narcosis in the rat, and this effect was antagonized by L-arginine.[213] On the other hand, local perfusion with ketamine into the rat hippocampus and striatum increased nitrite/nitrate concentrations and prolonged loss of the righting reflex; although the effect of ketamine-induced increases in hippocampal nitrite/nitrate concentrations was depressed by L-NAME, the righting reflex was not affected.[214] L-NAME reduced the depth and duration of behavioral depression after ketamine in rats in association with a decrease in blood and brain ketamine concentrations, suggesting that the decreased delivery of ketamine into the brain, perhaps due to L-NAME–induced alterations in blood flow, may explain the reduced behavioral response to ketamine.[215]

Propofol suppressed L-glutamate–, NMDA–, kainate–, and SNP–stimulated cGMP formation; ketamine suppressed L-glutamate– and NMDA–stimulated cGMP formation; and midazolam suppressed only kainate-induced cGMP formation.[216] The authors suggested that the inhibitory effects of these anesthetics on cGMP formation are due mainly to interaction with receptors for excitatory amines, and not due to the suppression of NOS or guanylyl cyclase activities.

Ketamine reduced lipopolysaccharide-induced tumor necrosis factor-$\alpha$ production without inhibition of nitrite release in mixed rat glial cells, astrocyte cultures, and microglial cultures, whereas propofol had no effect on lipopolysaccharide-induced nitrite or tumor necrosis factor-$\alpha$ production.[217] Ketamine, but not propofol, seems to inhibit some of the inflammatory responses of both lipopolysaccharide-treated astrocytes and microglial cells without causing nitric oxide release.

*Local Anesthetics.* L-NAME and diazepam decreased the incidence of lidocaine-induced convulsions in mice; in contrast, the L-arginine treatment increased the incidence of convulsions.[218]

**Pain.** Stimulation of ionotropic NMDA receptors causes intraneuronal elevation of $Ca^{2+}$, which stimulates NOS; nitric oxide synthesized and then diffused out from the neuron stimulates the formation of cGMP in neighboring neurons; depending on the expression of cGMP-controlled ion channels in the target neurons, nitric oxide may be excitatory or inhibitory.[219] Nitric oxide has been implicated in the development of hyperexcitability, resulting in hyperalgesia or allodynia, by increasing nociceptive transmitters at their central terminals. Intrathecal administration of magnesium sulfate as well as NMDA receptor antagonists reversed the hyperalgesia induced by $Mg^{2+}$ deficiency; the PKC inhibitor chelerythrine chloride and 7-NI induced an antihyperalgesic effect, suggesting that $Mg^{2+}$ deficiency induces sensitization of nociceptive pathways in the spinal cord, and PKC and nitric oxide play an active role in the intracellular mechanisms leading to hyperalgesia.[220] However, Bulutcu *et al.*[221] found that intraperitoneal or intrathecal administration of ketamine produced antinociceptive effects in the acetic acid–induced writhing and formalin tests in mice; pretreatment with intraperitoneal L-NAME, which produced no antinociception on its own, inhibited the antinociceptive effect of intraperitoneal ketamine, whereas L-NAME given intrathecally did not modify the antinociceptive effect of intrathecal ketamine. The authors suggested that the activation of the nitric oxide-cGMP pathway probably at the supraspinal level, but not the spinal level, contributes to the antinociceptive effects of ketamine.

In a model of local anesthetic tachyphylaxis in rats subjected to repeated sciatic nerve blocks by percutane-

ous injection of 2-chloroprocaine, L-NAME inhibited the development of tachyphylaxis.[222] L-NAME was evidently more potent in preventing local anesthetic tachyphylaxis given intrathecally than intraperitoneally, and intrathecal L-arginine augmented tachyphylaxis; spinalized rats exhibited tachyphylaxis to sciatic block.[223] Tachyphylaxis, like hyperalgesia, seems to be mediated *via* nitric oxide at least in part by a spinal site of action, and descending pathways may not be necessary for the development of tachyphylaxis.

**Peripheral Nervous System.** Halothane and isoflurane attenuated the relaxant response to nonadrenergic noncholinergic (NANC) nerve stimulation of canine cerebral arteries that was mediated through release of nitric oxide from nitrergic nerve terminals; halothane but not isoflurane reduced the relaxation induced by the nitric oxide donor *S*-nitro-*N*-acetylpenicillamine, suggesting that both anesthetics inhibit cerebroarterial dilatation mediated *via* the nitric oxide–cGMP pathway activated by NANC nerves, but the sites of action of halothane and isoflurane on the nitric oxide-cGMP pathway may differ.[224] In the isolated rabbit lower esophageal sphincter treated with atropine and guanethidine, ketamine and midazolam, but not thiopental, suppressed the NANC inhibitory response to potassium chloride that was inhibited in the presence of tetrodotoxin, L-NA, methylene blue, apamin, and glibenclamide, whereas SNP-induced relaxation was not affected by the anesthetics.[225] Neurogenic relaxation was suggested to be mediated by nitric oxide and by $Ca^{2+}$- and ATP-sensitive $K^+$ channels of smooth muscle, and the modulation of the nitric oxide-cGMP pathway seemed to be related, at least in part, to the inhibitory actions of ketamine and midazolam on the NANC relaxation in lower esophageal sphincter muscles. The ketamine-induced inhibition of the NANC relaxation was reversed by superoxide dismutase but not by catalase, but midazolam-induced inhibition of the response was reversed neither by superoxide dismutase nor by catalase; relaxations induced by nitric oxide donors were inhibited by ketamine but not by midazolam; the NOS activity was suppressed by midazolam but was not affected by ketamine.[226] It seems that ketamine blunts NANC relaxations by the extracellular production of superoxide anions and that midazolam inhibits it by the inhibition of NOS activity. In rats anesthetized with chloral hydrate, intracavernous administration of cocaine increased both the magnitude and the duration of intracavernous pressure, and this stimulating effect was blunted by previous treatment with L-NMMA, L-NAME, and methylene blue.[227] Cocaine may induce penile erection by increasing intracavernous pressure *via* a local action on the cavernous smooth muscle that is likely mediated through the release of nitric oxide and subsequent activation of guanylyl cyclase. Although penile erection is elicited *via* nitric oxide mainly from nitrergic nerves and also endothelial cells,[228] the authors did not determine the source of nitric oxide for cocaine-induced increase in cavernous pressure.

Bupivacaine induced apoptosis in the Schwann cell line in association with generation of ROS, which preceded the activation of caspase-3 and poly-ADP–ribose polymerase degradation, and blockade of ROS by antioxidants inhibited bupivacaine-induced cell death.[229]

## Summary and Conclusion

Interactions between nitric oxide and anesthetic agents in cardiovascular and nervous systems are summarized in this review. Volatile and intravenous anesthetics tend to facilitate the basal release of endothelial or neural nitric oxide as well as $PGI_2$ and EDHF and to inhibit the stimulated release of these substances in isolated blood vessels and vasculatures *in vivo*. These anesthetics with different molecular structures even in the same category (volatile or intravenous) do not always share the mechanisms of action. Preconditioning of volatile anesthetics prevents ischemia-reperfusion–induced tissue damages in the heart and brain; the beneficial effect is probably mediated by nitric oxide. NOS inhibitors potentiate actions of volatile and intravenous anesthetics. Accumulated information regarding the interactions of nitric oxide and anesthetics presented so far would contribute to constructing reliable, efficient methods of anesthesia and minimize untoward reactions during anesthesia. However, more quantitative and extensive studies in healthy individuals and patients with different diseases are required to determine whether it is indeed valid to extrapolate the findings from experimental animals to humans.

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