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Endothelium, Anesthetics, and Vascular Control

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ENDOTHELIUM-DERIVED relaxing factor/nitric oxide (EDRF/NO), first discovered in the vascular endothelium,[1] is now recognized as the signal transduction mechanism for the activation of soluble guanylyl cyclase.[2] Synthesized from L-arginine by NO synthase(s), NO is a novel cell messenger now implicated in wide-ranging physiologic and pathophysiologic actions in the cardiovascular, immune, and nervous systems.[2] In blood vessels, where EDRF/NO is produced by endothelium, it is a primary determinant of resting vascular tone through basal release, and causes vasodilation when synthesized in response to a wide range of vasodilator agents.[3,4] It also inhibits platelet aggregation and adhesion, and it may play a major role in disease states, such as atherosclerosis and hypertension, cerebral and coronary vasospasm, and ischemia-reperfusion injury.[2,5] In the immune system, it is an effector mechanism for macrophage-induced cytotoxicity,[6] and, in the brain, EDRF/NO appears to subserve multiple functions.[7] It is present in several specific neuronal pathways and is known to mediate the NMDA and acetylcholine receptor stimulated increases in neuronal cyclic GMP.[8,9–12] Nitric oxide has been implicated in longterm potentiation in the CA1 region of the hippocampus,[13,14] thus mediating an important step in learning and memory; it is the potential agent responsible for NMDA mediated cytotoxicity,[15] and is the mediator of nonadrenergic, noncholinergic neurotransmission.[16–18] The significance of the L-arginine to NO pathway in other cell types in which it is present (including bronchial epithelium, renal tubular, and juxtaglomerular cells, and the adrenal medulla) is now being explored.[8,19]

This paper reviews the biochemistry and cell and molecular biology involved in nitric oxide (NO) production and action; the role of NO signalling in the cardiovascular system; the evidence for, and importance of anesthetic interaction with this pathway; and likely sites and mechanisms by which anesthetics inhibit NO signalling.

Biochemistry, Molecular Biology, and Regulation of NO Synthesis

Current evidence indicates that there are at least two major NO synthase isoforms.[2,20] One requires calcium and calmodulin binding for activation, is expressed constitutively in neurons and in the vasculature, and is involved in cell communication. This constitutive enzyme is activated by a rise in cytosolic free calcium. In neural tissue, it is in a soluble form; in endothelium, it is membrane bound. The other isoform is only expressed after induction by cytokines or microbial products, such as endotoxin (lipopolysaccharide), and participates in host defense. This inducible isoform has calmodulin tightly bound as a subunit,[21] and produces NO continuously and in large amounts without a calcium requirement. The cytokine-induced isoform may also participate in pathophysiology associated with cytokine overproduction, such as in sepsis.[2,22,23] Although this inducible form of the enzyme is present in the macrophage under basal conditions, it is not normally found in the endothelial cell or vascular smooth muscle. It is present in these vascular tissues only after induction by cytokines.[22,23]

In contrast to their differences in location, expression, and function, the NO synthase isoforms appear to be biochemically similar. The inducible and constitutive NO synthases are active as homodimers with molecular sizes of 130 kDa (inducible) and 150 kDa (constitutive).[24,25] Both are members of a rare class of flavoproteins that contain both FAD and FMN as prosthetic

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groups.[3,25,26] The constitutive brain enzyme, the constitutive endothelial cell enzyme, and the cytokine-induced macrophage enzyme have been sequenced and cloned.[25,27-31] The deduced amino acid sequence of the endothelial cell NO synthase reveals 57 and 50% homology with the brain and macrophage enzymes, respectively.[25] In addition, the endothelial cell NO synthase contains a unique N-myristylation consensus sequence not shared by the brain and macrophage enzymes, which may explain its membrane localization. The primary sequences of these enzymes indicate that the monomers are comprised of an oxygenase and reductase domain. The reductase domain of the isoforms has significant sequence homology to all other FAD- and FMN-containing flavoproteins, including NADPH-cytochrome P450 reductase.[24] In addition to flavins, both isoforms contain bound tetrahydrobiopterin and a recently recognized heme moiety.[25,32,33]

These NO synthases are NADPH utilizing mixed function monooxygenases that oxidize L-arginine in a stepwise manner to form NO and citrulline as primary products.[24,25,33] NADPH serves as the electron donor, and oxygen is the electron acceptor. It is now recognized that the initial step in NO synthesis is an NADPH- and oxygen-dependent hydroxylation of arginine that forms N-hydroxyarginine. Enzymatic conversion of the intermediate N-hydroxyarginine to NO and citrulline also utilizes NADPH and O_2.[34,35] The oxygen atoms in both NO and citrulline are derived from O_2. Although not yet proven, it is proposed that NADPH passes electrons through the flavins, which subsequently reduce the iron in heme to its ferrous form, which can then bind oxygen and oxidize the substrate.[35] Limiting the availability of molecular oxygen is the likely mechanism by which hypoxia inhibits NO synthase activation and EDRF/NO-dependent vasodilation.[35]

All forms of the enzyme can be specifically and competitively inhibited by analogs of L-arginine in which a substitution is made at one of the guanidino nitrogen atoms.[36] These include N^G-monomethyl L-arginine (LNMMA), N^G-L-arginine methyl ester (LNAME), and N-imino-L-ornithine (L-NIO).[2,37] These inhibitors are proving to be of enormous benefit in elucidating the physiologic and pathophysiologic roles of the NO pathway.

After its production, the primary biologic function of NO is the activation of soluble guanylyl cyclase to increase the cyclic GMP content of several tissues, including VSM and brain.[38,39] It does so by binding to the heme moiety of soluble guanylyl cyclase. In vascular smooth muscle, the increase in cyclic GMP caused by guanylyl cyclase leads to relaxation, most likely through activation of cyclic GMP-dependent protein kinase and the subsequent phosphorylation of proteins involved in extrusion of calcium from the cytosol (also, see the article by Bosnjak in this issue of ANESTHESIOLOGY, page 1392).

Nitric oxide, a reactive free radical, has also been reported to have other, noncyclic GMP-mediated actions. It has been shown to activate ADP ribosyltransferase[40] and to inhibit a number of enzymes, including mitochondrial aconitase, electron transport chain complexes I and II, and ribonucleotide reductase.[41,42] These protein/enzyme interactions of NO have been suggested to be a result of its ability to form complexes with both heme and nonheme iron proteins.[43] Recently, White and Marletta demonstrated that both the inducible and constitutive NO synthases contain a heme moiety.[33] We recently reported the feedback inhibition of NO synthase by NO, a potentially important mechanism for regulation of this signalling pathway.[44] The binding of NO to the heme of NO synthase would be a likely mechanism of this observed inhibition.

Nitric oxide also interacts avidly with reactive oxygen species, resulting in its rapid inactivation.[45,46] It combines with superoxide radical to form peroxynitrite,[47] and with oxygen to form nitrite, nitrate, and nitric acid.[47,48] This explains the ability of high oxygen concentrations to inhibit EDRF/NO,[49,50] and of superoxide dismutase to markedly prolong its biologic half-life.[49,50] This is a potential mechanism of anesthetic inactivation of NO, because inhalational anesthetics have been shown to generate oxygen-derived radicals that would avidly combine with NO.[51,52]

An understanding of the regulation of EDRF/NO synthesis will form a basis for understanding the likely sites of inhalational anesthetic inhibition of NO signalling. Potential sites of regulation are indicated in figure 1, and include: (1) receptor activation and signal transduction; (2) calcium availability; (3) availability of other cofactors; (4) direct effects on NO synthase, including phosphorylation and feedback inhibition; (5) interactions with nitric oxide itself; and (6) interactions with guanylyl cyclase and cyclic GMP.

The availability of calcium is, perhaps, the most significant mode of regulation of NO synthesis in the endothelium. The constitutive NO synthase is activated by increases in cytosolic calcium.[55,59] The calcium de-

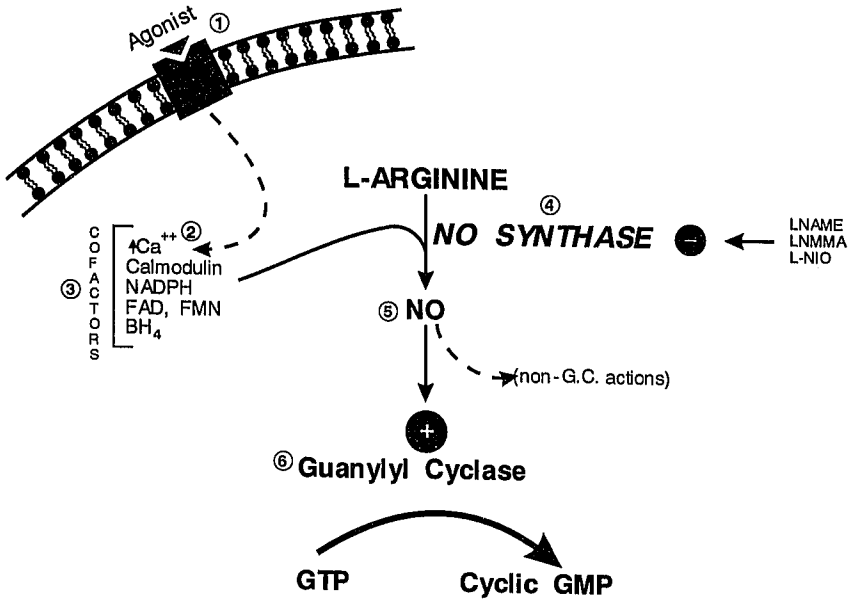

Fig. 1. The nitric oxide (NO) signalling pathway. In the brain and endothelium, NO is produced from L-arginine by similar constitutive enzymes called NO synthase(s). These enzymes are activated by the binding of calcium (Ca^{++}) and calmodulin, often in response to agonist–receptor interaction leading 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). Tetrahydrobioptein (BH_4) is another cofactor. After its production, NO binds to the heme moiety of guanylyl cyclase (GC), which catalyzes the production of cyclic 3′,5′-guanosine monophosphate (GMP) from guanosine triphosphate (GTP). Specific analogues of L-arginine, including nitro-L-arginine methyl ester, N^G-monomethyl-L-arginine, and N-imino ornithine, are competitive inhibitors of NO synthase. The numbers 1–6 represent potential sites of inhalational anesthetic interaction, as discussed in the text. (Modified with permission from Johns *et al.*[135])

pendence of the activation of NO synthase and production of NO has been studied extensively in the endothelial cell.[53,59] Endothelium-dependent responses are enhanced by the calcium ionophore A23187, and attenuated or abolished by the removal of extracellular calcium or pretreatment with calcium entry blockers (verapamil, nifedipine).[54,58] Although the removal of extracellular calcium consistently causes inhibition of EDRF-dependent responses,[55] calcium entry blockers do not inhibit endothelium-dependent relaxations in all vessels studied.[58,59] Both extracellular influx of calcium and release of calcium from intracellular stores are involved in EDRF release from endothelial cells. In the absence of extracellular calcium, bradykinin-stimulated endothelial cells release EDRF in an attenuated and transient manner.[54] An increase in endothelial cell intracellular calcium has been shown to accompany the release of EDRF in response to a wide variety of endothelium-dependent dilators, including histamine, bradykinin, ATP, melittin, thrombin, and norepinephrine,[56,59–68] implicating receptor-mediated translocation of intracellular calcium as the initial step in the production or release of EDRF. This increase in intracellular calcium associated with EDRF release correlates with an increase in the concentration of endothelial cell inositol-1,4,5-trisphosphate (IP3).[57,69–71] Inhibition of phospholipase C by gentamicin, thus blocking IP3 production, prevents EDRF release from cultured endothelial cells.[72]

Several endothelial cell receptor and signalling pathways have been related to EDRF/NO production. An increase in cytosolic calcium leads to calcium-calmodulin activation of NO synthase.[73–81] There are multiple mechanisms by which cytosolic free calcium may be increased in the endothelial cell (also, see the article by Bosnjak in this issue of ANESTHESIOLOGY, page 1392, for a more detailed discussion). Agonist binding to a receptor may lead to opening of receptor-operated calcium channels present in the plasma membrane.[73,74,82,83–87] Alternatively, receptor-mediated G protein activation of phospholipase C can lead to the cleavage of phosphatidylinositol-4,5-bisphosphate generating IP3 and diacylglycerol (DAG).[75,76,88] IP3 releases calcium from intracellular stores.[75,76,88] Although not definitively demonstrated in the endothelial cell, it is also possible that inositol 1,3,4,5 tetrakisphophate (IP4) can stimulate receptor-operated channels, allowing entry of calcium.[89] Both a calcium leak channel, dependent on the electrochemical gradient for calcium, and internal Na^+-dependent calcium entry (Na^+-Ca^{2+} exchange)

have been proposed, but not well demonstrated.[73,77,90] Although there are reports of voltage-operated calcium channels in endothelial cells, the overwhelming evidence indicates that L-type voltage-operated channels are not present.[76] The presence of other potential operated channels remains controversial. The rate of calcium entry can be modulated by the resting membrane potential, which may be regulated by two types of K^+ channels[73]: inwardly rectifying K^+ channels activated on hyperpolarization or shear stress, and a calcium-activated K^+ channel activated on depolarization, which may function to repolarize the agonist-stimulated endothelial cell.[78-81] ATP-sensitive K^+ channels have been demonstrated in endothelial cells.[76,77]

Phosphorylation is another likely regulatory mechanism for NO synthase. Study of the NO synthase amino acid sequence reveals recognition sites for protein phosphorylation, in addition to sites for NADPH, FAD, FMN, and calmodulin.[27,91,92] Indeed, NO synthase has recently been reported to be stoichiometrically phosphorylated by cyclic AMP-dependent protein kinase, protein kinase C, and calcium/calmodulin-dependent protein kinase,[26,91,93,94] each kinase phosphorylating a different serine site on the enzyme. The phosphorylation by protein kinase C resulted in a marked inhibition of the enzyme.

As mentioned, the receptor-mediated activation of phospholipase C (PLC), in conjunction with initiating the inositol phosphate cascade, produces diacylglycerol (DAG), which leads to protein kinase C activation. The DAG response to receptor stimulation in endothelial cells has two components. An initial peak correlates with PLC-mediated inositol-1,4,5-trisphosphate release. A secondary, sustained release of DAG may be related to the action of other lipases.[73] DAG, through activation of protein kinase C, may play an important physiologic role in modulating endothelial cell responsiveness to vasoactive agents.[72] Activation of protein kinase C by phorbol esters has been shown, by several investigators, to inhibit EDRF production stimulated by receptor-mediated agents, but not in response to the calcium ionophore A23187.[95,96] This is consistent with known protein kinase C inactivation of receptor/GTP binding proteins involved in PLC activation, a negative feedback pathway observed in other cell types. As mentioned above, the direct phosphorylation of NO synthase by protein kinase C has been demonstrated to inhibit enzyme activity.

The mechanisms of calcium sequestration by endoplasmic reticulum (a site of inhalational anesthetic interaction in other cell types) are poorly defined in endothelial cells. Currently, it is not known whether endothelial cells contain functional ryanodine receptors; however, this issue is being actively pursued. If a ryanodine receptor is localized in endothelial cells, this would be an obvious site for further investigation of anesthetic effects based on inhalational anesthetic interactions at this site in myocytes.[97-99]

Evidence for Inhalational Anesthetic Interaction with NO Signalling in the Vasculature

Several studies have examined the role of the endothelium in mediating the vascular responses of anesthetics or the effects of anesthetics on endothelium-dependent responses. Blaise *et al.*[100] demonstrated that isoflurane impairs the contractile response of canine coronary arteries induced by phenylephrine in an endothelium-dependent manner, and proposed that this might be caused by isoflurane-induced release of EDRF. Consistent with these observations, in an abstract report by Greenblatt *et al.*,[101] the microsphere technique was used to measure tissue-specific blood flow, indirectly indicating that isoflurane may stimulate EDRF/NO production in certain vascular beds. Several laboratories, including ours, have provided strong direct evidence, however, that anesthetics are not capable of stimulating EDRF release.[102-104] Rather, inhalational anesthetics appear to be potent inhibitors of EDRF-dependent vascular relaxation at clinically relevant doses. Muldoon *et al.*[105] demonstrated that halothane inhibits endothelium-dependent vasodilation in response to the receptor-mediated agonists, acetylcholine and bradykinin. Stone and Johns previously reported that a small vasoconstricting response observed with low concentrations of isoflurane, enflurane, and halothane requires an intact endothelium, and may be caused by the inhibition of EDRF production or action.[106] Recently, Uggeri *et al.* more directly and definitively demonstrated that these three volatile anesthetics can inhibit both receptor and nonreceptor-mediated EDRF/NO-dependent vasodilation.[107] Halothane, enflurane, and isoflurane inhibited the endothelium-dependent vasodilation induced by the receptor-mediated agent methacholine and that by the receptor-independent calcium ionophore A23187, but had no effect on the endothelium-independent vasodilation induced by sodium nitroprusside. Although this study demonstrated

that anesthetic inhibition of EDRF/NO vasodilation occurred distal to receptor activation of the endothelial cell, it did not rule out an additional effect on receptor mechanisms, as was suggested by Muldoon,[105] and which has been demonstrated in other cell types.[107-112] Toda *et al.* recently reported that both isoflurane and halothane inhibited acetylcholine-induced endothelium-dependent relaxation of rat aorta and simultaneously prevented acetylcholine-induced increases in cyclic GMP.[113,114] Consistent with the work by Uggeri *et al.*,[107] isoflurane was more potent than halothane in this regard. Unlike Uggeri *et al.*, however, these investigators did not demonstrate an inhibition of A23187-induced vasodilation.

In the studies by Muldoon[105] and Uggeri *et al.*[107] of inhalational anesthetic inhibition of EDRF-dependent vasodilation, it was shown that this inhibition was caused by an effect on the production, transport, or release of EDRF and was independent of any effect on guanylyl cyclase activation in the vascular smooth muscle. The evidence for this was that nitroglycerin- or sodium nitroprusside-induced relaxation, which is mediated by a direct activation of vascular smooth muscle soluble guanylyl cyclase after its breakdown to NO, was not affected by any of the anesthetics. A recent paper, also by the Muldoon laboratory, however, suggests that vasodilation induced by NO and by nitroglycerin is inhibited by halothane, and implies that inhalational anesthetics may inhibit guanylyl cyclase.[115] Thus, it is not clear from isolated vascular ring studies whether inhalational anesthetics are capable of interfering with NO signalling through an action in vascular smooth muscle involving guanylyl cyclase.

A recent report on the vascular actions of sevoflurane demonstrated the selective impairment of EDRF-dependent relaxation induced by acetylcholine, bradykinin, and the calcium ionophore, A23187, and the partial reversal of this effect by superoxide dismutase.[52] These authors demonstrated, with electron paramagnetic resonance spectroscopy techniques, that sevoflurane generated the superoxide free radical, and suggested superoxide inactivation of NO as a possible mechanism of sevoflurane's inhibition of EDRF.

Potential and Likely Sites for Anesthetic Interaction with NO Signalling

There are multiple sites at which inhalational anesthetics may, potentially, inhibit EDRF/NO production or release based on the known mechanisms of EDRF/NO synthesis and action and on the observed effects of these anesthetics in endothelial cells, neurons, and other cell types. Likely sites of interaction are indicated in figure 1 by circled numbers, which correlate with the discussion below.

1 and 2: Receptor Activation and Cytosolic Calcium Availability

As inhalational anesthetics have been shown to have profound and specific effects on calcium homeostasis in other cell types,[116] effects of inhalational anesthetics on calcium availability is a highly likely site of anesthetic interaction with EDRF/NO generation. As discussed above, inhalational anesthetics have been shown to impair receptor activation.[105] Halothane has been shown to shorten acetylcholine receptor kinetics,[108] and isoflurane has been shown to cause flickering of the acetylcholine receptor using patch clamp techniques.[109] Anthony *et al.*,[110] Dennison *et al.*,[111] and Aronstam *et al.*[112] investigated the mechanisms of inhalational anesthetic inhibition of muscarinic acetylcholine receptors in rat brain. They found both an increase in antagonist, but not agonist, binding affinity caused by a decrease in the rate of dissociation, and a decrease in the guanine nucleotide sensitivity of agonist binding. These effects were common to halothane, enflurane, isoflurane, diethyl ether, and chloroform, indicating that interference with muscarinic receptor-G protein interactions is a common property of volatile anesthetics, and may represent a general mechanism for the disruption of signal transmission between cells during anesthesia. Puil *et al.*,[117] studying calcium transients in response to NMDA receptor activation in rat hippocampal neurons, observed that both isoflurane and halothane inhibited the calcium response to glutamate. Although anesthetic inhibition of muscarinic receptor (or other receptor) activation may be a component of the mechanism by which anesthetics inhibit EDRF/NO production in response to those specific agonists, receptor activation is not likely to be the major site of anesthetic inhibition of NO signalling, because we have demonstrated significant anesthetic inhibition of calcium ionophore (A23187) stimulated EDRF/NO production that bypasses receptor effects.[107]

Effects of anesthetics on calcium homeostasis in endothelial cells have been reported in preliminary abstract form. Uhl *et al.*,[118] using fluorescent dye studies, showed that halothane modestly decreased basal intra-

cellular calcium and impaired the ATP-stimulated calcium transient in endothelial cells. Loeb *et al.*,[119] using the fluorescent calcium indicator dye FURA2, have reported that both halothane and isoflurane enhance basal calcium concentrations, and that halothane significantly inhibited the calcium transient evoked by bradykinin. Tsuchida *et al.*,[120] studying endothelium-denuded rat aorta, found a decrease in cytosolic calcium that correlated with halothane- and isoflurane-induced vasodilation. The most established actions of inhalational anesthetics on cytosolic Ca^{2+} concentration in other cell types have been through an effect on calcium movement into the cell, either by changing Ca^{2+} influx through receptor- or voltage-activated membrane Ca^{2+} channels, or by altering Ca^{2+} release from, or uptake into, the sarcoplasmic reticulum.[116,121,122]

EDRF/NO activity may be attenuated by an interaction with the phospholipase C-inositol phosphate pathway in the endothelial cell. Indeed, halothane has been shown to inhibit stimulated phosphatidylinositol-4,5-bisphosphate hydrolysis in RBL-2H3 cells.[123] Sill demonstrated that halothane inhibits serotonin-stimulated phosphatidylinositol-4,5-bisphosphate hydrolysis in vascular smooth muscle, and that isoflurane inhibits acetylcholine-stimulated phosphatidylinositol-4,5-bisphosphate hydrolysis in coronary smooth muscle.[124] This same group, in preliminary studies, demonstrated that halothane does not inhibit phorbol-12,13-dibutyrate-stimulated protein kinase C action in vascular smooth muscle, indicating that an effect through diacylglycerol is unlikely.[124] Thus, inhalational anesthetics are clearly capable of decreasing calcium availability for NO synthase activation, and may do so by altering calcium entry through the plasma membrane; through calcium release from, or reuptake into, intracellular stores; or through inhibiting phospholipase C and altering IP3-mediated calcium release.

3: Availability of Other Cofactors for NO Synthase

Halothane may interact with, and inhibit, calmodulin, perhaps by interacting with hydrophobic sites on the protein. Halothane potentiation of the antitumor activity of interferon is suggested to be mediated through inhibition of calmodulin. It was shown that halothane clearly mimicked specific calmodulin blocking agents.[125] Excess calmodulin has been shown to reverse the activating effects of halothane on sarcoplasmic reticulum calcium release in skeletal muscle, indicating that this action of halothane may be partially mediated through an inhibition of calmodulin.[126]

4: Direct Interactions with NO Synthase

Inhalational anesthetics have been shown to bind competitively to specific hydrophobic regions of proteins. For example, halothane, methoxyflurane, and chloroform caused a 50% inhibition of luciferase activity.[127] There appears to be a specific anesthetic binding pocket on this enzyme, the hydrophobicity of which (and, therefore, anesthetic sensitivity of luciferase activity) is modulated by ATP.[128] In the endothelial cell, NO synthase is 80–90% membrane associated,[32,129] providing an additional potential mechanism for anesthetic interaction. Inhalational anesthetics could directly impair endothelial NO synthase activity through interaction with a hydrophobic site on the enzyme, or by altering the fluidity or structure of enzyme-associated membrane.

5: Inactivation of NO

It is also possible that inhalational anesthetics may inactivate EDRF after its production, either *via* a direct interaction, or indirectly, by enhancing free radical activity within the endothelial cell, leading to the inactivation of EDRF by superoxide (see above discussion regarding sevoflurane).[8,51,52] Shayevitz *et al.*[51] showed that halothane and isoflurane increase the sensitivity of rat pulmonary artery endothelial cells to injury by oxygen metabolites by inhibiting processes involved in intracellular antioxidant defenses. Anesthetic mediated increases in oxygen radicals within endothelial cells would clearly be a means of inactivating NO.

6: Inhibition of Guanylyl Cyclase

Muldoon *et al.* recently suggested that, in contrast to their original work, halothane may inhibit endothelium-dependent vasodilation through direct inhibition of soluble guanylyl cyclase.[115] They suggest that halothane may interact with the heme moiety of guanylyl cyclase, as it was previously shown to interact with the heme of a cytochrome P450. Although our work in vascular rings indicates that this is not the major site of anesthetic inhibition of NO signalling, an additional action on guanylyl cyclase cannot be ruled out by such studies. A recent report by Eskinder *et al.*[130] studied the effects of halothane on the activity of isolated soluble and particulate guanylyl cyclases. Although halothane had no effect on the soluble cyclase (which is

involved in vasodilation by EDRF/NO), it significantly stimulated the activity of the particulate guanylyl cyclase (normally stimulated by atrial natriuretic peptide and some bacterial toxins, but not involved in EDRF/NO-dependent vasodilation).

Importance of Anesthetic Interaction with NO Signalling

An understanding of the mechanisms of the observed inhibitory interactions of inhalational anesthetics with NO signalling, and their potential stimulation of this pathway under certain conditions, is clearly of tremendous clinical importance, given the widespread role of this pathway in physiology and pathophysiology[7,8,19] and the extensive use of these anesthetics and their potent hemodynamic and central nervous system effects. The actions of NO have been most studied in the vasculature, the site at which anesthetics have clearly been shown to inhibit NO-dependent vasodilation. The pathway for NO production is present in all vascular beds and in large and small vessels, in a wide range of species.[2,5,32] EDRF/NO is a potent endogenous vasodilator and an inhibitor of platelet aggregation and adhesion.[2] Its activity is impaired in hypertension and atherosclerosis,[2] and its absence because of endothelial damage may play a role in cerebral and coronary vasospasm.[5] It is a mediator of flow-dependent vasodilation, and a modulator of the hypoxic pulmonary vasoconstrictor response.[132] Endothelial cell damage and impairment of EDRF/NO production may also contribute to acute and chronic pulmonary hypertension, and EDRF/NO may be responsible for the low resting tone of the pulmonary vasculature.[133] Inhaled exogenous NO is a potent, selective, and clinically useful pulmonary vasodilator.[48] The central nervous system functions of NO are just beginning to be explored, but it is clear that NO mediates excitatory amino acid receptor stimulation of neuronal cyclic GMP[8-12] (a pathway strongly implicated in mechanisms of anesthesia); that it mediates nonadrenergic, noncholinergic neurotransmission, through which it may control peristalsis of the gastrointestinal tract; and that it mediates relaxation of the corpora cavernosae of the penis.[16-18,134] Nitric oxide is also a mediator in synaptic plasticity, for example, in its role in long-term potentiation.[13,14] We recently demonstrated an exciting potential new role for the NO signalling pathway in modulating consciousness, and a possible involvement in the central mechanisms of anesthetic action, both of which clearly demand further exploration.[134]

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