Chapter 15

Nitric Oxide

Andrew A. Parsons

The importance of nitric oxide (NO) in modulation of biological pathways has been known for over 20 years. The seminal studies by Furchgott and Zwadzki that provided an initial identification of a labile relaxing factor released from endothelial cells was a stimulus for many investigators to identify the nature of the transmitter and explore its role in biology (13). The tremendous volume of research papers on NO over the last two decades is a testament to the key physiologic and pathophysiologic roles that have been attributed to this molecule.

The wealth of knowledge surrounding this molecule is represented by a variety of excellent reviews that provide a detailed account of the molecular and cellular effects of this agent (see Forstermann et al. [12], Ignarro [22]). The aim of this manuscript is to provide a brief overview of how NO is generated by enzyme systems and how it can produce its biological actions with respect to its potential role in migraine.

NITRIC OXIDE: A KEY ROLE IN BIOLOGY

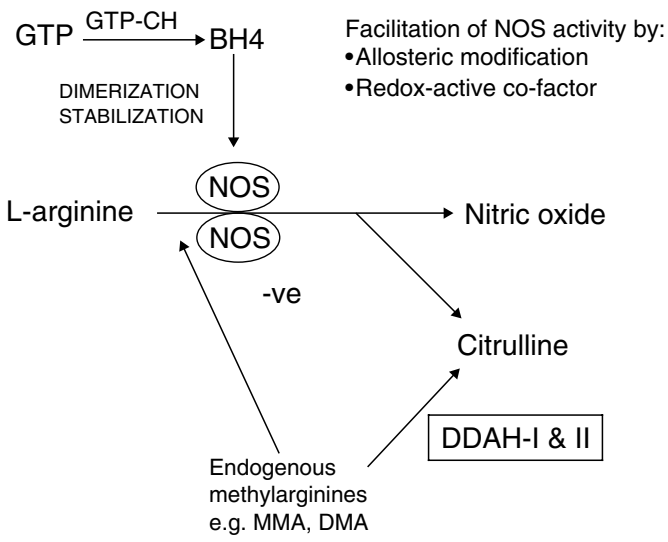
NO is a labile gas that has a short biological half-life, measured in seconds. It is a highly reactive and lipophilic molecule that has the ability to pass through biological membranes and interact with a number of target systems to produce its biological effects. The initial identification of NO as the endothelium-derived relaxing factor (13) produced by stimulating endothelial cells with agents such as acetylcholine or bradykinin focused attention on the role of NO in the cardiovascular system and control of blood pressure; however, there was a parallel development of our understanding of the potential role of NO in host defense (65) and as a neurotransmitter in the central nervous system (CNS) (55).

Perhaps one of the greatest initial breakthroughs came from the discovery that NO was produced by metabolism of the amino acid arginine by specific nitric oxide synthase (NOS) enzymes (4,5,22,34,55,65). Development of prototype tool inhibitor compounds that were simple analogs of arginine led to the use of these compounds to probe into the biological role of NO in physiology and pathophysiology. Many effects of NO were subsequently suggested and involved many organ systems and integrated biological pathways. NO is now believed to play a key role in many physiologic processes such as in the gastrointestinal tract, respiratory and cardiovascular systems, as well as the CNS. The first new medicines to modulate the NO pathway have also been developed for male erectile dysfunction.

CONTROL AND REGULATION OF NITRIC OXIDE PRODUCTION

A schematic representation of the steps involved in the enzymatic production of NO is shown in Figure 15-1. It has been established that NO can be produced from a series of enzymes (NOS) from L-arginine with concomitant production of citrulline. Neuronal NOS was first isolated and characterized from rat brain and was found to have similarities with NADPH-cytochrome P450 reductase (4,5). Other isoforms of NOS were quickly discovered with the original nomenclature based on the site of purification with neuronal (nNOS), endothelial (eNOS) [39, XXX], and inducible (iNOS) (65) forms being located in macrophage. More recent studies have shown that NOS enzymes are widely expressed, are found in a range of cell types, and are not located specifically to the original location. An alternative nomenclature has been suggested with nNOS being NOS1, eNOS being NOS2, and iNOS being NOS3 (Table 15-1).

A number of critical cofactors have been described for NADPH, flavin adenine dincucleotide (FAD), and flavin adenine mononucleotide (FMN), heme, zinc (Zn), and tetrahydrobiopterin. FAD, FMN, Zn, and heme play key roles in the oxidation-reduction activity of NOS (34) and tetrahydrobiopterin plays a crucial role in dimerization of inactive NOS monomers and stabilization of the resultant functional enzyme (60).



Regulation of NOS activity by GTP-CH and DDAH-I & II

FIGURE 15-1. Schematic representation of the steps involved in production of NO. L-Arginine is converted to NO and citrulline by the action of active NOS dimmers. Dimerization and stabilization of the active form occurs with tetrahydrobiopterin (BH4), which is synthesized from GTP by the action of GTP cyclohyrolase enzyme (GTP-CH). A number of critical cofactors have been described for NADPH, falvin adenine dincucleotide (FAD), and FMN, heme, Zn, and tetrahydrobiopterin. FAD, FMN, Zn, and heme play key roles in the oxidation–reduction activity of NOS. NOS activity is thought to be regulated by endogenous methylarginines, L-monomethyl arginine (L-NMMA), and ADMA. In normal circumstances the circulating concentration of L-NMMA and ADMA methylated arginines are maintained at low levels by the enzyme dimethylarginine dimethylaminohydrolase (DDAH I and II). (Adapted from Ignarro [3].)

Key properties of the NOS enzymes have been described in detail by other reviews (34,35) and are summarized in Table 15-1. It is striking that the NOS enzymes exhibit many similarities but with notable differences, particularly in the control of their expression and enzyme activity. For example, iNOS is not normally expressed but is under control of a variety of cellular stressors such as inflammatory cytokines and therefore is somewhat distinct from the other NOS isoforms. The distinct profile of iNOS in comparison to the other NOS isoforms is also supported by differences in sensitivity to intracellular calcium ion concentration. It is interesting to note that differences in the affinity of calmodulin, a calcium-binding protein, between iNOS and the other NOS isoforms are manifest in marked differences in the rate of NO production. Only small concentrations of NO are produced by the calcium-dependent NOS; NO production in these isozymes is reflected in the cellular calcium ion transient, whereas high concentrations can be produced by iNOS, as this enzyme activity is more related to transcriptional regulation.

Although transcriptional regulation of NOS activity was first identified in murine macrophage (65), it also appears possible for both nNOS and eNOS to be regulated transcriptionally, at least in some cell types (Table 15-1). Stressful stimuli such as hypoxia or increased glutamatergic activity have been shown to induce nNOS (8) and a variety of promoter sites have been identified that can control nNOS and eNOS expression. A key regulator for NOS expression

TABLE 15-1 Properties of NOS Isoforms

	Active Homodimer		
Parameter	***Neuronal NOS (NOS1)***	***Inducible NOS (NOS2)***	***Endothelial NOS (NOS3)***
Mass (kDa)	160	125–130	135
Expression	Constitutively expressed	Not normally expressed	Constitutively expressed
Calmodulin binding	Ca^{2+}/calmodulin dependent	Ca^{2+}/calmodulin independent	Ca^{2+}/calmodulin dependent
V_{max} (nmol/min/mg)	7	1000	5
Posttranslational modifications	Specific phosphorylation sites present	Specific phosphorylation sites present	Myristoylation, palmitolylation, phosphorylation sites present
Protein–protein interactions	PSD-95, caveolin 3, phosphofructokinase M		Cavelolin1, HSP90, bradykinin receptor
Factors that increase expression	Calcium, tissue injury, neuronal activity	Cytokines, bacteria, tissue injury, hypoxia, neuronal activity	Calcium, cytokines, bacteria, shear stress, tissue injury, hypoxia, neuronal activity
Factors that decrease expression	Cytokines, bacteria		
Transcription factors	AP-1/AP-2; NFκB, c-Fos, c-Jun, CREB	AP-1, NFκB, c-Fos, c-Jun, STAT1	AP-1, NFκB, STAT-1
Major biological role	Neurotransmission	Cytotoxicity/proinflammatory	Vasodilatation

Adapted from Forstermann et al. (2) and Ignarro (3).

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appears to be nuclear factor κ B (NFκB). This appears to be especially important for the regulation of iNOS because stimuli as diverse as bacterial endotoxin, interleukins, and inflammatory cytokines have been shown to induce iNOS expression by translocation and activation of the NFκB pathway. NFκB is a transcription factor that provides a link between extracellular signaling and changes in gene expression. Following membrane receptor activation, the NFκB heterodimer translocates, through phosphorylation and degradation of inhibitory (IkB) subunits, to the nucleus where it binds to specific domains on DNA and induces gene expression.

NO can be formed in many cell types and is under close transcriptional and molecular control, for example, via regulation of intracellular calcium ion concentrations. Other means of physiologic regulation of the NO pathways are possible via production of endogenous inhibitors of NOS. For example, the NOS inhibitor L-NG-monomethyl arginine (L-NMMA) is a naturally occurring amino acid that is produced by protein arginine methyltransferases (62). Additionally, asymmetric dimethylargine (ADMA) is a nonselective inhibitor of NOS (32,33). The circulating concentration of L-NMMA and ADMA methylated arginines are maintained at low levels by the enzyme dimethylarginine dimethylaminohydrolase (DDAH) (Fig. 15-1), which catalyzes their conversion to citrulline, dimethylamine, or monomethylamine.

THE MOLECULAR TARGETS OF NITRIC OXIDE

NO exhibits a range of unique features for a neurotransmitter or paracrine regulator with perhaps its most striking feature is the fact that it is a gaseous transmitter, highly reactive and unstable. NO does not interact with specific membrane-bound molecular targets in a similar manner as classical transmitters like acetylcholine at G-protein–coupled receptors or ion channels; NO acts via a series of redox reactions in a variety of proteins allowing marked diversity in the pathways linked to this molecule. These interactions may results in activation of biological pathways or inactivation of NO (Fig. 15-2). Perhaps the most well-characterized pathway is its binding to the heme group of guanylate cyclase resulting in activation of the enzyme and increased intracellular concentrations of cyclic guanosine monophosphate (cGMP), a key regulator of intracellular calcium ion concentrations and mediates cellular functions, such as vasodilatation. NO can therefore interact with heme-containing proteins. NO may also interact with non-heme–containing Fe and Fe-S–containing proteins as well as proteins with labile cysteine or tyrosine residues. NO can interact with a variety of other reactive oxygen or nitrogen species, which inactivate NO but can result in toxic derivatives such as peroxynitrite.

NO therefore has a potential multitude of effects and is involved in a number of key regulatory processes within the body. Some activities associated with NO are noteworthy in the consideration of the molecule as a mediator of migraine.

THE PHYSIOLOGIC EFFECTS OF NITRIC OXIDE

NO has a key role in regulation of cardiovascular tone and control of arterial blood pressure. The identification of endothelium-derived relaxing factor as NO, highlighted an autocrine role for this agent and implicated eNOS as

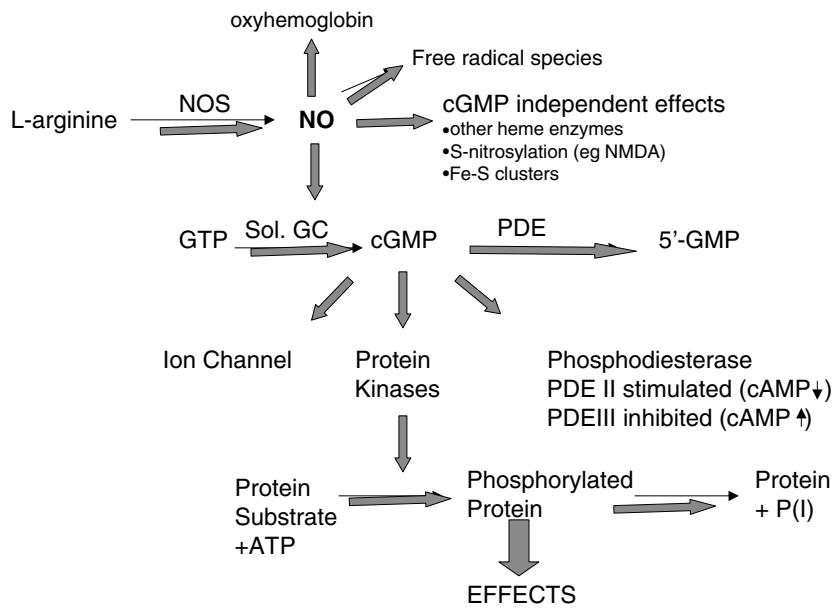

FIGURE 15-2. Schematic representation of potential fate of NO. NO may undergo a series of redox reactions that can inactivate the molecule and give rise to a diverse array of possible biological activities. NO may be inactivated by its binding to oxyhemoglobin or produce reactive peroxynitrite species. Activation of soluble guanylayte cyclase gives rise to a diverse array of possible effects in cells, by increasing the concentration of cGMP. cGMP levels in the cell are regulated by phosphodiesterase (PDE) enzymes that inactivate cGMP. cGMP may act directly to modulate channel function and other intracellular proteins phosphodiesterases. Activation of protein kinases produces phosphorylation of proteins and an array of biological effects. In addition, NO may bind directly to other heme-containing enzymes, nitrosylate other proteins (e.g., NMDA channel) or Fe-S–containing proteins. (Adapted from Ignarro [3].)

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a key source of NO in response to transmitters such as bradykinin or physiologic stimuli such as sheer stress. Work with genetically null mice in which the eNOS was deleted confirmed this role; there was marked hypertension and absence of acetylcholine-induced relaxation in isolated arteries (19). eNOS knockout animals are also more sensitive to cerebral ischemia (18) and have impaired cerebrovascular responses, highlighting the importance of eNOS-derived NO in control of physiologic processes.

NO clearly plays a modulatory role in a variety of systems and the complexity of these interactions is demonstrated by the neuroprotective effects of nitro-L-arginine in eNOS knockout animals (18). These indicated a detrimental role for non-eNOS–derived NO as the NOS inhibitor possessed neuroprotective effects in mice in which the beneficial effects of eNOS had been removed. These observations clearly support the concept of different NOS isoforms mediating opposing biological effects, in this case neuroprotection or neurotoxicity.

Within the CNS, NO plays a key role as a synaptic modulator and appears to play a central role in modulation of nociceptive pathways via a variety of mechanisms (see McMahon et al. [37] and Meller and Gebhart [38]). Studies show that NO can act as an retrograde transmitter facilitating glutamatergic transmission, possibly by the direct action of NO derivatives on the NR2B subunit of the *N*-methyl-D-aspartate (NMDA) channel (9) enhancing NMDA-evoked currents. NO plays a role in the modulation of pain pathways and learning and memory.

iNOS play a key role in mediating host defense and cellular immunity. Originally identified in mouse macrophages (65), targeted deletion of the iNOS gene has been used as an approach to explore its biological function. For example, a role in neurodegeneration (20), wound healing (54), and retinal degeneration (52) have been suggested.

NITRIC OXIDE AND HEADACHE

The headache-inducing effects of glyceryl trinitrate (GTN) have been known for many years; however, it has only recently been attributed to the ability of GTN to act as an exogenous NO donor and appears to be a key molecule in the development of migraine and other headaches (42). When administered intravenously to volunteers, GTN evoked an immediate mild to moderate headache that was dose related and appeared to resemble migraine headaches, because it was susceptible to treatment with sumatriptan, despite the absence of reported associated symptoms such as phono- or photophobia (23). GTN is rapidly metabolized in man and therefore the evoked headache is of a short duration in normal individuals, but can be prolonged by agents that prolong the effects of NO, such as *N*-acetylcysteine (22). Clearly, these results show that NO can mediate headache in volunteers; however, it has been shown that migraineurs exhibit exaggerated responses to administration of GTN. Studies have shown that when GTN is administered to migraineurs, they respond with an initially short-lasting headache proceeded after a number of hours by a second delayed headache of greater intensity. Therefore, migraine patients show a greater response to exogenous NO compared to controls, supporting the idea that migraine patients may be more sensitive to NO. Indeed, GTN evokes a greater arterial dilatation in migraineurs than in healthy volunteers (61). Dilatation of the larger cerebral vessels gives rise to pain, although this may not be the only mechanism by which NO can contribute to headache. Recent experimental studies have shown that orally administered sildenafil can produce headache (migraine attack) in patients with no evidence of arterial dilatation as measured by transcranial Doppler sonography (26). This study was the first to show that migraine-like headache can be induced by a cGMP-dependent mechanism in the absence of initial dilatation of the middle cerebral artery. Understanding of the potential pathways that NO/cGMP-mediated effects can produce its effects on the afferent nervous system may be critical to our understanding of the role of NO in headache.

The development of preclinical animal models of GTN on trigeminovascular pathways has provided some insight into the potential mechanisms by which GTN can produce these effects, especially on the delayed period of the second headache. For example, in studies of cortical superfusion of the NO donors, GTN and sodium nitroprusside over the feline cortex, they produced pial artery vasodilatation (64). These vasodilator effects of these agents were markedly attenuated by chronic unilateral trigeminal ganglionectomy and by the calcitonin gene-related peptide (CGRP) receptor antagonist, CGRP8-37 (64) in animals with trigeminal nerves intact. In rat isolated dura mater, superfusion with NO and NO donors stimulated the release of CGRP up to 160% of basal production (57). Furthermore, topical administration of NO solution or NO donors produced an increase in meningeal blood flow that was attenuated by the selective CGRP-receptor antagonist, CGRP8-37, supporting the concept of at least a partial role of CGRP in NO-induced meningeal vasodilatation (57). Some species differences may occur; studies in guinea pig isolated dura mater failed to show a modulatory effect of NO donors on CGRP release (11).

GTN therefore appears to be able to release CGRP from sensory nerves and it is therefore interesting to note that plasma CGRP concentrations correlate with migraine following GTN administration (25) and CGRP receptor antagonists are effective in migraine (41).

In addition, intravenous administration of GTN produces an initial pial arterial dilatation and concomitant elevation of cortical NO concentration. Following termination of the infusion, blood flow returns to control values

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GRBT050-15 Olesen- 2057G GRBT050-Olesen-v6.cls August 18, 2005 15:30

but cortical NO concentrations remain elevated (44,46), possibly by modulation of the local redox state and provide evidence for long-lasting increase of NO concentrations independent from the concentration of GTN. Studies with subcutaneous administration of GTN showed a biphasic response on monoamine concentrations in the brain, with an initial increase in norepinephrine concentration and a delayed increase in medullary and pontine serotonin concentration (58). Whereas the rapid rise in norepinephrine may reflect sympathetic reflexes to administration of GTN, it is tempting to speculate on the role of increased serotonin concentrations on regulation of nociception and its role in the generation of the delayed headache. Furthermore, GTN induces increases in immunoreactivity for a variety of markers such as c-*fos* (43), cGMP (59), and nNOS (43) in the nucleus trigeminalis caudalis. These effects may be of relevance for the perception and even central sensitization of nociceptive inputs in the trigeminal receptive area. Other studies using intravenous administration did not show marked differences in c-*fos* staining between GTN and vehicle, although GTN produced there was evidence of potentiation of activation of trigeminal neurons from cutaneous or dural pathways (24). Infusion of GTN-elevated interleukin (IL)-1b and IL-6 in the meninges and induced transcription and subsequent expression of iNOS for up to 10 hours postadministration, with the majority of the protein found in meningeal macrophages (48). These effects were associated with leakage of plasma protein into the dura mater, effects that were blocked by a selective iNOS inhibitor (48). GTN produces increases in macrophage NO production via activation of NFκB and induction of iNOS (49).

Collectively, these results show that administration of the NO donor GTN can initiate a variety of cellular responses that may activate nociceptive pathways directly and produce peripheral or central sensitization, possibly via transcriptional regulation of NFκB. This perhaps suggests that induction of NFκB by a variety of stimuli could give rise to similar responses. Therefore, induction of migraine-like symptoms with GTN allows the conclusion that NO can induce these effects, but this alone does not provide evidence for a causal link in a spontaneous attack.

NITRIC OXIDE AND MIGRAINE

Biochemical and high-performance liquid chromatography analysis of internal jugular blood samples during a migraine attack showed increases in nitrate, cGMP, neurokinin A, and CGRP concentrations that reached their greatest levels within 1 hour from onset, suggesting release of NO and vasoactive peptides that may play a role in the development of headache (50). Perhaps stronger evidence implicating a role of NO in acute migraine came from an innovative experimental clinical study using a double-blind design with some historical controls. The nonselective NOS inhibitor, L-NMMA, was administered intravenously during a migraine attack. Significant pain relief was observed at 2 hours postadministration in 10 out of 15 patients, compared to 2 out of 14 placebo controls. There was also some improvements in associated symptoms of photo- and phonophobia, supporting the concept that endogenous NO mediates headache pain during migraine (28).

Similarly, NO has been implicated in the generation of tension-type headache. Using a randomized double-blind crossover trial of 16 patients with chronic tension-type headache, infusion of L-NMMA (6 mg/kg) produced a small but significant reduction in pain score of 49 to 33, whereas no significant differences were detected in placebo-treated controls (44 to 40) (1). Collectively, a number of exploratory studies using the prototype NOS inhibitor L-NMMA indicate a role for NO in spontaneous migraine and other cephalalgias. Interestingly, headache can also be precipitated by other routes as L-NMMA had no effect on histamine-induced headache (51).

Although using the NOS inhibitor L-NMMA clearly supports the concept of a role for NO in the precipitation of migrainous headache, its nonselective effects do not allow conclusion of the relative contribution of each of the NOS isoforms. Furthermore, intravenous administration of L-NMMA triggers a broad spectrum of vascular changes (14,47), decreases cardiac output, and increases blood pressure and systemic vascular resistance (36). These systemic effects may suggest that L-NMMA can produce its effects by indirect effects, such as vasoconstriction, although L-NMMA–induced constriction of cerebral arteries has been discounted (29).

NITRIC OXIDE AND POTENTIAL PATHOLOGIC PATHWAYS IN MIGRAINE

The relative contribution of the NOS isoforms and the potential mechanisms that NO exerts its action are still unclear. The generation of a migraine attack could be considered to be composed of three main mechanisms: the trigger, relay of the nociceptive information, and a role for peripheral or central sensitization. NO may play a role in each of these areas.

NO and Migraine Initiation

In terms of the stimuli that may trigger initiation of migraine attacks, it is known that a variety of inflammatory stimuli that induced activation of NFκB could elevate iNOS and NO production in rodent macrophage. It is possible that a similar induction of iNOS could elevate NO in humans, although the precise cellular location is not clear

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GRBT050-15 Olesen- 2057G GRBT050-Olesen-v6.cls August 18, 2005 15:30

and may involve other cell types involved in the inflammatory response (49). One may hypothesize that a local meningeal inflammatory response could evoke induction of iNOS and thereby produce activation of trigeminal sensory fibers.

In addition to the possibility that NO could be involved in generation of migraine from peripheral stimuli, NO may be released following intrinsic brain activation. A CNS component in migraine has been hypothesized based on the range of premonitory signs that appear before headache (3). Interestingly, in a prospective study using electronic diaries, it was found that in patients who thought they could recognize premonitory signs, they were significantly able to predict the appearance of headache (15). Many individuals present with a variety of premonitory signs; tiredness, difficulty in concentrating, and stiff neck are the most frequent (15). One common premonitory sign is perhaps migraine aura. Recent understandings in the phenomenon of cortical spreading depression appear to suggest that a similar process occurs in humans and mediates migraine aura (17,30). Cortical spreading depression is a slowly spreading suppression of electroencephalographic activity in the CNS (31) that produces release of NO (45) and appears to stimulate upregulation of NOS and NO over a period of days (53). Cortical spreading depression therefore provides a coupling of cortical activity with reflex activation of sensory pathways that induce inflammation within the meninges (2), although the precise role of NO is yet to be established, NOS inhibition attenuates vascular responses to cortical spreading depression (16,45). In addition, NO appears to have a role in mediating repolarization of neurons after cortical spreading depression because NOS inhibitors lengthen the repolarization period (40). NO may play a role in the development of migraine with aura, although these patients have been largely excluded in many of the experimental studies reported.

NO and Nociception

Use of L-nitro-L-arginine methyl ester (L-NAME), another nonspecific inhibitor, has been shown to inhibit responses in the trigeminal nucleus caudalis, following electrical stimulation of the middle meningeal artery (10), highlighting a role for NOS-derived NO in responses to stimulation of the dura mater. Studies outlined with GTN show that NO can directly release CGRP (57,64), supporting a role for a direct effect on sensory nerve endings although NO and CGRP also appear to produce effects by some independent pathways as inhibition of NOS and CGRP receptor antagonism can have additive effects in the cerebral circulation (63).

NO may also modulate other nociceptive pathways. Within the trigeminal nucleus, iontophoretic administration of a NOS inhibitor suppressed activity at the first central synapse in response to electrical stimulation of the superior sagittal sinus, the facial receptive area, and to local application of glutamate (27). These observations support a modulatory role for NO in control of glutamatergic transmission. Therefore, NO may have actions at both the primary and second-order neurons of the trigeminal nociceptive pathways, clearly supporting the possibility of a key role of this gas in development of migraine and other headaches.

NO and Sensitization

There is increasing evidence for a role for central/peripheral sensitization in the development of a migraine attack (6,7). Furthermore, the appearance of central sensitization appears to be a factor in the magnitude of the treatment effect of triptans. For patients with cutaneous allodynia, triptan therapy was more likely to produce a pain-free state when the medicine was administered prior to establishment of allodynia (6). New therapies that limit the extent or attenuate sensitization may therefore offer new therapeutic advantages over the triptans. NO has a key role in sensitisation in nociceptive inputs into the spinal cord (9,38). Activation of glutamatergic pathways produces a facilitation of nociceptive reflexes in the spinal cord that are blocked by NOS inhibitors leading to the suggestion of a close interplay between glutamate and NO, although the precise nature of these interactions appears complex (9,38). It seems likely that similar regulation of synaptic activity can occur in the trigeminovascular pathway. Indeed, an invasive model recording electrical activity of trigeminal cells, a NOS inhibitor, reduced activations in response to superior sagittal sinus stimulation or to local application of glutamate (2). This may reflect some sensitization in the model from surgical trauma, but there is also behavioral evidence of a role for NO in development of tactile hypersensitivity, in the trigeminal receptive area. In a model of injury of the alveolar nerve in rats, hypersensitivity to von Frey filaments developed 5 days after surgery and persisted for up to 30 days with an associated increase in the numbers of nNOS-positive neurons in the trigeminal nucleus (66). Importantly, the allodynia was blocked by the NMDA channel blocker, MK-801 and the nonselective NOS inhibitor, L-NMMA. This supports a concept of NO and nNOS playing a role in activity-dependent modulation of nociceptive pathways in the trigeminovascular system. Induction of a meningeal inflammation with an inflammatory soup superperfused over the dura can also produce sensitization of meningeal sensory neurons (56), raising the possibility that induction of iNOS in the meninges may also produce a similar effect.

In summary, the ubiquitous role of NO and the regulation of NOS activity by numerous stimuli provides multiple potential pathways by which the NO system can modulate sensory nerve activity and sensitization, and may also

affect intrinsic brain activation. Given these complexities, understanding of the specific role of NO and NOS in migraine is still unclear; however, taken together it seems likely that NO is a key modulator in the generation of migraine and related headaches.

FUTURE DIRECTIONS

NO appears to play a key role in many biological and pathophysiologic processes and available evidence suggests it plays a key role in headache. The development of specific inhibitors may therefore give rise to new therapeutics for the treatment of migraine and related headaches and improve our understanding of the role played by the NOS isoforms in these conditions. However, the widespread localization and function of particularly nNOS and eNOS may limit the clinical investigation of new chemicals in development. Perhaps other approaches to regulate NO by specific targeting of tissues or cell types with approaches to modulate NOS activity may offer other approaches to develop new medicines, but a key issue is understanding the nature of the cellular and molecular targets on which to direct our interest. Attractive hypotheses can be put forward to support many different pathways by which NO can induce migraine and related headaches. NO may well be an important mediator in these conditions, but advances in the development of new treatments will be made when we have a better understanding of how a variety of stimuli can trigger activation of NO pathways and result in migraine in individual patients.

ACKNOWLEDGMENTS

The author thanks Ms. Charis Whitfield for her considerable skill and help in preparation of the manuscript and Dr. Paul Strijbos for helpful discussions concerning this complex neurotransmitter.

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GRBT050-15 Olesen- 2057G GRBT050-Olesen-v6.cls August 18, 2005 15:30

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