ANTIOXIDANTS & REDOX SIGNALING
Volume 8, Numbers 5 & 6, 2006

Forum Editorial

NO and Glial Cell Biology

NARAYAN R. BHAT[1] and DOUGLAS L. FEINSTEIN[2]

CERTAIN GASEOUS MONOXIDES and related products are increasingly recognized as important mediators of biological processes. This is nowhere more evident than in our brain where metabolically generated nitric oxide (NO), carbon monoxide (CO), and even the primitive hydrogen sulfide (H_2S) are considered among the novel modulators of neural functions. The classification of these cell-permeable, nonsynaptic vesicle-associated reactants as neurotransmitters has radically changed the definition of a neurotransmitter (2). However, true to their less desirable noxious nature, they may also assume pathophysiological roles. The "Nobel" gas, NO, in particular, can turn rebel and act as a neuroinflammatory and neurotoxic mediator in a number of central nervous system (CNS) disorders. In this brief overview, we will describe the latest developments in the neurobiology of NO from a 'glial' perspective, with particular reference to the articles compiled in this forum issue of ARS.

GLIA AS A MAJOR SOURCE AND THE TARGET OF NO IN THE BRAIN

The CNS is built of two major cell types: neurons, that are directly engaged in electrical communication and information processing; and glia, that in fact greatly outnumber neurons, and help support and maintain their normal functioning in a variety of ways. There are two 'macroglial' cell types: astrocytes which, besides providing structural and trophic support to neurons, regulate ionic and neurotransmitter balance in the neuronal microenvironment, participate in the formation and functioning of synapse, and, by interacting with capillary endothelium, maintain blood brain barrier (BBB) integrity; and oligodendrocytes, the most important function of which is to form and maintain myelin sheaths around the axons, thereby allowing fast electrical conduction. The third glial cell type, the microglia, can be considered the resident aliens of the CNS, that, being of monocytic origin, would have migrated into the brain during development. Under normal conditions, the 'resting' microglia perform homeostatic and surveillance roles. However, they are activated in response to injury, infection and trauma as well as under a number of disease settings: neurodegenerative (e.g., Alzheimer's and Parkinson's diseases) as well as neuroinflammatory (e.g., multiple sclerosis and HIV-associated dementia). Although transiently activated microglia are most likely beneficial for reparative processes, when chronically activated, they can produce toxic levels of inflammatory mediators including cytokines, chemokines, eicasanoids, reactive oxygen species, and, relevant to the subject at hand, plenty of NO. These proinflammatory functions of microglia are, to some extent, shared by astrocytes, which act as a second immune effector cell type of the CNS.

The interest in glial NO biology stems mainly from the perceived role of glial-derived NO in neuroinflammation under neurodegenerative/inflammatory situations mentioned above. Thus, the two glial cell types (astrocytes and microglia) respond to injury and inflammatory stimuli by an upregulation of inducible NO synthase (iNOS or NOS2) one of the three well-defined isoforms of the enzyme that produces NO as a byproduct of the reaction between L-arginine and molecular oxygen. While iNOS produces NO at a high rate and for a prolonged period of time, consistent with its role in host immunity, the two constitutively expressed members of the NOS family [i.e., the neuronal NOS (nNOS or NOS1) and the endothelial NOS (e-NOS or NOS3)] produce lower levels of NO in a regulated manner that can act as a transient signaling agent. Besides the fact that the low and high levels of NO produce dramatically different biological effects, the multiplicities of functions that NO plays in the brain are also influenced by the location and the timing of its release. As we will

[1]Department of Neurosciences, Medical University of South Carolina, Charleston, South Carolina.
[2]Department of Anesthesiology, University of Illinois, Chicago, Illinois.

see in the articles compiled in this volume of *Antioxidants & Redox Signaling*, NO plays a number of physiological and pathological roles that affect the functions of the brain in many different ways.

Although the expression of iNOS, nNOS, and eNOS was originally thought to be confined to glia, neurons, and endothelial cells, respectively, in actuality this distribution is not strict, and there is significant redundancy in the expression of NOS isoforms between different cell types. Thus, astrocytes constitutively express nNOS and can be induced to produce iNOS. Contrary to the long-held view that neurons are incapable of expressing iNOS, there is accumulating evidence that under certain conditions of brain injury or disease states (e.g., Alzheimer's disease) neurons can express this isoform and that primary neuronal cultures when presented with appropriate stimuli respond with iNOS upregulation (7). The articles by Kalinin *et al*. (9), and Madrigal *et al*. (10), provide *in vivo* and *in vitro* examples of neuronal iNOS expression and further implicate adrenergic and noradrenergic systems as endogenous regulators of neuronal iNOS expression. Similarly, the expression of iNOS by oligodendrocytes has been a subject of controversy, and is discussed in detail in the review by Boullerne and Benjamins (4). These authors offer an interesting explanation to reconcile discrepant observations in the literature, and suggest that iNOS expression in oligodendrocytes may depend on the developmental stage of the cells. Intriguing preliminary observations included in the review suggest the possible expression and function of the third isoform, eNOS, in oligodendrocytes.

Since L-arginine is the only known chemical source of NO, the availability of this amino acid in the brain is an important issue when considering how NOS activity is regulated. The review by Gensert and Ratan (5) focuses on the metabolic origin of brain/astrocytic NO and takes an in-depth look at the pathway of NO generation including the source and the metabolism of the amino acid itself, the y^+ transport system of cationic amino acid transporters (CATs) involved in arginine transport across the membrane, different NO synthases, and their regulation. Of particular interest is the delineation of the so-called 'arginine paradox' in molecular terms based on the authors' recent findings—an elegant example of metabolic regulation of gene expression occurring at the translational level. The review also describes neuron–glia (astrocyte) interactions relevant to arginine metabolism and the biology of NO within the brain.

The regulation of iNOS gene expression in glia is an active area of current research related to CNS inflammation. The review by Saha and Pahan (14) focuses primarily on this subject and presents an extensive survey of the regulation of iNOS gene expression in rodent and human glial cells, including the nature of the triggers and their signaling pathways, and a description of the iNOS promoter and of the transcriptional control mechanisms involved in the induction process. The differential inducibility and the nature of the inducers of rodent versus human iNOS genes point to the highly complex structure of the human iNOS promoter. It has been commonly observed that human cells respond poorly to stimuli that strongly induce iNOS expression in rodent cells. To address this issue, Vitek *et al*. (15) have created a double transgenic mouse that contains a functional human NOS2 gene including the human promoter and all of its exons and introns on a mouse NOS2 knockout background. *In vitro* studies with immune-activated microglia and peritoneal macrophages derived from these mice suggest an inherent, promoter-dependent weaker response of human iNOS relative to mouse iNOS, although species-selective effects occurring at posttranscriptional levels cannot be excluded. Details on these mechanisms of iNOS expression are also covered in the review by Saha and Pahan (14). Their discussion makes the case for iNOS gene expression as a prototype platform for the study of signal transduction pathways of epigenetic control of mammalian gene expression in general. It is also evident that iNOS/NO expression provides a standard readout for inflammatory cell signaling. The study by Pawate *et al*. (12) uses this strategy to define an isoform-specific proinflammatory role of c-Jun N-terminal kinase (JNK) in astrocytes. Clearly, induction of iNOS expression provides a convenient cell biological assay for confirming the actions and effectiveness of several antiinflammatory agents and strategies.

Work with astrocytic induction of iNOS/NOS2 has uncovered an interesting 'cross-talk' between the inducible and the constitutively expressed NOS isoforms. Thus, the expression of NOS2 seems to be tonically suppressed by low levels of NO released by NOS1 and NOS3 involving a suppression of NFκB activation critical for NOS2 induction. NOS2 inducers (e.g., LPS and IFNγ) remove this block as a prerequisite to their induction of NOS2 expression. The signaling pathways involved in these interactions have been the subject of recent work by Persichini and colleagues (13). The key mechanism involves tyrosine phosphorylation and inhibition of NOS1 as a result of increased arachidonic acid generation by phospholipase A2, which is activated as an immediate response to LPS/IFNγ treatment of astrocytes. Although speculative at this point, such a phenomenon may have implications for a counter-regulation of NOS2 expression in response to certain environmental stimuli that activate NOS1 and hence the release of low levels of NO [see Table 1 in Persichini *et al*. (13)].

As noted above, glial-derived NO has been implicated in neurotoxicity, associated with a wide range of degenerative and inflammatory diseases of the brain. Besides targeting of neurons, glial NO can also modulate the functions and physiology of glia themselves in many ways. Thus, like neurons, oligodendrocytes are the victims of NO insults. The review by Bollourne and Benjamins (4) discusses this issue extensively, including the mechanisms involved in NO-mediated oligodendrocyte cell death. The outcome with astrocytes is different, however, since these cells are rather tolerant to NO toxicity and in fact, modify their metabolic responses to compensate for NO-induced damage as discussed by Bolanos and Almeida (3). A key mechanism seems to involve signaling via AMP-activated protein kinase (AMPK), a cell energy sensor, which is activated (by phosphorylation) in response to the high AMP:ATP ratio resulting from an inhibition of the respiratory enzyme, cytochrome c oxidase, by

NO. AMPK, in turn, induces glucose uptake and activates certain rate-limiting enzymes in the glycolytic pathway to compensate for the energy failure in astrocytes experiencing a nitrosative stress.

CNS ROLES OF NO: PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL

In general, NO generated from nNOS and eNOS is involved in signaling events and physiological roles, whereas that derived from iNOS elicits harmful tissue-damaging effects. However, this demarcation is rather simplistic and the functional duality of NO very much depends on its steady state levels and the location of its release. At times, neuronally produced NO can also be neurotoxic as occurs during excessive stimulation of NMDA receptor under ischemic conditions.

Both physiological and pathological actions of NO are mediated by its targets that become structurally modified by reactive NO or its derivatives (peroxynitrite and *S*-nitroso L-glutathione (GSNO)). Peroxynitrite, an extremely reactive and potently toxic molecule, can interact with cysteine residues found on numerous proteins resulting in the formation of *S*-nitrothiols and thereby regulate thiol-dependent enzymatic activities and protein functions. In fact, a consensus sequence for *S*-nitrosylation (i.e., K/R/H/D/E–C–D/E) is present in a number of proteins, suggesting that *S*-nitrosylation is an important posttranslational modification affecting protein function (8). Peroxynitrite also modifies tyrosine residues to produce 3-nitrotyrosine, perhaps in a reversible manner, thus providing another means to regulate enzymatic activities. Besides a range of structural proteins, proteases, and respiratory enzymes, several of NO targets are important signaling molecules including tyrosine kinases, Ras, ion channels, and, of course, guanylyl cyclase (8). The two isoforms (i.e., vascular $\alpha1\beta1$ and neuronal $\alpha2\beta1$) of guanylyl cyclase, a heme containing protein, act as intracellular 'receptors' for NO leading to the production of the second messenger, cGMP, and regulation of a wide variety of physiological functions including vascular tone, platelet aggregation, penile erection, gastric emptying, hormone release, inflammation, neurotransmission, and learning and memory.

The paradoxical roles of NO (i.e., pathological *vs.* beneficial) may perhaps, be explained by its differential dose-dependent effects and the engagement of distinct signaling pathways. An important recent development with respect to pathological consequences of NO modification of proteins is the finding that *S*-nitrosylation of certain proteins (i.e., glyceraldehydes 3-phosphate dehydrogenase) leads to precisely regulated apoptotic pathways associated with neurodegeneration (1). In these instances, the shift to NO-dependent apoptotic signaling involves a specific set of *S*-nitrosylation targets which seems to depend on the exposure of neurons to stress stimuli (i.e., cytokines, toxins, and excitatory amino acids). As noted by Bolanos and Almeida (3), astrocytes, in contrast to neurons (and perhaps, oligodendrocytes), can handle nitrosative and oxidative stress better due to higher levels of glutathione and antioxidant enzymes, in association with an adaptive modulation of glucose metabolism. The adaptive changes elicited by astrocytes may also involve an upregulation of protective genes such as the CO-producing hemoxygenase-1, as might happen under conditions of hypoxic stress (6).

With multiple physiological and pathological roles played by NO, therapeutic targeting of NO actions is not quite a 'NO' brainer. Again, it is important to keep in mind that the intracellular environment in which NO is released can dictate its ultimate role. Under conditions of increased inflammation, injury, and oxidative stress, the role of NO is shifted towards being a pathological mediator; under such conditions, suppression of NO production or scavenging of it should be beneficial. Finally, a new twist in NO-based therapy combines the 'good' nature of low NO with existing drugs, including steroids, statins, and NSAIDs (11). This combinatorial approach of 'just adding NO to drugs', designed primarily to target cardiovascular indications, may find applications in inflammatory and neurodegenerative conditions as well.

REFERENCES

1. Benhar M and Stamler JS. A central role for *S*-nitrosylation in apoptosis. *Nat Cell Biol* 7: 645–646, 2005.
2. Boehning D and Snyder SH. Novel neural modulators. *Annu Rev Neurosci* 26: 105–131, 2003.
3. Bolaños JP and Almeida A. Modulation of astroglial energy metabolism by nitric oxide. *Antioxid Redox Signal* 8: 955–965, 2006.
4. Boullerne A and Benjamins JA. Nitric oxide synthase expression and nitric oxide toxicity in oligodendrocytes. *Antioxid Redox Signal* 8: 967–980, 2006.
5. Gensert JM and Ratan RR. The metabolic coupling of arginine metabolism to nitric oxide generation by astrocytes. *Antioxid Redox Signal* 8: 919–928, 2006.
6. Guo G and Bhat NR. Hypoxia/reoxygenation differentially modulates NF-κB activation and iNOS expression in astrocytes and microglia. *Antioxid Redox Signal* 8: 911–918, 2006.
7. Heneka MT and Feinstein DL. Expression and function of inducible nitric oxide synthase in neurons. *J Neuroimmunol* 114: 8–18, 2001.
8. Hess DT, Matsumoto A, Kim SO, Marshall HE, and Stamler JS. Protein *S*-nitrosylation: purview and parameters. *Nat Rev Mol Cell Biol* 6: 150–166, 2005.
9. Kalinin S, Polak PE, Madrigal JLM, Gavrilyuk V, Sharp A, Chauhan N, Marien M, Colpaert F, and Feinstein DL. Beta-amyloid-dependent expression of NOS2 in neurons: prevention by an α2-adrenergic antagonist. *Antioxid Redox Signal* 8: 873–884, 2006.
10. Madrigal JLM, Russo CD, Gavrilyuk V, and Feinstein DL. Effects of noradrenaline on neuronal NOS2 expression and viability. *Antioxid Redox Signal* 8: 885–892, 2006.
11. Napoli C and Ignarro LJ. Nitric oxide–releasing drugs. *Ann Rev Pharmacol Toxicol* 43: 97–123, 2003.
12. Pawate S, Shen Q, and Bhat NR. C-Jun N-terminal kinase (JNK) regulation of iNOS expression in glial cells: pre-

dominant role of JNK1 isoform. *Antioxid Redox Signal* 8: 903–909, 2006.
13. Persichini T, Cantoni O, Suzuki H, and Colasanti M. Cross-talk between constitutive and inducible NO synthase. An update. *Antioxid Redox Signal* 8: 949–954, 2006.
14. Saha RN and Pahan K. Regulation of inducible nitric oxide synthase gene in glial cells. *Antioxid Redox Signal* 8: 929–947, 2006.
15. Vitek MP, Brown C, Xu Q, Dawson H, Mitsuda N, and Colton CA. Characterization of NO and cytokine production in immune-activated microglia and peritoneal macrophages derived from a mouse model expressing the human NOS2 gene on a mouse NOS2 knockout background. *Antioxid Redox Signal* 8: 893–901, 2006.

Address reprint requests to:
Narayan R. Bhat
Department of Neurosciences
Medical University of South Carolina
171 Ashley Ave
Charleston, SC 29425
E-mail: bhatnr@musc.edu

Date of first submission to ARS Central, November 29, 2005; date of acceptance, December 10, 2005.

This article has been cited by:

1. Garima Dutta, David S. Barber, Ping Zhang, Nicholas J. Doperalski, Bin Liu. 2012. Involvement of dopaminergic neuronal cystatin C in neuronal injury-induced microglial activation and neurotoxicity. *Journal of Neurochemistry* **122**:4, 752-763. [CrossRef]