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Forum Review

The Metabolic Coupling of Arginine Metabolism to Nitric Oxide Generation by Astrocytes

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ABSTRACT

Arginine, the only known precursor of nitric oxide, enters the brain parenchyma from the blood through the endothelial cells or from the cerebral spinal fluid through the ependymal cells. Astrocytes, whose processes abut the endothelium and ependymum, take up arginine through cationic amino acid transporters and release arginine through this transport system to the synapses that astrocytes shield. Some of these synapses are excitatory, and liberate glutamate into the synaptic cleft. Glutamate induces arginine release from astrocytes, making it available to the neuron. Neurons can take up arginine to be used in nitric oxide-mediated processes, such as neurotransmission. Thus, neural and nonneural cells act in concert to affect neuron physiology in an elegantly integrated system. This review focuses on the components of the interaction between astrocytes and neurons in nitric oxide biology. *Antioxid Redox Signal.* 8, 919–928.

INTRODUCTION

The brain is a complex organ containing a variety of neural and non-neural cell types engaged in intimate interactions characterized by intercellular and intracellular communication, stimulation, inhibition, buffer, protection, and even death and destruction. Despite a prevailing neurocentric view of the brain, the brain could not function without all its cells working in concert. Microglia are instrumental in maintaining homeostasis by secreting various compounds that facilitate normal neuronal processes and in restoring homeostasis during times of stress by destroying pathogens and removing debris. Macroglia (astrocytes and oligodendrocytes) significantly influence neuronal function, with astrocytes buffering the synapses and nodes of neurons, and oligodendrocytes insulating axons with myelin enabling transmission of action potentials. Normal brain function also could not proceed without endothelial cells that form the blood vessels, or ependymal cells that line the ventricular system, and probably not without satellite cells, pericytes and progenitors, whose roles are not so well defined.

Of all the interactions among the different cell types in the brain, however, there is none so provocative in its potential effect on healthy neuronal function as the astrocyte. With endfeet on blood vessels and at synapses and nodes of Ranvier, the astrocyte is strategically poised to participate in neuronal function on several levels (Fig. 1). The astrocyte, while helping maintain the blood brain barrier in part by inducing endothelial cells to form tight junctions, receives cues from endothelial cells, which are subject to systemic change. The astrocyte also communicates with, and buffers the communication received by and sent by, the neuron. Astrocytes protect the signals received by a neuron by shielding synapses controlling extrasynaptic diffusion, and facilitate the sending of neuronal signals by buffering potassium (K^+) at the node. Clearly, normal neuronal function is highly dependent on normal astrocyte function. This review will focus on arginine metabolism in the astrocyte and how it contributes to nitric oxide (NO) biology in the astrocyte and neuron.

INTRODUCING NITRIC OXIDE

Nitric oxide (NO) claims a very decorated history; it was declared the "Molecule of the Year" by the journal *Science* in 1992, inducted into York University's "Chemistry Hall of

Burke/Cornell Medical Research Institute, White Plains, New York, and Weill Medical College of Cornell University, New York, New York.

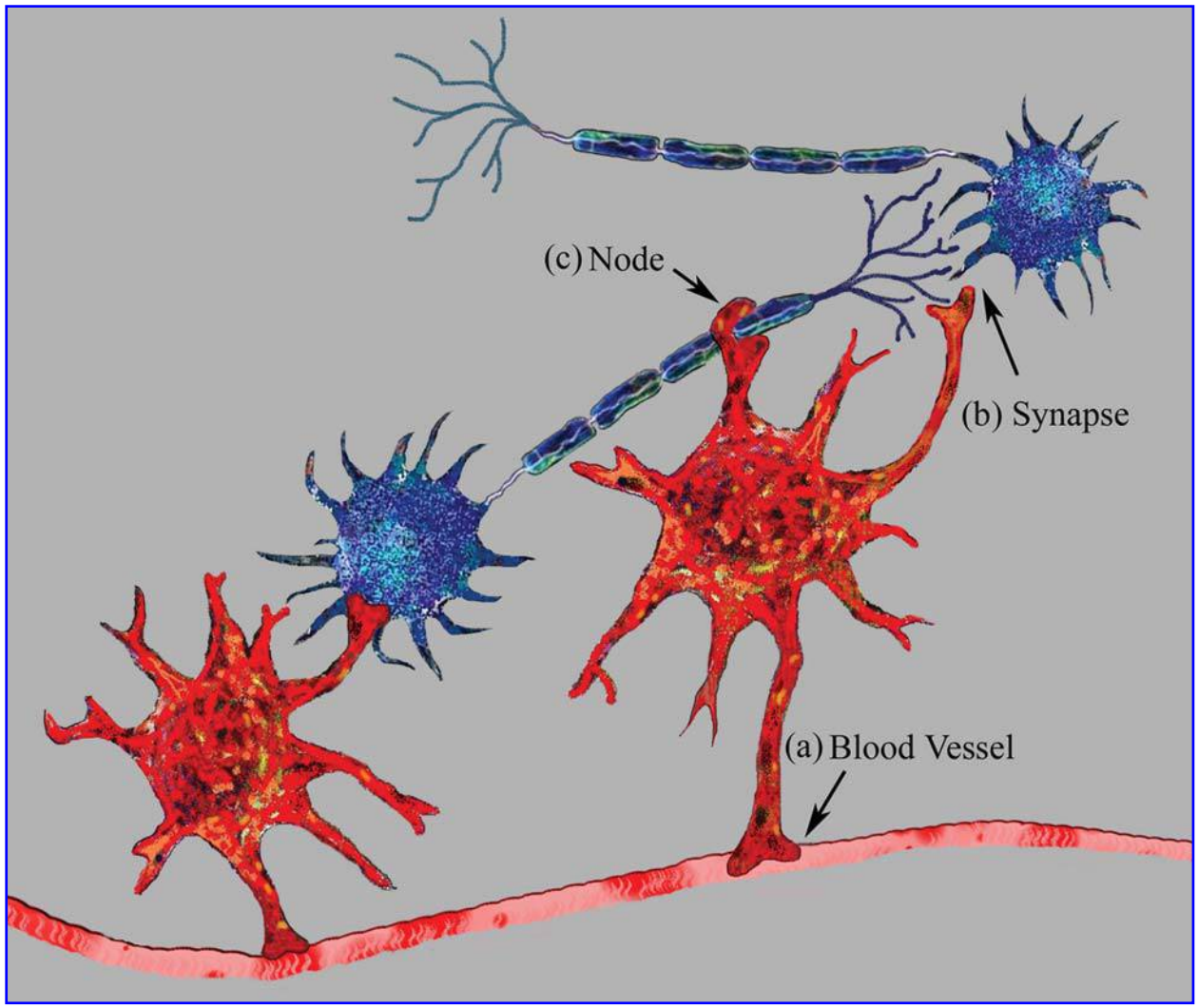

FIG. 1. The strategic position of the astrocyte. Astrocytes (*red*) have intimate contact with blood vessels (**a**) and with neurons (*blue*) at their synapses (**b**) and nodes of Ranvier (**c**). Arginine enters the brain parenchyma from the periphery through the vasculature (**a**). Endothelial cells, which line the vasculature, partner with astrocytes in maintaining the blood brain barrier. Arginine is transported into and out of the endothelial cell through the y^+ transport system. It enters the astrocyte through CAT-1 transporters (and the reactive astrocyte through CAT-2 transporters). At excitatory synapses (**b**), glutamate is released into the synaptic cleft. Glutamate is taken into the astrocyte that shields the synapse through astrocyte glutamate transporters. Glutamate induces release of arginine from the astrocyte through CAT. The y^+ transport system transports arginine into the neuron, where it can mediate NO-regulated neuron biology. One such biological process is neural transmission, where information is transmitted along an axon via action potentials. Astrocytes facilitate neural transmission by buffering potassium at the nodes of Ranvier (**c**), where potassium levels are critical in action potential conductance. Whether potassium induces arginine release from astrocytes is not known, but would provide a mechanism for regulating NO-mediated processes in the axon.

Fame" in 1995, and was the focus of the 1998 Nobel Prize in Physiology/Medicine. This prize was awarded to Robert F. Furchgott, Louis J. Ignarro, and Ferid Murad, for their discoveries vis-à-vis NO (previously called endothelium-derived relaxing factor, EDRF) as a signaling molecule in the cardiovascular system, where it facilitates blood vessel relaxation. Indeed, NO participates in a plethora of biological functions, such as memory and learning (25, 63, 91); inter- and intracellular communication, as in neurotransmission (16, 54, 59); smooth muscle relaxation, for example, in penile erection (11, 43, 44); cardiovascular response to a host of stimuli, such as exercise (37); and immune function, in which NO mediates the inflammatory response and is enlisted to fight infection (15, 56, 58).

Nitric oxide synthase isoforms

Nitric oxide is generated as a byproduct in one of three major pathways of arginine metabolism (discussed below and depicted in Fig. 2). In this pathway, nitric oxide synthase (NOS) catalyzes the deamination of arginine to generate citrulline and NO. Although all three NOS isoforms generate

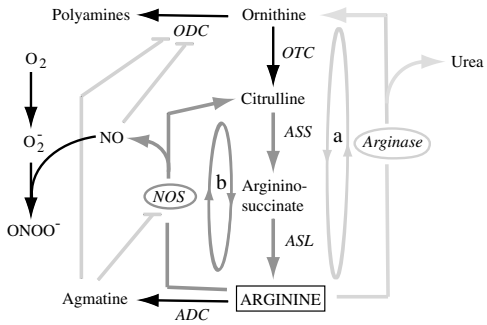

FIG. 2. Metabolic pathways of arginine. Arginine is converted to citrulline and NO by NOS, to agmatine by ADC or to ornithine and urea by arginase. When O_2^- is available, as in conditions of arginine depletion, NO generates $ONOO^-$. Agmatine and NO inhibit ODC, eliciting cytostasis via inhibition of polyamine synthesis. Agmatine also inhibits NOS activity. Two pathways for recycling arginine are denoted by *a* and *b*. ADC, arginine decarboxylase; ASL, argininosuccinate lyase; ASS, argininosuccinate synthase; NOS, nitric oxide synthase; ODC, ornithine decarboxylase; OTC, ornithine transcarbamylase.

NO from the metabolism of arginine to citrulline, they are encoded by three distinct genes, generally located in different cell types, activated by different extracellular cues, linked to different intracellular pathways, and produce different functional results (21). In endothelial cells, eNOS (endothelial NOS, NOS-3) is tethered to the cell membrane by caveolin, and generates NO when the acetylcholine receptor becomes activated, inducing the inositol triphosphate (IP_3) pathway, leading to increased intracellular calcium (Ca^{++}); Ca^{++} and tetrahydrobiopterin (BH_4) participate in the activation of calmodulin that activates eNOS. As a result, NO and cGMP are produced, and blood vessel relaxation is regulated (48, 53). In neurons, glutamate activates the *N*-methyl-D-aspartate (NMDA) receptor triggering Ca^{++} entry and Ca^{++} and BH_4 activation of calmodulin. Subsequent activation of nNOS (neuronal NOS, NOS-1), which is tethered to the cell membrane by a member of the membrane-associated guanylate kinase (MAGUK) family of proteins, postsynaptic density 95 (PSD95), leads eventually to the production of NO and cGMP. As a result, long-term potentiation (LTP), neurotransmission, and even ischemic injury are regulated (1, 48, 53). The third isoform binds calmodulin without requiring Ca^{++} concentrations above basal levels. Once induced, this NOS isoform remains active for prolonged periods, producing large sustained quantities of NO (48, 53). This is the inducible form of NOS (NOS-2 or inducible NOS, iNOS). Induction of iNOS occurs in many cell types, but in neural tissue, the most prominent induction occurs in the reactive cells: astrocytes and microglia (33, 45).

Induction of iNOS in astrocytes

There are several extracellular cues for iNOS induction and activation in astrocytes, including proinflammatory cytokines, such as interleukins (IL) IL-1 beta (IL-1β) and IL-6, interferon gamma (IFNγ), tumor necrosis factor alpha (TNFα), lipopolysaccharide (LPS), and various combinations of stimuli (26, 36, 74, 75). A variety of pathways can mediate iNOS induction through these stimuli in astrocytes. Several protein kinases, including serine/threonine kinases, and the atypical isoforms of protein kinase C (ϵ, η, and δ) are involved in iNOS induction (55, 73, 75). In rat astrocyte cultures, induction of iNOS by LPS, but not by IFNγ and IL-1, is attenuated by the tyrosine kinase inhibitor, tyrphostin; the more promiscuous tyrosine kinase inhibitor, genistein, prevents iNOS induction by LPS and by IFNγ and IL-1 (75). Serine/threonine kinases are required in both pathways of iNOS induction in rat astrocytes (75). Epidermal growth factor receptor (EGFR) and the mitogen-activated protein kinase (MAPK), p38, also are involved in iNOS activation (57, 73). In human optic nerve astrocytes, iNOS induction by cytokines (IFNγ and IL-1β) is mediated through p38, and iNOS induction by increased hydrostatic pressure is mediated through the EGFR; the nuclear factor-κB (NF-κB) pathway is implicated in the induction both by cytokines as well as by increased hydrostatic pressure (57).

Transcriptional regulation of iNOS in astrocytes

In rat astrocytes, LPS and IFNγ induce iNOS through activation of NF-κB through IκB kinase (IKK), and activation of signal transducer and activator of transcription (Stat1) through janus kinase (JAK) (44, 62, 73). The transcription factor NF-κB is sequestered in the cytoplasm by IκB. When IκB is phosphorylated by IKK, IκB is targeted for degradation and NF-κB is translocated into the nucleus where it can bind the promoters of target genes (40). LPS/IFNγ induces degradation of IκB by increasing the activity of IKK; as a result, NF-κB translocates into the nucleus where it binds the iNOS promoter inducing iNOS expression (40, 73). The transcription factor Stat also mediates LPS/IFNγ-induced iNOS expression in astrocytes. LPS/IFNγ induces the phosphorylation of JAK, which phosphorylates Stat enabling translocation of Stat into the nucleus where it binds the iNOS promoter at gamma-interferon-activated sites (GAS) thus inducing iNOS transcription (27, 73).

Regulation of iNOS is primarily transcriptional. The iNOS promoter contains GAS, IFNα and γ response elements (IREs), and response elements for NF-κB, NF-IL6, and activating protein-1 (AP-1) (14, 22, 38, 46, 88). Jana and colleagues show that distinct promoter elements are involved in the activation of iNOS in human astrocytes (38). In cytokine-induced expression of iNOS, CCAAT/enhancer-binding protein beta (C/EBPβ) is required. When low levels of iNOS are induced, as in IL-1β treatment, AP-1 is involved, whereas GAS is not. The GAS, and not the AP-1 site, however, is involved when high levels of iNOS are induced, as when astrocytes are treated with both IL-1β and IFNγ (38). Precise combinations of inducers of iNOS and the time course and mechanistic pathways for activation may vary by cell type and species; indeed, human and murine iNOS promoters differ slightly (14, 22, 36). Furthermore, although induction of iNOS is primarily transcriptional, translation of iNOS requires uptake of arginine from the extracellular milieu (discussed below) (44).

ARGININE, NITRIC OXIDE'S ONLY KNOWN PRECURSOR

Arginine metabolism

Although arginine is the only known precursor of NO, there are several arginine metabolic pathways. Arginine can be converted to citrulline and NO by NOS as mentioned above, but it also can be converted to agmatine by arginine decarboxylase (ADC), or to ornithine and urea by arginase (Fig. 2).

Arginine is converted to agmatine by the removal of a carboxyl group by ADC. Agmatine can inhibit iNOS and polyamine synthesis (7, 64, 67). Agmatine inhibits polyamine synthesis through its effects on antizyme translation. Agmatine induces a translational frameshift of antizyme mRNA resulting in full-length antizyme protein (67). Antizyme protein prevents the dimerization of ornithine decarboxylase (ODC), precluding its function. Antizyme also inhibits the putrescine transporter, depriving the cell of substrate required for polyamine synthesis resulting in the biological effect of growth arrest (7, 67). The mechanism by which agmatine inhibits iNOS is unknown.

Arginine also can be converted to citrulline and NO by NOS. Like agmatine, NO inhibits the putrescine transporter and ODC function, albeit by a different mechanism (7). The cytostatic effect of NO is mediated through its effect on ODC and the putrescine transporter, possibly via a mechanism involving nitrosylation, which prevents ODC dimerization (5). N^G-hydroxy-L-arginine (NOHA), an intermediate in the iNOS pathway, inhibits arginase in astrocytes (10).

The main metabolic pathway for arginine is responsible for disposing the toxic metabolite ammonia. This is the urea cycle, in which the conversion of arginine to ornithine and urea is catalyzed by arginase (Fig. 2). This pathway occurs mainly in hepatic tissue and other nonneural tissues. Arginine is synthesized in the kidney from citrulline, which is supplied to the kidney by the small intestine where it is produced from NH_3, CO_2, and ornithine.

Endogenous arginase expression in healthy uninjured neural tissue is not clear. Arginase expression has been detected, however, in both injured and diseased spinal cord (J.M. Gensert, unpublished observations) (89). Arginase is upregulated in the spinal cords of mice exposed to experimental allergic encephalomyelitis (EAE), a model for the demyelinating disease multiple sclerosis (MS); the arginase expressing cell type, however, has not been reported (89). We have observed arginase expression in lesioned spinal cord both in axons and in macrophages that invade the lesion (J.M. Gensert, unpublished observations). Arginase expression and activity also has been detected in astrocytes that are activated *in vitro* (44, 89).

The arginase pathway provides a mechanism to attenuate NO production by drawing arginine away from the NOS pathway (Fig. 2) (51). Ornithine then can be used to recycle arginine (which does not occur in neural tissue due to the absence of ornithine transcarbamylase, OTC), or to generate polyamines via the action of the putrescine transporter and ODC. While in unperturbed human and murine brain, ODC is localized primarily to neurons, during reactive gliosis, ODC is upregulated in reactive astrocytes (6, 52). Polyamine expression is detected in astrocytes, and astrocytes contain the necessary machinery for uptake of polyamines and polyamine precursors (6, 42). Generation of polyamines would have the biological effect of inducing proliferation, while also facilitating regeneration as has been demonstrated in an *in vitro* model (13, 67).

The arginase and NOS pathways appear to compete for the common substrate arginine. The competition for arginine and the reciprocal nature of iNOS and arginase induction have been well established in macrophages *in vitro* and *in vivo* (35). Type 1 cytokines, which include IFNγ, TNFα, and IL-1 induce iNOS but not arginase, whereas the type 2 cytokines IL-4, IL-10, and IL-13, and granulocyte macrophage colony stimulating factor (GM-CSF) and prostaglandin E2 (PGE2) suppress iNOS and induce arginase (35, 50). Simultaneous application of inducers of both enzymes leads to the reduction of both iNOS and arginase (50). Furthermore, exposure of macrophages to iNOS inducers prior to exposure to arginase inducers exhausts the cells' ability to respond to the subsequent exposure to arginase inducers; the reciprocal also holds true.

Arginase is activated by agents that increase intracellular concentrations of cAMP, and when cAMP is increased in macrophages, LPS-induced iNOS is down regulated (17). Similarly, increases in cAMP in neural systems also activate arginase. The elevation of cAMP enables neurons to overcome myelin-induced inhibition of axon regeneration/outgrowth in models of spinal cord injury and regeneration *in vitro* and *in vivo* (13, 28, 76). This "regeneration" results from the upregulation of arginase in a transcription-dependent mechanism involving the activation of the transcription factor cAMP response element binding protein (CREB), which is required for the neurons to overcome the inhibitory effects of myelin (28). Upregulation of endogenous arginase in regenerating neurons *in vivo* and the effect of increased cAMP on iNOS in this model have not been reported. The role of neither astrocytes (nor macrophages) in this paradigm has been delineated.

The three arginine pathways involve various sets of enzymes whose components possess differences in regulation, regional expression and cellular and subcellular localizations (86). It is provocative to consider the possibility of intercellular trafficking of substrates or induction of enzymes as means of integrating pathways whose components are otherwise fragmented. Indeed there is evidence to suggest that intercellular trafficking of at least some of the substrates does occur. For example, citrulline can be taken up by astrocytes and microglia (68) and arginine can be transported into cultured glial cells (70), and released from glia (84). An interglial NO–citrulline cycle between immunostimulated astrocytes and microglial cells has been proposed (68, 69).

As the only known precursor of NO, arginine's availability is critical to NO-mediated biology. Arginine is one of the rate limiting factors in NO production (51). Arginine can be made available via synthesis, recycling, or import. *De novo* synthesis of arginine requires OTC and carbamylphosphate synthetase I (CPS I), both of which are located in the mitochondrial matrix of select cell types in the liver and small intestine, but not in neural cells (86). Their absence in neural cells precludes the synthesis of arginine from citrulline gen-

erated from ornithine (Fig. 2). Similarly, the recycling of arginine in the arginase pathway through ornithine by OTC, argininosuccinate synthase (ASS), and argininosuccinate lyase (ASL) (*a* in Fig. 2) is not possible without induction of OTC. However, CNS cells do contain other enzymes in that pathway, ASS and ASL, and recycling of arginine through the NOS pathway requires ASS and ASL, but not OCT (*b* in Fig. 2). Although ASS and ASL often exhibit differential cellular localization patterns in many brain regions and are often expressed by neurons, both ASS and ASL can be induced in astrocytes in culture (8, 69, 86). Argininosuccinate synthase expression is induced in astrocytes stimulated with LPS, IFNγ, or a combination of both (69). Treatment of astrocytes with LPS and IFNγ also induces NO production through iNOS, supporting the likelihood of a recycling mechanism for arginine through the NOS pathway in astrocytes. *In vivo*, however, ASS is induced in reactive microglia by injection of LPS and IFNγ into striatum; only rarely, is ASS observed in reactive astrocytes *in vivo* (34). Notwithstanding, this still suggests a plausible mechanism for induction of components of these pathways under conditions of arginine demand, and for endogenous arginine production in astrocytes. Indeed, of the neural cells, astrocytes have the highest intracellular concentration of arginine (3). In cultured astrocytes, the concentration of arginine was measured at 45.6 nmol/mg protein, a value exceeded by only taurine and glutamine (90). Intracellular arginine levels are well above the K_m value for iNOS (10–16.5 μ*M* for arginine); however, extracellular arginine is nevertheless required for the production of iNOS protein. This is referred to as the "arginine paradox" (26, 44, 47, 77, 83). As a function of extracellular arginine concentration, the K_m value for iNOS activity in astrocytes is approximately 50 μ*M* (77). Induced membrane transport system y^+ activity also has a K_m of 50 μ*M* (77).

Inducible NOS translation requires exogenous arginine (44). Uptake of exogenous arginine leads to the charging of tRNAs, and the reduced phophorylation and activation of the serine/threonine protein kinase, general control of nutrition 2 (GCN2). GCN2 regulates translation initiation through phosphorylation of the alpha subunit of eukaryotic initiation factor-2 (eIF2α). Decreased GCN2 phosphorylation is accompanied by decreased eIF2α phosphorylation, which results in translational initiation of iNOS (44). The translational control of iNOS expression by arginine may have specific adaptive functions. Indeed, arginine depletion in the presence of iNOS can lead to superoxide generation. By coupling iNOS protein synthesis to arginine levels, the cell provides a scheme for ensuring that in arginine depleted cells iNOS is not synthesized and toxic superoxide cannot be produced.

Arginine uptake

Extracellular arginine is required for biological processes that require arginine despite endogenous arginine synthesis. Neural cells therefore must be able to take up arginine, and extracellular arginine must be made available.

Kidney-derived arginine is available to the brain and spinal cord, and gains entry to the nervous system from the blood and cerebrospinal fluid (CSF) through the capillary endothelial cells and the choroid plexus epithelial cells, respectively (71, 80, 82, 87). Circulating arginine enters the brain parenchyma through the y^+ system transporter on endothelial cells, which partner with astrocytes in maintaining the blood brain barrier (80). Arginine immunoreactivity has been detected in the astrocyte processes that enwrap the endothelial cells of the vascular suggesting uptake of arginine from the endothelium (2). In addition, astrocytes also express the y^+ system transporter.

Arginine is transported primarily through the y^+ transport system, present in both neurons and glia (47, 77). This system is sodium- and pH-independent, inhibited by high extracellular concentrations of potassium, and also can transport lysine and ornithine, which inhibit arginine uptake. Arginine uptake by the y^+ system also is inhibited by methylated arginine analogues such as *N*-monomethyl-L-arginine (NMMA) and asymmetric dimethylarginine (ADMA).

The y^+ transport system consists of three related cationic amino acid transporter (*Cat*) genes. The three transporter isoforms, CAT-1, CAT-2, and CAT-3, all are expressed in CNS and have a high affinity for arginine. Induced membrane transport system y^+ activity has a K_m of 50 μ*M* (77). As noted above, iNOS activity as a function of extracellular arginine has a K_m of approximately 50 μ*M*. In the absence of extracellular arginine uptake via the y^+ transport system, iNOS activity is abolished (77). An alternatively spliced form of CAT-2, CAT-2a shows a low arginine affinity and is expressed primarily in liver (77, 84).

The major system y^+ arginine transporter in rodent astrocytes that have not been activated is CAT-1 (77). CAT-2 either is not expressed or marginally expressed in untreated astrocytes, but is induced in astrocytes by a variety of cytokines and IFNγ, and LPS; this induction results in increased arginine uptake and increased NO production through iNOS (77). Schmidlin and Weisinger demonstrated astrocyte arginine uptake that was stimulated by LPS and inhibited by a host of compounds that affect NOS, including histidine, asparagines, glutamine, citrulline, creatine, N^G-nitro-L-arginine, NMMA, or L-canavanine (70). In *Cat2* null mice, there is a significant reduction in astrocytes of iNOS-mediated NO production in response to IFNγ/LPS treatment (47). This reduction is due at least partly to diminished L-arginine uptake and decreased iNOS expression.

Arginine release

Release of endogenous arginine has been demonstrated *in vitro* (72, 84, 85), *in situ* (32), and *in vivo* (23, 41). A host of stimuli can induce arginine release from astrocyte cultures. For example, glutamate, through activation of glial glutamate receptors (ionotropic non-NMDA receptors) induces arginine release (29, 30). Peroxynitrite ($ONOO^-$) stimulates the L-arginine transport system y^+ in astrocytes and also the release of arginine from astrocytes in an arginine transport-dependent mechanism; the arginine transport inhibitors L-lysine and NMMA inhibit $ONOO^-$ induced arginine release (84, 85). Superoxide anion (O_2^-) induces arginine release from astrocytes, but via an arginine transport-independent mechanism (85). Although Vega–Agapito and colleagues reported that a variety of NO donors and H_2O_2 do not significantly induce

arginine release from astrocytes, a later study shows NO-induced arginine release from cultured cerebellar astrocytes (72, 85).

Selective electrical stimulation of cerebellar white matter (mossy fibers) or parallel fibers *in situ* induces delayed arginine release from the Purkinje cell compartment, which in this system is separated from the stimulated areas (32). Purkinje cells in the cerebellum are surrounded by astrocytes. In histochemical studies, arginine has been detected in these Bergmann glia and not in the neighboring basket cells or Purkinje cells, suggesting that astrocytes may release arginine in response to neuron stimulation (3). Whether astrocytes are responsible for the arginine release in this system, however, is unclear. Grima and colleagues also induced arginine release from brain sections and cultured astrocytes, but not cultured neurons, by activation of non-NMDA ionotropic glutamate receptors located on astrocytes (30). *In vivo* stimulation of striatum with NMDA or kainate and afferent stimulation in thalamus also induces arginine release (23, 41). Given the predominant localization of arginine in astrocytes in brain *in situ* (3), the high intracellular concentration of arginine in astrocytes *in vitro* (90), and the release of arginine from cultured astrocytes (30, 32, 72, 84, 85), it is likely that astrocytes are the responsible cell type for this neuronal activity-dependent arginine release *in vivo*.

Astrocyte to neuron transfer of arginine (Fig. 2)

Kidney-derived arginine can enter the brain parenchyma from the blood and the CSF through the endothelium and epithelium, respectively. Astrocytes that contribute to the blood–brain barrier or that abut the endothelium can take up arginine. Can those astrocytes serve as reservoirs of arginine, releasing arginine and making it available to neighboring neurons? Do neurons have the capacity to take up arginine?

Several laboratories have suggested neuronal uptake of arginine that is released from astrocytes (23, 24, 31, 39, 84). In the most convincing report, Vega–Agapito and colleagues use a coculture system to demonstrate that arginine released from astrocytes by $ONOO^-$ stimulation is taken up by neurons (84). A transfer of arginine from astrocytes to neurons *in situ* is inferred in the work of Grima and colleagues who used NMDA treatment of brain sections to induce neuronal NO production and glial arginine secretion (31). The glial arginine secretion could be blocked by the ionotropic non-NMDA receptor antagonist, CNQX, presumably by acting on receptors resident on only astrocytes. Accordingly, NO production could be blocked also by CNQX as well as by the NOS inhibitor, N^G-nitro-L-arg; exogenous arginine was able to overcome this inhibition. In fact, arginine uptake was required for NMDA-induced NO production as L-lysine, which blocks arginine uptake by competitively inhibiting its transport, also blocked NMDA-induced NO production. The role of (glial-derived?) arginine in neuronal function is suggested *in vivo* as well. Neuronal synaptic activity was influenced by exogenous arginine application to the thalamus (23) and the visual cortex (39) via an NO production mechanism.

ASTROCYTIC NITRIC OXIDE—FRIEND OR FIEND?

Experimental systems

Reactive astrocytes are present in every neuropathological condition studied. Inducible NOS has been detected in reactive astrocytes that surround pathological neurons in disease as well as in injury. It therefore behooves us to study the effect of astrocytic NO production on the astrocytes as well as on their neuronal neighbors.

Bradykinin, glutamate, norepinephrine, cytokines, arginine, and LPS and IFNγ, all have been shown to induce astrocyte production of NO *in vitro* (9, 70). The effect of NO on the astrocytes appears to be inconsequential–a rapid, yet reversible, inhibition of astrocyte cellular respiration (9). However, astrocyte derived NO damages the neuronal mitochondrial respiratory chain *in vitro* (78). Whether this damage is reversible or irreversible depends at least in part on the length of time the neurons are exposed to the NO-secreting astrocytes. In a coculture system, when NO-producing astrocytes were removed from the neurons after 24 h, but not after 48 h, the neuron mitochondrial respiratory chain recovered completely (79).

Astrocyte-derived NO can be protective both to neurons and astrocytes *in vitro*. Pretreatment of astrocytes with IFNα/β prevents neuronal mitochondrial respiratory chain damage (78, 81). Also, induction of iNOS prior to an *in vitro* model of oxidative stress attenuates peroxide-induced astrocyte death (65, 66). This protection is negated by the NOS inhibitor L-NAME. Iron status of the astrocyte appears to play a role in the astrocyte response to oxidative stress as iron chelation with desferrioxamine (DFO) delays peroxide-induced death, whereas iron loading exacerbates it (65, 66).

Buskila *et al*. demonstrate the interrelationship between astrocytes and neurons in NO production *in situ* (12). In acutely prepared sections of mouse brain, astrocytes and neurons were distinguished by fluorescence kinetics and patterns of the NO sensitive fluorescent probe, 4,5 diaminofluorescein-2 diacetate (DAF-2DA), cell morphological features, electrophysiological signatures, and responses to selective NOS inhibitors. Astrocyte NO production required iNOS activity in the astrocyte, as well as nNOS activity in the neuron, and transfer of NO or $ONOO^-$ from the neuron to the astrocyte was not involved in the astrocyte response. It is hypothesized that the astrocyte NO production is in response to a stimulus from the neuron, but the nature of that signal and what function the NO production would serve warrant further study.

Astrocytic iNOS in disease and injury

Astrocytes express iNOS in neural disease (Alzheimer's disease, Parkinson's disease, multiple sclerosis, and amyotrophic lateral sclerosis) and injury (stroke/ischemia and spinal cord injury). Inducible NOS is localized to a subpopulation of reactive astrocytes in acute MS and diffuse-sclerosis-type lesions of human MS tissue (61). Inducible NOS is observed in reactive astrocytes in brain and spinal cord in Theiler's murine encephalomyelitis virus (TMEV) infected mice and in EAE, two rodent models of demyelinating disease (20, 60). Although correlative expression of iNOS in ac-

tive demyelinating disease in the EAE model has been interpreted as iNOS participating in the damage, an equally plausible interpretation is that iNOS is upregulated in reactive astrocytes to protect against further damage in an already highly damaged environment (18, 19). In fact, Cross and colleagues' earlier work suggests a protective or restorative role for astrocytic iNOS as iNOS inhibition (with aminoguanidine) in the EAE model for MS ameliorated MS spinal cord pathology (inflammation, demyelination, axonal necrosis) (20). Many other studies also demonstrate a protective role for iNOS in EAE (89). Arginase also is upregulated in the spinal cords of EAE mice (89). When mice were treated with an arginase inhibitor, amino-6-boronohexanoic acid (ABH) during the inductive and effector phases of disease, milder EAE (based on behavioral scores), delayed onset of pathology, reduced disease pathology, and overall better outcome were observed. If arginase and iNOS indeed function reciprocally in this system, then it would follow that iNOS upregulation would be associated with an improved disease outcome. Alas, iNOS expression was not reported and which cell type is responsible for the increased arginase expression in EAE spinal cord is unclear. What is clear is the improved outcome with arginase inhibition and the protective role of iNOS.

CONCLUSION

The regulation of iNOS, NO, and arginine metabolism in astrocytes is complex. First, astrocytes are just one cell type with intricate interactions with neurons and endothelial cells (Fig. 1) as well as with several other cell types, whose interactions with each other are just as relevant, though not considered in this review. Second, although most literature is comprised of snapshots, these snapshots are used to dissect and describe a component of a biological system, which is both dynamic and extensive. Finally, timing, duration, and concentrations and combinations of inducers or target molecules all are biological strategies aimed at modulating a *specific* response. For example, one may treat astrocytes with LPS and IFNγ to study NO production; however, LPS and IFNγ treatment of astrocytes also induces release of glutamate and ATP, which elicit their own effects (4). To illustrate: by releasing glutamate, astrocytes may modulate synaptic transmission and plasticity (49). However, astrocytic glutamate also may kill neurons by excitotoxic mechanisms. Also, the components of each event are subject to regulation. For example, the NO donor SNAP (*S*-nitroso-*N*-acetylpenicillamine) induces glutamate and arginine release from cerebellar astrocytes (72). Glutamate further stimulates arginine release through an autocrine mechanism. Arginine then stimulates more NO release . . . to the system's benefit or detriment.

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ABBREVIATIONS

AP-1, activating protein-1; ABH, amino-6-boronohexanoic acid; ADC, arginine decarboxylase; ASL, argininosuccinate lyase; ASS, argininosuccinate synthase; ADMA, asymmetric dimethylarginine; CAT, cationic amino acid transporter; C/EBPβ, CCAAT/enhancer-binding protein beta; CREB, cAMP response element binding protein; CPS I, carbamylphosphate synthetase I; CSF, cerebrospinal fluid; DFO, desferrioxamine; DAF-2DA, 4,5 diaminofluorescein-2 diacetate; eNOS, endothelial NOS; EDRF, endothelium-derived relaxing factor; EGFR, epidermal growth factor receptor; eIF2α, eukaryotic initiation factor-2 alpha; EAE, experimental allergic encephalomyelitis; GAS, gamma-interferon-activated sites; GCN2, general control of nutrition 2; GM-CSF, granulocyte macrophage colony stimulating factor; IKK, IκB kinase; iNOS, inducible NOS; IP_3, inositol triphosphate; IL, interleukin; IFNγ, interferon gamma; IREs, IFNα and γ response elements; JAK, janus kinase; LPS, lipopolysaccharide; LTP, long-term potentiation; MAGUK, membrane-associated guanylate kinase; MAPK, mitogen-activated protein kinase; MS, multiple sclerosis; NO, nitric oxide; NOS, nitric oxide synthase; NOHA, N^G-hydroxy-L-arginine; NMDA, *N*-methyl-D-aspartate; NMMA, *N*-monomethyl-L-arginine; nNOS, neuronal NOS; NF-κB, nuclear factor-κB; ODC, ornithine decarboxylase; OTC, ornithine transcarbamylase; PSD95, postsynaptic density 95; PGE2, prostaglandin E2; Stat1, signal transducer and activator of transcription; SNAP, *S*-nitroso-*N*-acetylpenicillamine; BH_4, tetrahydrobiopterin; TNFα, tumor necrosis factor alpha.

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