Neurointegrity and europhysiology: astrocyte, glutamate, and carbon monoxide interactions

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Abstract

Astrocyte contributions to brain function and prevention of neuropathologies are as extensive as that of neurons. Astroglial regulation of glutamate, a primary neurotransmitter, is through uptake, release through vesicular and non-vesicular pathways, and catabolism to intermediates. Homeostasis by astrocytes is considered to be of primary importance in determining normal central nervous system health and central nervous system physiology – glutamate is central to dynamic physiologic changes and central nervous system stability. Gasotransmitters may affect diverse glutamate interactions positively or negatively. The effect of carbon monoxide, an intrinsic central nervous system gasotransmitter, in the complex astrocyte homeostasis of glutamate may offer insights to normal brain development, protection, and its use as a neuromodulator and neurotherapeutic. In this article, we will review the effects of carbon monoxide on astrocyte homeostasis of glutamate.

Key words: carbon monoxide; astrocytes; glutamate metabolism in the brain; neuroprotection by carbon monoxide; astrocyte control of glutamate metabolism; gasotransmitters; neurotherapy; neuroprotection; GABA

Introduction

Central nervous system (CNS) progenitor cells differentiate into three main cell types – neurons, astrocytes, and oligodendrocytes. Cell proliferation, survival, migration, differentiation, and apoptosis are regulated by multiple signals including neurotransmitters that are critical for normal brain development and function.[1-7] Composed of electrically excitable neuronal networks that are connected by chemical synapses, CNS homeostasis is primarily provided by the non-externally excitable astrocytes. The latter, identified by an algorithm based on cytological features,[8] supplies neurons with glutamate, the conjugate base of glutamic acid and the major neurotransmitter of the CNS. Astroglial regulation of the neurotransmitter is through uptake, release through vesicular and non-vesicular pathways, and catabolism to intermediates[9-16] and requires that glutamate is adequately and constantly replenished and that anaplerosis and cataplerosis are balanced.[17-24] Directed to several different biochemical pathways, glutamate is important to glutamate-glutamine cycling. Net formation of glutamate from glucose is required for glutamatergic neuronal signaling in the CNS, requires astrocytic pyruvate carboxylase (PC) and astrocytic tricarboxylic acid (TCA) cycle activity and is required for CNS function. Return of glutamate to neurons and continued release of glutamate depend on astrocyte glutamine synthetase (GS) activity. The dynamic metabolic fluxes in astrocytes and cellular localization of metabolic processes determine metabolic formation and degradation of glutamate.

Astrocyte contributions to brain function and prevention of neuropathologies are as extensive as that of neurons. Homeostasis of glutamate by astrocytes is considered to be of primary importance in determining normal CNS health and physiology.[25-32] A major metabolite connecting glucose and amino acid metabolism *via* the tricarboxylic acid cycle, glutamate is an immediate precursor of γ-aminobutyric acid (GABA), initially excitatory and later inhibitory, and is required for normal development of the brain. It acts as a trophic factor that influences proliferation, migration, differentiation, synapse maturation, and cell death during CNS development.[33,34] Excitation by GABA is a result of high intracellular concentrations of chloride and is followed by sequential formation of GABA-releasing and glutamatergic synapses. Giant depolarizing potentials, generated in part by excitatory actions of GABA, result in the generation of large oscillations of intracellular calcium and activity-dependent synapse formation (glutamate and GABA synapses) and neuronal growth. A chloride-extruding system develops later and results in the inhibitory effects of GABA.[35-38] GABA is, thus, inhibitory in the developed human brain, glutamate excitatory, and GABA is required for glutamate synapse development in the CNS.

The physiologic changes noted above depend on the biochemical requirements of the developing brain for normal functioning. These constantly change and evolve in all age groups. While the TCA cycle is important for glutamate synthesis in both the astrocyte and the neuron, the shuttle of glutamate from neurons to astrocytes is limited in the neonatal brain. This is compensated for by astrocytic anaplerosis *via* increased pyruvate carboxylation.[20,24,39] Fewer astrocytes are present in the newborn brain compared to the adult brain and PC activity is lower. Gliogenesis occurs mainly during the first few weeks after birth. Glutamate levels double from week 32 gestational age and continue to increase in the first year of life.[40] The major role of neurotransmitters, including glutamate, in neuronal differentiation, migration, and survival in the developing brain is mainly through facilitating the entry

of Ca^{2+} [41,42] and the astroglial cradle (formed by perisynaptic processes) that is necessary for synaptogenesis, maturation, isolation, and maintenance of synapses and is dependent on the homeostasis of glutamate. Synaptic connectivity, synaptic plasticity, and information processing of the CNS proceeds only with an intact and working system.[43-45] Astrogenesis is required for the majority of functional synapses – morphologic and functional diversity occurs during development.[46,47] Glutamate is central to these dynamic changes and resulting clinical outcomes – gasotransmitters may affect these diverse interactions positively or negatively. The effect of carbon monoxide, an intrinsic CNS gasotransmitter, in the complex astrocyte homeostasis of glutamate may offer insights to normal brain development, protection, and its use as a neuromodulator and neurotherapeutic. In this article, we will review the effects of carbon monoxide on astrocyte homeostasis of glutamate.

METABOLISM OF GLUTAMATE IN THE ASTROCYTE AND NEURON

Glutamate functions as a neurotransmitter, as a precursor of neuronal neurotransmitters, as an energy substrate and buffer, and as a nitrogen buffer. Most CNS glutamate is produced within the brain as the blood-brain-barrier effectively excludes most of the blood-borne glutamate and there is net removal of glutamine. The neurotransmitter is not uniformly distributed and, potentially neurotoxic, low extracellular fluid concentrations of glutamate are necessary to avoid excitotoxicity. The required levels for normal function vary in different regions of the brain and during different times of life. Concentration influences its dual role as neurotransmitter and energy source. A concentration gradient of CNS glutamate, vesicles > cytosol/mitochondria > extracellular fluid, is a result of the effectiveness of astrocyte glutamate synthesis, glutamate catabolism, and glutamate transporters. De novo synthesis of TCA cycle intermediates from glucose catalyzed by PC results in the formation of oxaloacetate, essential for the de novo synthesis of glutamate and the glutamine that is transferred from astrocytes to neurons to maintain the pool of glutamate. Complete and/or partial pyruvate recycling *via* oxidation of glutamate is important in balancing the PC anapleurotic reaction. These processes are dynamic and change with brain activity and the release of K^+ by synaptic terminals during depolarization.[39,48] Metabolism of glutamate is affected *via* the GS reaction, glutamate/α-ketoglutarate-linked aminotransferases coupled to the glutamate dehydrogenase (GDH) reaction, decarboxylation to GABA, and glutathione (GSH).

Glutamine synthetase reaction

The glutamate-glutamine cycle (**Figure 1**) describes the transfer of glutamate from neurons to astrocytes and subsequent return of glutamine from astrocytes to neurons. There is not a stoichiometric relationship between glutamate flux out of the presynaptic neurons and the flux of glutamine from astrocytes back to the presynaptic terminals. Important to the process, astrocytes represent a heterogenous population of cells and GS, an astrocyte marker.[49] Three types of GS have been identified, GSI, GSII, and GSIII. Humans have the GSII type.[50] Located primarily in the cytoplasm of astrocytes, human GS is impor-
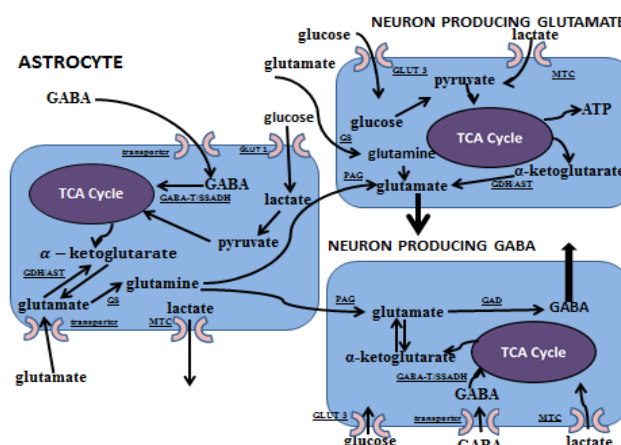

**Figure 1: Glutamate-glutamine-GABA cycle.**
Note: Astrocyte and neuron roles in metabolic processes and the release and reuptake of neurotransmitters. Gln: Glutamine; GAD: glutamate decarboxylase; GABA-T: GABA transaminase; GDH: glutamate dehydrogenase; SSADH: succinic semialdehyde dehydrogenase; AST: aspartate aminotransferase; PAG: phosphate-activated glutaminase; GABA: γ-aminobutyric acid.

tant for removal of ammonia (NH_3) and is a major route for removal of glutamate from the CNS.

Net synthesis of the non-toxic glutamine, precursor of glutamate and GABA, occurs only in astrocytes and requires cellular compartmentalization of GS and PC.[22] Catalyzed by GS, L-glutamate combines with ammonia and adenosine triphosphatase (ATP) to form L-glutamine, adenosine diphosphate (ADP), and phosphate:

L-Glutamate + NH_3 + ATP ↔ L-Glutamine + ADP + Pi.
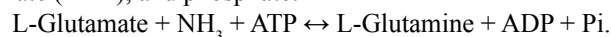
Glutamine is a major source of CNS glutamate *via* the glutaminase (present in neurons and astrocytes) reaction[51]:

L-Glutamine + H_2O → L-Glutamate + NH_3.
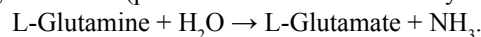
The glutamate-glutamine cycle loses intermediates to other pathways and de novo synthesis of glutamine in astrocytes is needed to continue its operation. Anaplerosis is mediated by PC and ranges from 6 to 35% of the rate of the TCA cycle which meets this requirement[52] with the resulting oxaloacetate entering the Krebs cycle:
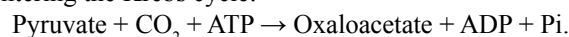
Pyruvate + CO_2 + ATP → Oxaloacetate + ADP + Pi.

Glutamate/α-ketoglutarate-linked aminotransferases coupled to the glutamate dehydrogenase reaction

Catabolism of glutamate to carbon dioxide (CO_2) requires access to the TCA cycle and pyruvate recycling. The coupling of an aminotransferase reaction to the GDH reaction generates ammonia or incorporates ammonia into an amino acid. The TCA cycle-derived α-ketoglutarate provides the carbon-skeleton for glutamate and maintains glutamate nitrogen levels.

The glutamate-glutamine cycle is coupled to the α-ketoglutarate cycle *via* the mitochondrial GDH:

L-Glutamate + $NAD(P)^+$ + H_2O ↔ α-Ketoglutarate + NH_4^+ + NAD(P)H.
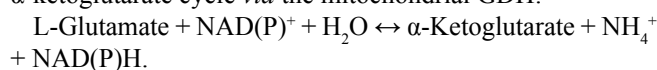
Present as two isoforms in humans (GDH1 and GDH2), the enzymes differ greatly with regards to allosteric regulation.[53] GDH is the major route of glutamate oxidation in astrocytes.[26]

As a co-substrate in all aminotransferase catalyzed reactions, glutamate also has a major role in astrocyte amino-acid interconversion. α-Ketoglutarate-linked aminotransferases are

important for the transport of reducing equivalents from cytosol to mitochondria. Three aminotransferases are important players in the conversion of glutamate to α-ketoglutarate – the aspartate, alanine, and branched chain amino acid aminotransferases. The first is the major enzyme responsible for production of α-ketoglutarate that can be oxidatively metabolized in the TCA cycle to form oxaloacetate and then aspartate.

Mitochondrial aspartate aminotransferase (AAT) catalyzes the following reaction:

L-Glutamate + Oxaloacetate ↔ L-Aspartate + α-Ketoglutarate.

The reaction proceeds readily in both directions and is important in the TCA cycle. AAT, present in all tissues, has the highest specific activity in the brain.

Located in the cytoplasm and requiring the cofactor pyridoxal-5-phosphate, alanine aminotransferase catalyzes the interconversion of alanine, α-ketoglutarate, pyruvate, and glutamate. In the neuron,

L-Glutamate + Pyruvate → L-Alanine + α-Ketoglutarate.

In the astrocyte,

L-Alanine + α-Ketoglutarate → L-Glutamate + Pyruvate.

This nitrogen shuttle requires uptake of alanine by astrocytes and transamination of alanine in the astrocytes.[54]

The essential branched chain amino acids valine, leucine, and isoleucine rapidly cross the blood-brain barrier and are taken up by astrocytes. CNS uptake requires that an equivalent amount of nitrogen exits the brain to maintain nitrogen homeostasis. Intracellular amino acid nitrogen is incorporated into glutamine which then moves to the extracellular compartment. In astrocytes, the mitochondrial isozyme of the branched chain amino acid aminotransferase isozymes catalyzes the metabolism of valine, leucine and isoleucine by transamination with α-ketoglutarate to form α-ketoisovalerate, α-ketoisocaproate, and α-keto-β-methylvalerate, respectively:

L-Valine + α-Ketoglutarate ↔ L-Glutamate + α-Ketoisovalerate

L-Leucine + α-Ketoglutarate ↔ L-Glutamate + α-Isocaproate

L-Isoleucine + α-Ketoglutarate ↔ L-Glutamate + α-Keto-β-methylvalerate.

The 3 branched chain amino acids are sources of glutamate in the astrocyte.[26,55-58]

Decarboxylation to γ-aminobutyric acid

Decarboxylation of glutamate by glutamic acid decarboxylase (GAD) results in the inhibitory neurotransmitter GABA (**Figure 1**). Two isozymes, GAD65 and GAD67, are restricted to neurons and primarily GABAergic neurons and catalyze the reaction:

L-Glutamate → GABA + CO_2.

GABA is released by the neuron and taken up by astrocytes. Metabolism of GABA in the astrocyte to the TCA cycle intermediate succinate is catalyzed by GABA-transaminase and succinic semialdehyde dehydrogenase, the GABA shunt:

GABA + α-Ketoglutarate ↔ Succinic Semialdehyde + L-Glutamate

Succinic Semialdehyde + NAD^+ → Succinate + NADH.

Glutamate is a product of the first reaction.

Glutathione

GSH is a major water soluble antioxidant of the brain, is a major source of peptide-bound glutamate and is more prevalent in astrocytes than in neurons. Uptake of cysteine is the major determinant of its production in the CNS and is mediated by the excitatory amino acid transporter 3 and the alanine, serine and cysteine system that transports alanine, serine, and cysteine.[59] Synthesis requires 2 ATP-dependent steps, the first catalyzed by γ-glutamylcysteine ligase (rate limiting step) and the second by glutathione synthetase:

L-Glutamate + L-Cysteine + ATP → L-γ-Glutamylcysteine + ADP + Pi

L-γ-Glutamylcysteine + Glycine + ATP → GSH + ADP + Pi.

Astrocytic GSH released to the interstitial space is hydrolyzed to γ-glutamycysteine and glycine. γ-glutamycysteine is then hydrolyzed to glutamate and cysteine that are then actively transported into neurons by excitatory amino acid transporter 3 and excitatory amino acid transporter 2.

Other sources of astrocyte glutamate

5-Oxoproline (5-OP), pyroglutamic acid, is ubiquitous in nature and is the cyclic lactam of glutamate, a reservoir of glutamate. It is found as a free metabolite in living cells and as an N-terminal modification in antibodies, enzymes, structural proteins, neuronal peptides and hormones (including the accumulating peptides in Alzheimer's disease and familial dementia). The γ-glutamyl cycle describes the enzymatic formation of 5-OP from glutathione by γ-glutamyl cyclotransferase and degradation of 5-OP to L-glutamate by 5-oxoprolinase. 5-Oxoprolinase, a pyro-glutamic acid-cleaving enzyme, acts on 5-OP to form L-glutamate[60,61]:

5-OP + ATP + $2H_2O$ → L-glutamate + ADP + Pi.

PHYSIOLOGY OF GLUTAMATE IN THE CENTRAL NERVOUS SYSTEM

Glutamate is the major neurotransmitter in the CNS and mediates the fast excitatory signaling needed for motor/sensory/autonomic processing, is an energy substrate, and is important for neurometabolic and neurovascular coupling. Astrocytes sense local glutamate activity and respond to this by elevations in the intracellular Ca^{2+} concentration. Also capable of releasing glutamate, proposed mechanisms of release include hemichannels, anion channels, and exocytosis.[62-68] Functional integrity of astrocytes and neurons is necessary for transfer and processing of information determined by glutamate release and concentrations. Involvement of glutamate in the pathogenesis of CNS diseases due to excessive release, reduced uptake, and/or alteration of receptor function has been postulated.[69-80]

Critical to maintaining CNS integrity and preventing neuropathology, glutamate homeostasis is an important function of astrocytes. Initially considered to be "glue" keeping the nerve cell elements together and involved in various housekeeping functions, astrocyte function in the tripartite synapse (synapse between a pre- and post-synaptic neuron with bidirectional communication with one astrocyte) emphasizes astrocyte participation in synaptic signaling. This is done through exchanging information with the pre- and postsynaptic neurons, responding to synaptic activity, and regulating synaptic activity.[81-85] Energy substrates and transmitter precursors are provided and waste products are removed. Close proximity to large numbers of neurons enables single astrocytes to pro-

mote synchrony of neuronal action potential firing resulting from glutamate release.[86-89] Adaptive to their environment, astrocytes are able to change the expression of large numbers of proteins affecting CNS function. Heterogeneity in astrocyte receptor expression, gap junction coupling, membrane currents and morphology occur between and within brain regions responding to and resulting in dynamic physiology.[90-94] Interplay between neuronal signaling to astrocytes and astrocytic signaling to neurons involve ATP and glutamate which coordinately activate astrocytes through mobilization of their internal Ca^{2+}. This triggers release of neuroactive molecules including ATP and glutamate from astrocytes. Ca^{2+} waves are generated in astrocytes and neuronal synaptic transmission and neuronal excitability are modulated.[95]

Presynaptic factors (release probability, quantal content, vesicle composition), modulation of the concentration and longevity of glutamate in the extracellular space (diffusion, actions of glutamate transporters), and postsynaptic factors (types and locations of ionotropic glutamate receptors, their numbers, and nature and locations of associated intracellular signaling systems) modulate excitation at glutamatergic synapses. Molecular components include synthesis and release of glutamate, glutamate-gated ion channel receptors (iGluRs), glutamate G-protein-coupled receptors (GPCRs), and glutamate transporters. Membrane receptors for most major neurotransmitters and neuromodulators are expressed by astrocytes and are important for neural communication, memory formation, learning, and regulation. Neurotransmitter receptors form ion channels to mediate rapid membrane potential changes, exert their action through slower intracellular signaling pathways, and serve as synaptic scaffolds to regulate synapse formation and maintenance (non-ionotropic function). These receptors usually are not specific for glutamate and receptors (classified based on mammalian pharmacology and identified as being ionotropic or metabotropic, responsible for the glutamate-mediated postsynaptic excitation of neural cells, and functions include modulation of synaptic plasticity) result when sixteen functional subunits assemble in tetrameric complexes to form GluR1–GluR4 for α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) receptors(occur in 2 alternatively spliced versions, flip and flop), GluR5–GluR7 and KA1–KA2 for kainate receptors, and GluN1, GluN2A-GluN2D, and GluN3A–B for N-methyl-D-aspartate (NMDA) receptors.[90] AMPA receptors, kainate receptors, and NMDA receptors are ionotropic glutamate receptors in humans. Metabotropic glutamate receptors (mGluRs), GPCRs that bind glutamate within a large extracellular domain and transmit signals through the receptor protein to intracellular signaling receptors, are classified into three groups based on their sequence homology, pharmacological profile, and coupling to intracellular transduction pathways – Group I mGLuRs consist of mGlu1, mGlu5, and their splice variants; Group II includes mGlu2 and mGlu3; and Group III mGlu4, mGlu6, mGlu7, mGlu8, and some splice variants.[91,92] While functional heterogeneity of these components between astrocytes within and across brain regions exists, impact on physiological and clinical presentations remains undefined.[93,94,96-100]

The intracellular signaling cascades depend on astroglial activation occurring within milliseconds to neuronal activity and neurotransmitters released at synapses. Fast responses occur as changes in intracellular free Ca^{2+} concentration occur.[101-103] Neurotransmitters released from neurons are thought to activate receptors at the astroglial membrane inducing activation of phospholipase C and the concomitant production of IP3 which triggers release of endoplasmatic reticulum Ca^{2+}.[104,105] Hemichannels open and other Ca^{2+} dependent neurotransmitter release mechanisms are activated. Hemichannel opening allows release of glutamate into the extracellular space which can activate purinergic or NMDA receptor channels in astrocytes and induce changes in free Ca^{2+} concentration.[106,107] Low glutamate concentrations result in short-acting Ca^{2+} oscillations in single astrocytes. Higher concentrations result in astrocyte-to-astrocyte propagating Ca^{2+} waves.[108] Different neurotransmitters result in different Ca^{2+} responses in astrocytes and may be able to activate entire astroglial networks.[109] Because astrocytes envelope synapses, release of glutamate also activates neighboring pre- and post-synaptic neurons and, therefore, modulate surrounding synaptic activity.[110,111]

Synthesis and release of glutamate

Glutamate has to be present in the right concentration, in the right place, and for the right amount of time to regulate brain development, cellular survival, differentiation, glutamate elimination, and formation and elimination of synapses. There are no special or unique synthetic or degradative enzymes for L-glutamate – the amino acid is synthesized from α-ketoglutarate and glutamine. Loss of glutamine may result, but its replenishment is ensured by other biochemical pathways noted above.

Spontaneous release of glutamate from astrocytes helps to set the basal probability of neurotransmitter release *via* metabotropic glutamate receptor activation.[112] K_{2P} channels in astrocytes (isoforms TWIK1, TREK1, TREK2, TASK1, and TASK3) are also critical for glutamate release.[113-115] In astrocytes that are part of a tripartite synapse, calcium oscillations cause proximal and distal release of glutamate to surrounding neurons.[116] After release of glutamate from neurons, transport of glutamate to astrocytes, conversion of glutamate to glutamine in astrocytes, transport of glutamine from astrocytes to neurons and reconversion of glutamine to glutamate in neurons completes the glutamate/glutamine cycle. Astrocytic regulation ensures that greater than 60% of synaptic glutamate is processed by the associated astrocyte. Swelling and shrinking of astrocytes may allow reduction of leakage and/or prevent spillover of glutamate.

Glutamate-gated ion channels

iGluRs, integral membrane proteins, are important to CNS synaptogenesis, neuronal pathfinding, neuronal viability, and regulation of synaptic efficacy.[117] These are ligand-gated nonselective cation channels that result in flow of K^+, Na^+, and Ca^{2+} with glutamate binding. Excitatory postsynaptic current results and is depolarizing. Excitatory synaptic transmission in the human brain depends on a high concentration of glutamate at the postsynaptic membrane after its release from presynaptic terminals. iGluRs are the major mediators of excitation

and activation of AMPA receptors, NMDA receptors, and/or kainate receptors and require proximity of the amino acid. Ion channel pores formed by these receptors activate with binding of glutamate. Overstimulation results in neurodegeneration and neuronal damage due to excitotoxicity. Synaptic plasticity and molecular mechanisms of learning and memory are also dependent on iGluRs.

Major neurological and neurodevelopmental disease processes are associated with iGluR mutations, misexpression, misregulation, or deficits in signaling.[118-125] Encoded by 18 genes which coassemble to form 3 major families (AMPA, NMDA, and kainate), these receptors form tetrameric ligand-gated channel pores that allow influx of Na^+ and Ca^{2+}. AMPA iGluR subunits include GluA1, GluA2, GluA3, and GluA4, NMDA subunits GluN1, Glun2A, GluN2B, GluN2C, GluN2D, GluN3A, and GluN3B, and kainate subunits GluK1, GluK2, GluK3, GluK4, and GluK5. A fourth family is formed by the delta subunits GluD1 (GluRδ1) and GluD2 (GluRδ2). In the brain, GluA1/GluA2 (AMPA receptor subtype), GluN1/GluN2A/GluN2B (NMDA receptor subtype) and GluK2/GluK5 (kainate receptor subtype) are the most widely expressed. Composed of 4 large subunits, iGluRs contain 4 discrete semiautonomous domains – the extracellular amino-terminal domain, the extracellular ligand-binding domain, the TMD – harbors the ion channel, and an intracellular C-terminal domain. Binding of glutamate to the extracellur ligand-binding domain allows the influx of ions and generation of synaptic current in the brain. Various ionotropic glutamate receptors on postsynaptic cells determine potentiation or depression of the cell. Glutamate, acting on glutamate receptors, causes Ca^{2+} to influx into the postsynaptic cell resulting in a cascade of cell processes that can result in damage to cell structures, and, possibly, cell death. Clinical neuropathology associated with glutamate excitotoxicity include traumatic brain injury, cerebral ischemia, hyperalgesia, attention deficit hyperactivity disorder, autism, Huntington's disease, multiple sclerosis, Parkinson's disease, Rasmussen's encephalitis, schizophrenia, seizures and, possibly, acquired immune deficiency syndrome dementia complex, Alzheimer's disease, amyotrophic lateral sclerosis, combined systems disease (vitamin B12 deficiency), depression, anxiety, drug addiction/tolerance/dependency, glaucoma, hepatic encephalopathy, hydroxybutyric aminoaciduria, hyperhomocysteinemia, homocysteinuria, hyperprolinemia, lead encephalopathy, Leber's disease, mitochondrial encephalopathy, lactic-acidosis, and stroke-like episodes syndrome, myoclonic epilepsy with ragged-rebfibers, mitochondrial abnormalities, neuropathic pain syndromes, nonketototic hyperglycinemia, olivopotocerebellar atrophy, essential tremor, Rett syndrome, sulfite oxidase deficiency, Wernicke's encephalopathy, and depersonalization disorder.

α-Amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid receptors

Widely expressed in the adult brain, AMPA receptors are the first iGLuRs to appear during CNS development.[126-128] Most excitatory synaptic transmission in the CNS is mediated by glutamate through activation of AMPA receptors. Interaction of postsynaptic proteins with AMPA receptor subunits control AMPA receptor insertion, location, pharmacology,

synaptic transmission, and neuroplasticity.[129] Altering the number of AMPA receptors is the basis of synaptic neuroplasticity – synapses become stronger with the addition of AMPA receptors.[130,131] Subunit composition varies depending on the brain region and the functional properties of AMPA receptors and their trafficking depend on subunit composition.[132,133] The majority of AMPA receptors in the CNS contain GluA2 which is important for changing Ca^{2+} permeability and resulting synaptic transmission and intracellular signaling.[134] AMPA receptor Ca^{2+} permeability depends on the GluA2 subunit mRNA and mediates fast excitatory synaptic transmission in the CNS. Incorporation of the GluA2 subunit reduces the Ca^{2+} permeability and channel conductance and prolongs the decay kinetics of a synaptic current.[135] A switch in AMPA receptor phenotype during physiological functioning and under pathological conditions results in a change in information processing and Ca^{2+}-dependent signaling at synapses.[136]

N-methyl-D-aspartate receptors

Astrocyte AMPA receptors and NMDA receptors differ in their sensitivity to glutamate as well as their desensitization rate and provide information about the concentration and duration of glutamate in the synaptic cleft. The latter form glutamate-gated ion channels that are highly permeable to Ca^{2+} (main source of calcium responsible for glutamate-induced excitotoxicity), relay physiological signals into neurons, and trigger intracellular signaling cascades that can lead to cell death.[16] NMDA receptor activation resulting in increased Ca^{2+} concentrations triggers synaptic plasticity and amount and kinetics of the calcium influx determine physiology.[137] Essential but potentially harmful, subunit composition of NMDA receptors determines functionally distinct CNS properties.[138,139] Seven subunits have been identified – GluN1 (8 isoforms), GluN2A, GluN2B, GluN2C, GluN2D, GluN3A, and GluN3b – and are brain region-specific. All of the subunits have 4 domains, the N-terminal domain, the agonist binding domain, the TMD, and the intracellular C-terminal domain. N-terminal domain, agonist binding domain, and TMD are targeted by endogenous or synthetic allosteric modulators.[140]

Ongoing modification of subunit composition with resultant changes in biochemical interactions and physiology continues throughout life. Composition of their heterotetrameric assembly, usually two GluN1 and two GluN2 subunits, determines function of the NMDA receptors. GluN1 and GluN2-containing NMDA receptor complexes are abundant throughout the CNS. GluN2 subunits define channel properties and subcellular localization of the receptor. Diheteromeric (GluN1/GluN3) or triheteromeric (GluN1/GluN2/GluN3) complexes are formed if GluN3 subunits are incorporated.[140] Combinations of subunit assembly results in functionally different NMDA receptors and physiologic differences in the CNS. Although GluN1 is ubiquitously expressed in the CNS, developmental and regional variations depend on the type of GluN1 isoform.[140] GluN2A–D, forming glutamate-gated ion channels that are heterogeneous and widely permeable to calcium ions, result in normal neurophysiology as well as neuropathology depending on the receptors functional properties. GluN2B-NMDA receptors have a higher affinity for glutamate.[140] Extrasynaptic NMDA receptors have higher

levels of GluN2B-containing heterodimers, are distinct, and probably serve a specific function.[16] The GluN3-containing NMDA receptors are not as common as those composed only of GluN1 and GluN2 subunits.[139] In the CNS, they are important in delaying synapse maturation until the arrival of sensory experience and in targeting non-used synapses for pruning. Reactivation of GluN3A expression at the wrong time may result in neuropathology.[141]

Kainate receptors

Another member of the ionotropic glutamate receptors, kainate receptors, form from 5 subunits (GluK1, GluK2, GluK3, GluK4, and GluK5) into tetrameric ion channels. GluK1–3 can form homomeric and heteromeric receptors whereas GluK4–5 must form heteromeric receptors with one of the GluK1–3 subunits to form functional channels.[142-146] The former are low-affinity kainate receptors and the latter high-affinity kainate receptors.[147] GluK5 is widely distributed through the CNS and in heteromeric complexes with GluK2 constitutes the most abundant kainate receptor subtype in the brain.[146,147]

Located at pre- and postsynaptic membranes of neurons and astrocytes in the CNS, kainate receptors are constituents of excitatory synaptic transmission and modulation of excitability by regulating neurotransmitter release. In an experimental mode of temporal lobe epilepsy, Vargas et al showed an increase in astrocyte expressed kainate receptor subunits (GluK1, GluK2, GluK3, GluK4, and GluK5) following status epilepticus. The authors suggested that this may serve as a sensor for excess glutamate.[148] Astrocyte response to extracellular glutamate is propagation of intracellular Na^+ waves resulting in activation of glial Na^+/K^+-ATPase which increases energy demand inside the astrocyte.[149] Increased energy demand and glucose consumption inside the astrocyte provides metabolic substrates to neurons through the astrocyte-to-neuron lactate shuttle and provide energy substrates for injured surviving neurons.[150] Functioning kainate receptors may also trigger release of gliotransmitters – glutamate release from astrocytes has been implicated in hippocampal slice models of seizure activity.[158,159] Increased Ca^{2+} signaling, hypothesized to result from pathological expression of kainate receptors in reactive astrocytes, may cause synchronization of neurons due to glutamate and result in hyperexcitability. Both may contribute to the pathophysiology of epilepsy[151,152] and differences in NMDA receptors and kainate receptors in astrocytes are associated with delayed neuronal death in the injured brain – functional astrocyte loss is considered a cause of neuronal death in the injured CNS as vulnerability of astrocytes to NMDA and kainic acid are associated with delayed neuronal death.[153] Partially Ca^{2+}-permeable, kainate receptor activation also results in an influx of Ca^{2+} ions and the resulting Ca^{2+} waves within the astrocytic syncytium may lead to the release of gliotransmitters.[154,155] In hippocampal neurons, released glutamate triggers a slow, transient, current which can activate NMDA receptors[156] and/or activates GluK5-containing kainate receptors on interneurons and increases inhibitory postsynaptic currents.[147]

Delta receptors

GluD1 and GluD2 form this iGluR sub-family because of sequence homology. Recent animal studies show that these receptors are upregulated during postnatal development and play a role at excitatory synapses in neuronal networks in the adult brain.[157] GluD1 is predominately expressed in the inner ear and is important for high-frequency hearing and GluD2 is predominately expressed in cerebellar Purkinje cells and is important for motor coordination and motor learning. GluD2 contribute to synaptic formation through interaction with cerebellin precursor protein 1 and presynaptic Neurexin. The latter binds to the N-terminal domain of GluD2 and regulates formation and maintenance of parallel fiber-Purkinje cell synapses. Both receptors are widely expressed in other brain regions.[158-161]

These delta receptors possess functional ion channel pores, but ligand binding at the ligand-binding domain may only result in desensitization-like conformation channels. Receptor domains, except the ligand-binding domains, of GluD1 and GluD2 are capable of ligand-gated ion channel function.[162] GluD1 regulates the connectivity of parallel fiber-interneurons synapses and this results in differentiation and/or survival of molecular layer interneurons. This is done with the help of GluD2 and results in cerebellar synaptic wiring.[163,164] Glutamate binding to mGlu1 has also been shown to result in GluD2 ion channel activity and contributes to slow excitatory postsynaptic currents in Purkinje cells.[165] An allosteric interaction between amino-terminal domain and ligand-binding domain layers for GluD2 is thought to be due to anchoring of GluD2 to the Nrx-Cbln1 complex which limits motion of the amino-terminal domain layer and allows D-serine-induced conformational changes to be transmitted to the TMD and C-terminal domain.

Glutamate G-protein-coupled receptors

As the most abundant receptor gene family in the human genome, GPCRs are membrane bound proteins activated by extracellular ligands through interactions with G proteins – a family of proteins that act as molecular switches inside cells and are regulated by factors that control their ability to bind to and hydrolyze guanosine triphosphate to guanosine diphosphate. Conformational changes of the GPCR result in activation of the G protein which is composed of a heterotrimeric complex of α, β, and γ subunits. Activation of G protein subunits then modulates the function of effector molecules. The GPCR family has several subgroupings, the majority of classical neurotransmitter GPCRs belonging to family A. mGluRs belong to Class C GPCRs which also includes GABAB receptors, calcium-sensing receptors, pheromone receptors, and taste receptors.[166,167]

Eight mGluR subtypes have been identified and are subclassified into three groups based on sequence homology, G-protein coupling, and ligand selectivity. mGluRs 1 and 5 are in Group I, mGluRs 2 and 3 in Group II, and mGluRs 4, 6, 7, and 8 in Group III. Groups I and II are present in neurons with mGluR3 and mGluR5 also being present in astrocytes. Group III mGluR are not present in astrocytes, are predominantly presynaptic, and mGluR6 expresses its effects in retina. Signaling pathways of Group I mGluR include phospholipase C stimulation, stimulation of adenylyl cyclase, and MAP kinase phosphorylation, that of Group II include inhibition of adenylyl cyclase, activation of K^+ channels, and inhibition

of Ca^{2+} channels, and that of Group III include inhibition of adenylyl cyclase, activation of K^+ channels, and inhibition of Ca^{2+} channels (The mGluR6 in the retina initiates a signal transduction cascade used by depolarizing bipolar cells to convey the light-induced decline in photoreceptor glutamate release to retinal ganglion cells). mGluRs contain a large extracellular N-terminal domain, the Venus flytrap domain (VFD), has two lobes and bind glutamate in a cleft between them. This dimer may be open-open (inactive), open-closed, or closed-closed. The latter two are induced by binding of ligand. VFD's can also bind divalent cations which can potentiate or activate the receptor. The signal induced by ligand binding is transmitted from the VFD's through cysteine-rich domains. A disulfide bridge linking the VFD and cysterine-rich domain may be required for propagation of signals induced by agonist binding to mGluRs. The heptahelical domain of the mGluRs may regulate G protein coupling specificity. Modulators of mGluRs affecting glutamate activity bind within the hepatohelical domain and may positively or negatively influence glutamate activity. Modulation of G protein coupling also occurs at the C-termini of mGluRs.[167]

Activation of mGluR by glutamate may require binding of both protomers, may depend on the mGluR group, and result in Ca^{2+} mobilization and activation of protein kinase C. Ion channels on the plasma membrane are indirectly activated through a signaling cascade involving G proteins. Binding of glutamate to mGluR1 results in an increase in Ca^{2+} in the cytoplasm, that of mGluR5 release of K^+ from the cell by activating K^+ channels, that of mGluR2 and mGluR3 result in inhibition of adenylyl cyclase causing shutdown of the cAMP-dependent pathway and thereby decreasing the amount of cAMP, and that of mGluR4, mGluR6, mGluR7, and mGluR8 result in activation of Ca^{2+} channels allowing more Ca^{2+} to enter cells.[168-172]

Glutamate transporters

Excitatory signaling, neurovascular coupling, glutamate/metabolic pathways, glycolytic enzymes/glycolysis, mitochondria/oxidative phosphorylation, glycogen phyosphorylase/glycogen synthetase, Na^+/Ca^{2+} exchangers, and Na^+/K^+-ATPase depend on glutamate transporters that are present on astrocytes and neurons. Critical for normal brain function, the family of brain glutamate transporters includes excitatory amino acid transporters 1 to 5 (EAAT1–5). All transport L-glutamate and, during one transport cycle, the transporter binds one extracellular molecule of glutamate, three sodium ions, and one proton. Conformational changes result in these substrates being released into the cytoplasm. To complete the cycle, a potassium ion in the cytoplasm is bound to the transporter and conformational changes result in the potassium being released into the extracellular space. EAAT's can also function as chloride channels and may be considered inhibitory glutamate receptors.[173-175] EAAT1 (GLAST) and EAAT2 (GLT-1) are mainly located on astrocytes. EAAT3–5 are exclusively neuronal.

Synaptic transmission is dependent on extracellular glutamate concentration. Impaired GLT-1 results in an increase in extracellular glutamate levels and, in GLT-1 knockouts, result in hyperactivity and severe seizures.[176,177] In animal models, astrocytic GLT-1 and neuronal GLT-1 are different and abnor-

malities result in distinct clinical patterns.[177] GLAST-deficient mice display longer seizure duration. Human mutations are associated with ataxia and seizures.[178-180] While neuron-specific glutamate transporters are not important for survival, behavioral abnormalities have been observed.[181,182] Although diffusion is the major mechanism governing synaptic glutamate concentration, glutamate transporters are also key regulators of synaptic transmission and termination of transmission. Glutamate existence in the synaptic cleft is short, about 1.2 ms, and concentration is high, 1 mM. While AMPA- and NMDA-glutamate receptors become desensitized with prolonged exposure to the neurotransmitter, glutamate transporters actively control duration of excitatory transmission by competing for glutamate in the synapse and is related to astrocytic coverage of synapses – controls presynaptic glutamate receptor activation through glutamate uptake. Critical during high activity and/or multisynapse transmission, glutamate transporters control glutamate receptor activation and spillover of glutamate between synapses and, thus, maintain specificity of synaptic transmissions.

WHAT HAPPENS WITH TOO MUCH GLUTAMATE IN THE CENTRAL NERVOUS SYSTEM?

Glutamate excitatory signaling is needed for most motor, sensory, and autonomic processing, memory formation, and normal brain development – physiologic activity being determined by the balance of synaptic excitation and inhibition (the E/I balance). Glutamate, the predominant excitatory neurotransmitter in the CNS, is normally restrained by inhibitory mechanisms, but an increase in the number of glutamate synapses, enhanced glutamate synaptic function, and/or increased extracellular glutamate concentrations result in an imbalance and the excess glutamate effect may result in massive neuronal death and brain damage due to excitotoxicity. Control of glutamate clearance which results in low extracellular glutamate and less excitotoxicy is an important function of astrocytes.[183] Astrocytes have receptors regulated by the neurochemical environment that allow them to determine neuronal transmission. Activation of these receptors results in changes of ion concentrations (mainly Ca^{2+} and Na^+) in the cytoplasm of the astroglia which regulate astroglial functions and act as a substrate for astroglial excitation.[184-188] Complex interactions between the blood vasculature, interposing cerebrospinal fluid, and surrounding interstitial fluid are facilitated by astrocytes as well and influence neurotransmitter concentrations.[188] Glutamate homeostasis determined by astrocytes in these dynamic processes is not well defined.

Although synaptogenesis and synaptic maturation proceed through formation of an initial contact between the terminal and postsynaptic neuron, maturation of the synapse, stabilization and maintenance, and elimination, survival time and contribution of synapses varies. Control of extracellular glutamate and neuronal glutamate pools by astroglia regulate extracellular glutamate concentration by balancing uptake through Na^+-dependent astroglia-specific glutamate transporters (SLC1A3/EAAT1 and SLC1A2/EAAT2) and release mainly through the cysteine-glutamate exchanges of systemic Xc- (SLC7A11). The astroglial cradle regulates these processes

and isolates synapses within the territory of a single astrocyte and prevents neurotransmitter spill-in and spill-over. While point-to-point transmission of glutamate across a synapse has been the conventional view of glutamate neurophysiology, glutamate spillover due to escape of glutamate from the synaptic cleft results in glutamate accumulating in the extrasynaptic space as well. Glutamate concentrations vary greatly between plasma, cerebrospinal fluid, intracellular fluid, and extrasynaptic fluid. Computational models suggest that extracellular glutamate concentrations are regulated differently in the synaptic, perisynaptic, and nonsynaptic subcompartments and regulates or results in pathology of synaptic transmission, synaptic plasticity, synaptic crosstalk, nonsynaptic neurotransmission, neuronal survival, gliotransmitter release, and/or hemodynamic responses.[189-193]

E/I imbalance contributes to acute and chronic CNS neurodevelopmental and neurologic disorders. If extracellular glutamate rises to above normal, glutamate receptors are overactivated and intracellular reactions in the postsynaptic neurons result in cell injury and possibly death due to excitotoxicity. Astroglial glutamate transporters coordinate this excitatory signaling and brain energetics. These transporters co-localize with, form physical interactions with, and couple to energy-generating systems (Na^+/K^+-ATPase, Na^+/Ca^{2+} exchanger, glycogen metabolizing enzymes, glycolytic enzymes, and mitochondria/mitochondrial proteins). Glutamate uptake regulates these processes and the process may regulate glutamate uptake.[191] Transporters remove the extra extracellular glutamate *via* a sodium-potassium couple uptake mechanism. If not removed, excess glutamate has been associated with neural damage and results in high levels of Ca^{2+} to influx into the postsynaptic cells. Cell degradation due to proteases, lipases, nitric oxide synthetase, and enzymes that affect cell structures results. A cycle of positive feedback cell death can lead to neurodegeneration. Oxidative stress is also affected. High synaptic glutamate levels reverse cysteine/glutamate antiporter (transports cysteine into the cell and glutamate out). Glutathione, an antioxidant, is not synthesized at the appropriate levels. This leads to more ROS that injure the cells which then cannot process glutamate. Nitric oxide synthase is also activated by the high concentration of glutamate with a resulting over production of nitric oxide – damages mitochondria. Astrocyte homeostasis of glutamate is important in regulating these complex interactions.[181-185]

POSSIBLE BIOCHEMICAL EFFECTS OF CARBON MONOXIDE ON ASTROCYTE GLUTAMATE HOMEOSTASIS

CO is a gasotransmitter: 1) It is a small molecule of gas; 2) It is freely permeable to membranes; 3) It can have endocrine, paracrine, and autocrine effects; 4) It is endogenously and enzymatically generated and its production is regulated; 5) It has well defined and specific functions at physiologically relevant concentrations; and 6) Its cellular effects may or may not be mediated by second messengers, but have specific cellular and molecular targets. CO is cytoprotective and at least 86% originates from heme metabolism. Heme-independent sources include auto- and enzymatic-oxidation of phenols, photo-oxidation of organic compounds, iron-ascorbate catalyzed

lipid peroxidation of microsomal lipids and phospholipids, and reduction of cytochrome b5. Endogenous CNS CO is critical for the normal functioning, neuroprotection, and neurorepair of the brain. Synthesis, transactions, and disposition are clinically relevant for neurodevelopment, neuropathology, and neurotherapy. A neurotransmitter, endogenous CO is dependent on brain heme oxygenase isozymes, heme oxygenase-1 (HO-1) (primarily localized in the endoplasmic reticulum) and heme oxygenase-2 (HO-2) (primarily anchored to the endoplasmic reticulum). These catalyze the first and rate-limiting step in the degradation of heme to iron, biliverdin, and CO. CNS cytoprotective effects are attributed to CO[194] and are mimicked by low dose exogenous CO (inhaled CO and CORM's). Increased CO production by upregulation of heme oxygenases has been targeted for neuroprotective and neurotherapeutic effects and exogenous administration of CO or CORM's and/or targeting of heme oxygenases to increase the production of CO have been suggested as treatment for multiple CNS disease processes.[195-205]

CNS CO signal transduction pathways result in vasoactive, antithrombotic, anti-proliferative, neurotransmission, anti-inflammatory, and/or anti-apoptotic effects.[206] CO interaction with soluble guanylate cyclase (sGC) *via* the sGC/cGMP pathway is associated with vascular effects (platelet aggregation, dilation, profibrinolysis, and anti-inflammation) and downregulation of cell proliferation. In the latter, CO upregulates the p38 mitogen activated protein kinase (p38β MAPK) and p21 resulting in downregulation of cell proliferation. CO effects on p38β MAPK affect apoptosis (through antiapoptotic genes and caspase activation) and inflammation (through heat shock factor-1, interleukin-10, interleukin-1β, interleukin-6, tumor necrosis factor-α, and macrophate inflammatory protein1β).[206] Induction of the heme oxygenases has resulted in elevated CO levels and are thought to be neuroprotective as well as neurotherapeutic.

As astrocytes are critical for the homeostasis of glutamate and resultant excitotoxicity and neuropathology, further evaluation of CO effects on astrocytes and the biophysiological interactions affecting glutamate homeostasis is needed. CNS astrocytes are now targeted for treating neurological disorders as non-neuronal cells (which include astrocytes) outnumber neurons, vesicle-based release of transmitter is present in astrocytes although the kinetics is slower than in neurons, and astrocytes contain glycogen which is converted into L-lactate, a fuel and signalling molecule important to cognition and neuroprotection and an alternative energy source during excitotoxic brain injury. CO is thought to increase L-lactate.[207] Exposure of cortical neurons to glutamate increases HO-2 activity and CO production. Upregulation of CNS HO-1 also results in an increase of CO. Astrocyte heme oxygenases can also be upregulated thereby increasing the production of CO. Diffusion of the gas to different cell types and cell organelles result in effects on biochemical pathways. The effects of CO on astrocyte homeostasis of glutamate and prevention of excitotoxicity with resultant neuropathology, most likely, involve multiple pathways as well as intra and extracellular structures that result in communication between cells and within cells.[206,208-216] Important for CNS homeostasis, astrocyte glutamate metabolic pathways, uptake of glutamate by astro-

cytes, and release of glutamate from astrocytes are affected by the presence or absence of CO.

Glutamate metabolic pathways
Cellular energy

Mitochondrial energy production is modulated based on cellular needs and bioenergetic constraints. ATP is responsible for most of the energy in cells. Mitochondrial respiration determines, to a large extent, this cellular energy, depends on oxidative phosphorylation (consists of the electron transport chain (ETC) and ATP synthase), and plays an important role in energy production and apoptosis.[217-219]

Regulation of the mitochondrial ETC and ATP synthase (result in the majority of cellular energy through cell signaling) limit reactive oxygen species (ROS) responsible for cell damage and the triggering of death processes, oxidative phosphorylation activity, mitochondrial membrane potential hyperpolarization, release of CytC, formation of the apoptosome, and execution of apoptosis *via* caspase activation. Bioenergetic defects, respiratory chain-induced oxidative stress, defects of mitochondrial dynamics, increased sensitivity to apoptosis, and the accumulation of damaged mitochondria with unstable mitochondrial DNA have been associated with energy defects. Mitochondria regulate Ca^{2+} signals and ionic homeostasis, synaptic development, blood flow, glucose metabolism, glutamate clearance, plasticity, proliferation, coordinate local metabolism, provide energy, are dynamic, and integrate survival and death biochemical changes. There is extensive fission and fusion of these organelles, directed movement within cells, and controlled degradation. The former is critical for development of the CNS and determine the shape, connectivity, distribution, and density of mitochondria within neurons and, probably, astrocyte processes. In astrocytes, mitochondria are seen within the finer processes of the cell, movement is regulated, and they contribute to local Ca^{2+} signaling within the astrocyte. Protoplasmic astrocytes have highly branched processes and veil-like structures resulting in a very large surface area to volume ratio and intimate association with synapses (may exceed 2 million synapses in the human brain).[220] Astrocyte mitochondria are mobile (arrested by glutamate and Ca^{2+}), change speed and direction, and movement depends on adaptor proteins and motor proteins.[221-227] Mitochondrial movement in the astrocyte promotes distribution of mitochondria into cellular processes, allows retrograde movement out of the cell processes and into the cell body where degradation of mitochondria takes place, improves fusion and fission processes which allow exchange of proteins and genetic materials, promotes the regulation of transfer of mitochondria between adjacent cells (horizontal transfer), and redistribution of mitochondria to sites needing increased energy production is facilitated.[228-232]

Astrocyte mitochondrial presence and activity are related to neuronal activity and, especially, glutamatergic activity. Inhibition of neuronal activity increases activity of mitochondria in astrocytes. Mitochondria are not uniformly distributed in astrocytes and their location at synapses and active regions is associated with neuronal activity – about 15% of the astrocytic mitochondria are mobile. The addition of glutamate decreases the percentage of mobile astrocyte mitochondria in prepara-

tions. Glutamate, considered to be partly responsible for the mobility of astrocyte mitochondria, is cleared into astrocytes *via* GLAST and GLT1 (EAAT1 and EAAT2). These catalyze glutamate and aspartate transport against a concentration gradient with three Na^+ ions and a H^+ with the counter-transport of a K^+ ion. Na^+/K^+ ATPase is activated which results in reversal of the Na^+/Ca^{2+} exchanger leading to increases in Ca^{2+}.[233-235] Mitochondria movement is regulated by glutamate uptake and is directly related to the concentration of Ca^{2+}. Inhibition of glutamate uptake increases mobile mitochondria in astrocyte processes. The movement of astrocyte mitochondria depends on cellular transport machinery, adaptor proteins that link mitochondria to motor proteins. Mitochondrial Rho proteins and trafficking kinesin binding proteins are thought to be important in the positioning of mitochondria to active processes in astrocytes.[236-238] Mitochondrial Rho proteins respond to Ca^{2+} signaling – Ca^{2+} results in the arrest of mitochondria within astrocyte processes in response to neuronal activity and glutamate.[226,227,237] Under normal conditions, mitochondria approach endoplasmic reticulum allowing Ca^{2+} flow between organelles – close apposition is needed. Formation of tethers that link the mitochondria with endoplasmic reticulum may be dependent on Miro proteins[238-240] and affect other cellular processes.

CO targets mitochondria, including astrocyte mitochondria, at several levels: 1) Mitochondrial ROS, signaling molecules for CO-induced pathways, are increased with physiological amounts of CO; 2) CO limits mitochondrial membrane permeabilization and thereby inhibits the release of pro-apoptotic factors into the cytoplasm; 3) CO increases the ability of the mitochondria to take up Ca^{2+}; 4) CO modulates mitochondrial metabolism by increasing TCA cycle rate, oxidative phosphorylation and mitochondrial biogenesis which increases ATP; and 5) CO prevents excitotoxicity-induced cell death and modulates cell proliferation by limiting the activity of T-type and L-type Ca^{2+} channels.[241-246] CO, therefore, affects ROS signaling and anti-oxidation, Ca^{2+}-induced mitochondrial membrane permeabilization, programmed cell death, Ca^{2+} buffering, and metabolism. CO targets cyclooxygenase (COX), the final electron acceptor of the mitochondrial electron transport chain – catalyses oxidation of ferrocytochome c by gaseous oxygen. At high levels, CO results in high levels of carboxyhemoglobin which exceeds the limit of toxicity. However, at lower levels, COX-specific activity is dependent on oxygen concentration and may activate hypoxia-inducing factor-1 which is involved in the regulation of COX subunits for optimization of mitochondrial respiration. Hypoxia and CO presence have a synergistic effect on COX inhibition when the ETC is in a more reduced state. Low concentrations affect the coupling between ATP production and respiration. Cytoprotection is associated with mitochondrial mild uncoupling stimulation thus decreasing mitochondrial ROS production and is dependent on its concentration – at lower concentrations, increased respiratory control ratio and mitochondrial transmembrane potential are seen whereas, at higher concentrations of CO, a decrease in respiratory control ratio and mitochondrial membrane potential is seen. Brief exposure of astrocytes isolated from cortex increased cellular oxygen consumption with an improvement of mitochondrial respiratory chain and

oxidative metabolism. CO also stimulates mitochondrial bio-genesis, important for cell metabolism and cell protection, and regulates mitochondrial respiratory complexes.[247-252]

Changes in astrocyte intracellular calcium occur with exposure to CO and/or glutamate. Two different pathways have been identified. With glutamate, the metabotropic response results in an initial Ca^{2+} spike that can propagate rapidly from cell to cell and probably involves IP3. This can produce oscillatory intracellular waves that propagate within cells and are sustained only if external Ca^{2+} is present. The second response, the ionotropic response, results in sustained elevation in Ca^{2+} and is associated with receptor-mediated Na^+ and Ca^{2+} influx, depolarization, and voltage-dependent Ca^{2+} influx. CO, involved in Ca^{2+} signaling and in the modulation of Ca^{2+} channels, inhibits L-type Ca^{2+} channels and may be cytoprotective. This has not been demonstrated in astrocytes. CO has also been implicated in preventing activity of T-type Ca^{2+} channels, and regulation of intracellular organelle Ca^{2+} stores. The direct effect of CO on Ca^{2+} signaling in the astrocyte mitochondria is not known, but glutamate-induced calcium signals stimulate CO production in astrocytes.[235,246,253-256] Further studies are needed to evaluate the role of CO in mitochondria and glutamate homeostasis.

Mitochondrial glutamate versus cytosol glutamate and carbon monoxide

Transport of keto acids, amino acids, nucleotides, inorganic ions, and co-factors across the mitochondrial inner membrane is dependent on the mitochondrial carrier family - mitochondrial glutamate pathways being controlled by mitochondrial enzymes and transmembrane carriers (especially GDH and mitochondrial glutamate carriers, respectively). Biochemical pathways between the cytosol and the mitochondrial matrix result and are essential for synthesis of ATP from oxidation of sugars and fats, synthesis of heme and iron sulphur clusters, production of heat, macromolecular synthesis, and the synthesis, degradation, and interconversion of amino acids. Regulation of GDH activity has been suggested as a neurotherapeutic approach – neuronal mitochondrial activity is improved and excitotoxic risk reduced with increased GDH activity.[256] Similar studies on astrocytes have not been done. The mitochondrial glutamate carriers, also important to glutamate homeostasis, consist of six trans-membrane α-helices and three matrix helices arranged with threefold pseudo-symmetry – substrate binding site is accessible from the intermembrane space of the mitochondrion. Required for normal CNS function, two aspartate-glutamate carriers (AGC: aralar and citrin) – opening and closing of the carrier and substrate binding may depend on disruption and formation of two salt bridge networks – and two glutamate/hydroxyl carriers (GC1 and GC2) are present in astrocytes.

The aforementioned AGC's are classified as strict exchangers or uniporters and are electrogenic.[257] Precursor proteins are synthetized in the cytosol and then are transported to mitochondria membranes. Astrocytes express both aralar and citrin – AGC1 (aralar) is important to glutamate-mediated excitotoxicity in the mature CNS and muscles. Both supply aspartate that is produced in the mitochondrial matrix to the cytosol in exchange for cytosolic glutamate plus a proton[258]

and is important for the control of mitochondrial respiration and calcium signaling and antioxidant defenses. Ca^{2+} on the external side of the inner mitochondrial membrane stimulates transport activity and the Ca^{2+}-binding sites are localized and are independent of Ca^{2+} entry into the mitochondria by calcium uniporter and activates the AGCs from the external face of the mitochondria.[259,260] Glutamate delivered into the mitochondria through AGC's is transaminated with intramitochondrial oxaloacetate to form aspartate and is not available for GDH. GC1 and GC2, unlike the AGC's, function in forward or reverse mode and provide substrate for GDH. GC1 and GC2 are equally expressed in the brain. GC's are important in ureogenesis, glutamate derived from GC's produce NH_3. Direction of transport of glutamate is dependent on the energy state of the mitochondria and is controlled by mitochondrial metabolism.

Aspartate, important to the homeostasis of astrocyte glutamate biochemical pathways, can only exit the mitochondria in exchange for glutamate. The former is expressed in the mitochondrial matrix of astrocytes. Normally, astrocytes metabolize glutamate *via* cytosolic GS resulting in glutamine.[259] When extracellular glutamate exceeds 0.5 mM, the amount metabolized *via* the TCA cycle in the mitochondria increases significantly and, if energy is needed, glutamate is metabolized primarily by GDH – glutamate enters the TCA cycle as α-ketoglutarate after deamination catalyzed by GDH and, ultimately, catabolism of glutamate to CO_2 with the production of ATP. Transport of glutamate from the cytosol across the mitochondrial membrane is by the AGC and the GC - AGC requires aspartate in exchange of glutamate and GC glutamate is transported with a proton. AGC requires a concentration gradient of solutes and/or electrochemical potential across the inner mitochondrial membrane and is a Ca^{2+} sensor[259] which may be involved in Ca^{2+} signaling. In addition to their role in glutamate and aspartate exchange, AGC 1 (Aralar1) and AGC 2 (citrin carriers) are part of the malate-aspartate shuttle that is needed for transfer of reducing equivalents from cytosolic NADH into mitochondria (and activates ETC) leading to ATP generation.

Calcium signaling is necessary to the function of GDH and the mitochondrial glutamate carriers. Matrix Ca^{2+} is known to be important to mitochondrial function and the regulation of glutamate transport by extramitochondrial Ca^{2+} may further determine their roles. AGC's are activated by low extramitochondrial levels of Ca^{2+}. CO affects Ca^{2+} concentrations and, therefore, probably GDH and mitochondrial glutamate carriers. Further studies are needed.

Precursors and intermediates

In the CNS, neurovascular coupling regulates energy supply required for fluctuating energy needs. Astrocyte-neuron metabolic cooperation result in spatial and temporal delivery, production, utilization, and storage of energy for the brain – astrocytes, glutamate, and CO are essential to this process. Astrocytic processes cover approximately 63% of capillaries, allow detection of neuronal activity (and, therefore, energy requirements), and affect local vascular supply which increases or decreases availability of energy substrates. Afferent activity results in release of excitatory neurotransmitters from presynaptic sites and results in an increase of astrocyte glutamate.

Glutamate stimulates CO production by astrocytes which is needed for activating transient K_{Ca} currents and single K_{Ca} channels in myocytes in contact with astrocytes resulting in dilation of the vessel, increase in blood flow, and subsequent increased availability of energy sources when needed.[261-263] While glucose has been considered the obligatory energy substrate in adult brains *via* oxidative metabolism, other substrates from the blood (ketones, lactate, *etc*.) are also used as energy sources and may be preferred and are transported into both neurons and astrocytes. Nonoxidative metabolism for energy production may be important as well.[264-266] The metabolism of glucose to CO_2 and water may or may not result through glycolysis, the pentose phosphate pathway, and/or glycogenolysis as metabolic intermediates can also be oxidized as an energy source (*i.e.* lactate, pyruvate, glutamate, or acetate).

Glycogen, an energy substrate found predominantely in astrocytes, is broken down to lactate and is affected by neurotransmitters and glucose concentration and provides some neuroprotection during hypoglycemia and high levels of CNS activity. Lactate is considered to be neuroprotective and may be the preferred CNS energy substrate. Interestingly, glutamate stimulated CO production by astrocytes with intact heme-oxygenase 2 also affects L-lactate levels in astrocytes suggesting an association between L-lactate and heme oxygenase neuroprotective systems. The cell specific metabolic profile of astrocytes is different from that of neurons and there are different expressions of enzymes that regulate cell metabolism including glycogenolysis and glycolysis. Intercellular lactate shuttles transferring lactate from lactate-producing cells to lactate-consuming cells exist between astrocytes and neurons – a lactate gradient from astrocytes to neurons allows for a carrier-mediated lactate flux from astrocytes to neurons and astrocyte-derived lactate can be used as an energy substrate for neurons.[267,268] Anaerobic glycolysis results when pyruvate is acted on by lactate dehydrogenase to form lactate and aerobic glycolysis when a cell specific gene expression profile that affects conversion of pyruvate to lactate as opposed to pyruvate being used in the TCA cycle. Transport of glucose across the blood brain barrier is ten times higher for glucose than for lactate and astrocytes (in an activity dependent manner) determines CNS lactate formation by an uncoupling between glycolysis and the TCA cycle. Studies are needed to further define the role of lactate in neuroprotection, effect of CO on production of lactate and other metabolic substrates in astrocytes, use of lactate as an energy substrate by astrocytes and neurons, transport of lactate from astrocytes to neurons, and metabolism of various metabolic substrates to lactate.

Uptake of glutamate by astrocyte

Failure of sodium homeostasis, due to reduced ATP, results in extra- and intracellular changes of potassium, calcium, and protons and, therefore, changes in excitotoxicity. Astrocyte metabolism and sodium homeostasis is dependent on glutamate uptake and is critical to maintaining sodium homeostasis and energy levels of surrounding neurons. A major determinant of uptake of glutamate by astrocytes is the transmembrane Na^+ gradient controlled by NaK-ATPase (NKA). The latter has three subunits – α, β, and γ with 4 isoforms of the α subunit. Astrocytes express α1 and α2 subunits and, in the brain, the

α2 isoform is mainly expressed in astrocytes. CO regulates NKA through cGMP and protein kinase G (PKG) and glutamate regulates CO through mGluRs.[263,265] NKA has been estimated to be responsible for nearly 50% of the cellular ATP hydrolysis linking cellular sodium regulation and energy metabolism in the CNS. Decreased NKA activity results in energy deficiency and has been associated with neurodegenerative diseases.[265-267,269-273] Upregulation or downregulation of astrocyte production of CO to affect sodium homeostasis and energy levels of neurons needs further evaluation as the interactions between astrocytes, glutamate, and CO directly affects neurons, CNS health, and pathologies of the CNS.

Release of glutamate from astrocyte
Ca^{2+}-dependent exocytosis

Exocytosis of glutamate, one of the mechanisms of astrocytic glutamate release, is largely dependent on soluble N-ethylmaleimide-sensitive-factor attachment protein receptor proteins (regulators of exocytosis) and is Ca^{2+}-dependent. Extracellular signaling molecules result in an increase in Ca^{2+} concentration in the cytosol and, triggered by an increase of cytosolic Ca^{2+} (mainly from endoplasmic reticulum stores), exocytosis of glutamate results with the merger of gliosignal-containing vesicles with the plasma membrane and membrane associated molecules.[274-278] Released astrocytic glutamate reacts with receptors on synaptic terminals and excitability of neurons is seen. Mitochondria have a Ca^{2+} uniporter that transports Ca^{2+} into the mitochondrial matrix when cytosolic Ca^{2+} is greater than about 0.5 μM which, when blocked, increases cytosolic Ca^{2+} and glutamate release.[241,279] CO decreases the activity of L- and T-type Ca^{2+} channels, probably affects Ca^{2+} signaling between endoplasmic reticulum and mitochondria, regulates the mitochondrial calcium uniporter and regulates mitochondrial Ca^{2+} concentrations.[241-243] Ca^{2+} regulation by CO, therefore, affects Ca^{2+}-dependent exocytosis and requires further study.

Intracellular calcium concentration

Astrocytes, activated during physiological functioning of the CNS, release glutamate in response to an increase in Ca^{2+}, but Ca^{2+} concentration in astrocytes does not result in on/off responses but rather in different and graded functions depending on the need of the cell - the type of neuronal input is important to the type of astrocytic Ca^{2+} response. Reactions vary in the different organelles. Ca^{2+} enters the cell through Ca^{2+} channels in the plasma membrane or store-operated Ca^{2+} channels. Sources include the extracellular space and the endoplasmic reticulum/sarcoplasma reticulum. Mitochondria Ca^{2+} uptake is efficient and is mainly *via* a mitochondria Ca^{2+} uniporter (MCU or CCDC109A).[278,279] Ca^{2+} crosstalk with the plasma membrane is important for cellular functions. Within mitochondria, Ca^{2+} activates three dehydrogenases (pyruvate dehydrogenase, oxoglutarate dehydrogenase, and isocitrate dehydrogenase) in the mitochondrial matrix, stimulates production of ROS, results in opening of mitochondrial permeability transition pores and possibly regulates Fl-Fo ATP synthase.[280-283] Efflux of the ion from mitochondria is primarily determined by Na^+-dependent and Na^+-independent pathways. Astrocyte mitochondrial Na^+-Ca^{2+} exchanger is important for store-operated Ca^{2+} en-

try indicating communication between mitochondria and the plasma membrane. CO is essential to the observed effects of intracellular Ca^{2+} concentration on cellular functions – CO stimulates astrocytic mitochondrial biogenesis *via* L-type Ca^{2+} channel-mediated peroxisome proliferator-activated receptor-gamma coactivator -1alpha/estrogen-related receptor α activation and through potentiation of L-type Ca^{2+} channel activity increases hypoxia-inducing factor-1α-independent vascular endothelial growth factor expression *via* an AMP-dependent protein kinaseα/sirtuin1-mediated peroxisome proliferator-activated receptor-gamma coactivator -1alpha/estrogen-related receptor α axis.[283,284] Other effects of CO on intracellular Ca^{2+} need further evaluation.

Potassium channels

Astrocytes may serve as excitatory interneurons that affect synchrony of a set of neurons.[45,47] Modulation of excitability and synchronization of neuronal activity and network oscillations through the effect of K^+ may be partially regulated through Ca^{2+}-dependent release of glutamate (activate extrasynaptic NMDA receptors) that depends on K^+. Extracellular K^+ concentration and astrocyte depolarization also affect glutamate uptake through glutamate transporters – inverse glutamate uptake and vesicular glutamate release may affect synchrony of neurons and is affected by K^+ buffering by astrocytes.[285,286] Uptake of excess extracellular K^+ released during neuronal discharge is important to the regulation of extracellular K^+ that regulates neuron membrane potential and subsequent excitability. Diverse astrocyte K^+ channels mediate these K^+ currents.

Astrocytes express various K^+ channels that depend on CNS development and region of the brain with specific subcellular types that include inward rectifying K^+ (Kir) channels (Kir4.1, Kir5.1, and Kir6.1 present in astrocytes), two-pore-domain K^+ (K_{2P}) channels (TASK1, TREK2, TREK1, and TWIK1), Ca^{2+} activated K^+ channels, and Kv channels (Kv1 to Kv12 which include channels affected delayed rectifier (K_{DR}) and transient (K_A) currents).[285-290] The channels are involved in processes that depend on transmembrane voltage, *i.e.*, Kir4.1 sets the resting potential of astrocytes and is responsible for the large inward currents recorded at negative membrane potentials, TASK1 may inhibit cellular swelling under ischemic conditions,[291] BK channels depend on membrane potential and intracellular Ca^{2+} concentration, and Kv channels are expressed perinatally. Astrocyte K^+ conductance is dominated by the Kir channels.[292-297]

Outside of the CNS, CO interacts with K^+ channels.[298,299] In vascular smooth muscle K (Ca) channels are activated and a specific target of CO is the histidine residue. Activation of ATP-sensitive K^+ channels results in the peripheral antinociceptive effect of the HO/CO pathway. CO inhibits the voltage-gated K^+ currents in the medulloblastoma cell line DAOY and reverses the oxidant-induced increase in K^+ activity.[300] CO results in inhibition of recombinant Kv2.1 expressed in HEK293 cells. These K^+ channel effects have not been studied in astrocytes and deserve further evaluation.

Sodium dependent glutamate transporters

Astrocytic, not neuronal, glutamate transporters are important to maintaining extracellular glutamate concentrations,

are needed for brain development through regulation of extracellular glutamate concentrations, and modify metabolic communications between neurons and astrocytes. The majority of these physiologic processes (neurovascular coupling, excitatory signaling, Na^+/Ca^{2+} exchangers, Na^+/K^+ ATPase, glycogen phosphorylase/glycogen synthase, mitochondria/oxidative phosphorylation, glycolytic enzymes/glycolysis, and glutamate/metabolic pathways) are mediated by GLT-1 and GLAST (EAAT1 and EAAT2) – astroglial glutamate transporters – and co-localize with, form interactions with, and couple to energy-generating systems. Primary functions of EAATs (sodium-dependent glutamate transporters) include maintaining extracellular glutamate concentration in the low micromolar range which allows glutamate to be used as a signaling molecule while preventing cytotoxic effects and controlling antioxidant defenses and metabolic roles of transported substrates. Binding of glutamate is rapid and buffering after entry into the extracellular space results. Responses are dynamic and depend on the location of the transporters. Uptake of glutamate is *via* co-transport of three sodium ions and one proton with counter-transport of one potassium ion. Energy is released and active transport of glutamate results. If extracellular glutamate uptake by astrocytes is reduced, excitotoxicity occurs. After uptake, glutamate is directed into different enzymatic pathways and may result in energy production and glutamine production. Activity of EAATs is closely coupled to glutamine release by astrocytes.[301-305]

Astrocyte GLAST and GLT-1 are responsible for the clearing of extracellular glutamate. In a hippocampal astrocyte culture from adult and aged rats, decreases in GLAST and GLT-1 were associated with decreases of nuclear factor E-2 related factor 2 (Nrf2) – regulates the transcription of HO-1 and, therefore, CO production which probably mediates neuroprotection.[306-309] Further studies are needed to evaluate the relationship between Nrf2, HO-1, CO, GLAST and GLT-1.

Glutamate-gated ion channel receptors

Astrocyte glutamate signaling follows binding of glutamate to iGluRs and/or mGluRs and can lead to calcium signaling and subsequent glial excitability. Although the former is ubiquitously expressed in all neural cell types, iGluR subunit composition (18 iGluR receptor subunits) and subtypes differ between species, CNS cell types and brain areas, and change during development. Presence is not all or none, but rather quantitative, and developmental patterns are unique. A primary function is sensing and responding to neuronal release of glutamate – differences exist between astrocytes, types of iGluRs present, area of the brain, and other biochemical reactions present in the area. Heterogeneity in the function of iGluRs may result from differential expression of subtypes. AMPAR are mainly responsible for fast excitatory synaptic transmission and synaptic plasticity, NMDAR for excitatory synaptic transmission, plasticity, and excitotoxicity, and kainate receptors for regulating activity of synaptic networks and synaptic plasticity in the hippocampus and sensory cortex, but delta receptors (belong to the iGluR family because of sequence homology) function as ion channels only after interaction with other types of membrane proteins.[310-317]

Apolipoprotein E (ApoE) affects these iGluR functions.

ApoE, the major apolipoprotein in the CNS interaction with other types of membrane proteins (predominantly synthesized by astrocytes), reduces the level of extracellular glutamate and iGluR activation and, possibly, glutamate induced excitotoxic damage and protects astrocytes from hypoxia and glutamate-induced apoptosis. Nrf2 is increased in the presence of ApoE. The downstream target of Nrf2, heme oxygenase (and, therefore, CO production), is essential for cytoprotection.[318-323] Studies have not been done evaluating the effect of ApoE on astrocyte glutamate and iGluR activation, Nrf2 production, and upregulation of heme oxygenase and CO production and their interrelationships and are needed to further define the role of upregulation of CO by ApoE on neuroprotection.

Calcium permeability of α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid receptors and GluA2

Subunit composition of AMPA receptors is dynamic and changing. Their Ca^{2+} permeability and resulting action potentials are reflected in the output of neurons, normal synaptic functions and disease. Functional differences result in differences in excitatory signaling. Ca^{2+} permeability is related to the GluA2 subunit which may be subjected to RNA editing by the ADAR2 enzyme – edited GluA2 subunits render the AMPA receptor impermeable to Ca^{2+}. Change in number of AMPA receptors and GluA2 inclusion results in a change in AMPA receptor Ca^{2+} permeability and, therefore, synaptic transmission and intracellular signaling. GluA2 mRNA and GluA2 subunit expression are downregulated in neurons prior to cell death. The "GluA2 hypothesis" suggests that reduction of GluA2 results in more Ca^{2+} influx through newly synthesized AMPA receptors and, thereby, results in neurotoxicity secondary to glutamate. Unedited GluA2 results in AMPA receptors that are permeable to Ca^{2+}. Blocking the formation of GluA2-less receptors, blocking GluA2-less receptors, and blocking of resulting apoptosis are approaches used to reduce neurotoxicity. As in neurons, Ca^{2+} influx results in astrocytic Ca^{2+} signaling. AMPA-evoked Ca^{2+} influx into astrocytes has been associated with a weak expression of GluA2.[324-330] CO has a pivotal role in calcium signaling in both neurons and astrocytes. The role of astrocytic GluA2 in glutamate related neurotoxicity and calcium signaling and CO on these interactions needs further study.

Summary

Our understanding of the role of astrocytes in protecting the brain continues to result in methodologies in prevention and treatment of disease processes of the CNS. Astrocyte homeostasis of glutamate is crucial to CNS development, neurophysiology, and neuroprotection and has been targeted for neurotherapy. The role of CO in this homeostatic function is complex and is now being seen as obligatory. Upregulation of heme oxygenases and/or treatment with CO are being suggested as therapies for a wide range of neuropathologies. Limited knowledge of the effects of this important gasotransmitter on CNS biophysiology affects our ability to intervene on our patient's behalf. Research, however, is suggesting that astrocytes, glutamate, and CO are interacting and affect the CNS. Preconditioning with CO may lead to a change in sub-

strate utilization in the brain of newborn piglets undergoing deep hypothermic circulatory arrest.[331] Loss of CO production and pial arteriolar dilation following astrocyte injury suggests that astrocytes may employ CO as a gasotransmitter for glutamatergic cerebrovascular dilation.[320] Inhibition of synaptosomal glutamate uptake by CO in a time-, dose-, and temperature-dependent manner was shown in the striatum and hippocampus.[332] In newborn pigs, the astrocyte component of the neurovascular unit is responsible for the vasodilation response of pial arterioles to topically applied glutamate *via* iGluRs functionally liked to activation of constitutive heme oxygenase.[333] Glutamate elevates Ca^{2+} (i) in astrocytes leading to Ca^{2+}- and calmodulin-dependent HO-2 activation, and CO production.[334] These and other effects of CO on astrocyte homeostasis of glutamate requires further evaluation and delineation. Results may lead to a simpler and better understanding of the role of CO in normal and abnormal astrocyte/glutamate interactions and result in medical interventions that limit or cure disease processes in the CNS.

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