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

Forum Review

Modulation of Astroglial Energy Metabolism by Nitric Oxide

JUAN P. BOLAÑOS[1] and ANGELES ALMEIDA[1,2]

ABSTRACT

Activated astroglial cells produce large amounts of nitric oxide (NO) which, through the binding to soluble guanylyl cyclase, rapidly increases cyclic GMP concentrations. In addition, through the binding with the a–a_3 binuclear center of cytochrome *c* oxidase, NO rapidly decreases the affinity of this complex for O_2, hence reversibly inhibiting the mitochondrial electron flux and ATP synthesis. Despite promoting a profound degree of mitochondrial inhibition, astrocytes show remarkable resistance to NO and peroxynitrite, whereas neurons are highly vulnerable. Recent evidence suggests that the inhibition of mitochondrial respiration by these nitrogen-derived reactive species leads to the modulation of key regulatory steps of glucose metabolism. Thus, upregulation of glucose uptake, the stimulation of glycolysis and the activation of pentose-phosphate pathway appear to be important sites of action. The stimulation of these glucose-metabolizing pathways by NO would represent a transient attempt by the glial cells to compensate for energy impairment and oxidative stress, and thus to emerge from an otherwise pathological outcome. *Antioxid Redox Signal* 8, 955–965.

BACKGROUND

Nitric oxide (NO) plays essential functions in the modulation of vascular tone (61, 88), neurotransmission (49), and the immune system (58). Formed by a family of nitric oxide synthases (NOS), NO mainly binds to soluble guanylyl cyclase and mitochondrial cytochrome *c* oxidase, and thus mediates cellular signaling cascades. The interactions with these targets suggest that NO plays critical pathophysiological roles that only recently are beginning to be understood.

Within the brain, astrocytes play critical roles in brain energy homeostasis, in part because they are strategically localized in the brain network (50). They channel metabolic substrates between the blood and neurons; through their end-feet processes, astrocytes surround blood vessels (80), and can readily take up the glucose arriving from the blood through endothelial cells. Moreover, the astrocytic processes that surround synapses (13) contribute to modulating energy metabolism as a function of neuronal synaptic activity. In view that astrocytes, when activated, become a major source of NO, we shall describe here the cellular consequences derived from the interaction of NO with cytochrome *c* oxidase and other mitochondrial constituents in these cells. We shall study how these interactions indirectly modulate brain energy metabolism under normal and pathological situations.

FORMATION OF NITRIC OXIDE IN NEURAL CELLS

All brain cells are able to synthesize NO (83, 84). In postsynaptic neurons, NO is formed after glutamate-mediated activation of its receptors, mainly the *N*-methyl-D-aspartate (NMDA) subtype. After this activation, Ca^{2+} is transiently increased in the cytosol and forms a complex with calmodulin that binds to—and activates—constitutive neuronal NOS (nNOS or NOS1) (Fig. 1) (66). Glial cells (astrocytes, microglia, and oligodendrocytes) synthesize NO after the transcriptional expression of a calcium-independent inducible NOS isoform (iNOS or NOS2) (Fig. 1). *In vivo* factors that promote iNOS induction encompass a number of inflammatory conditions, including ischemia (5, 78, 84). Experimentally, iNOS induction in glial cells can be promoted by incubation with the endotoxin lipopolysaccharide (LPS) or

[1]Departamento de Bioquímica y Biología Molecular, Universidad de Salamanca/Instituto de Neurociencias de Castilla y León, Centro Nacional de Investigaciones Cardiovasculares, Edificio Departamental, Campus Miguel de Unamuno, and [2]Unidad de Investigación, Hospital Universitario de Salamanca, Salamanca, Spain.

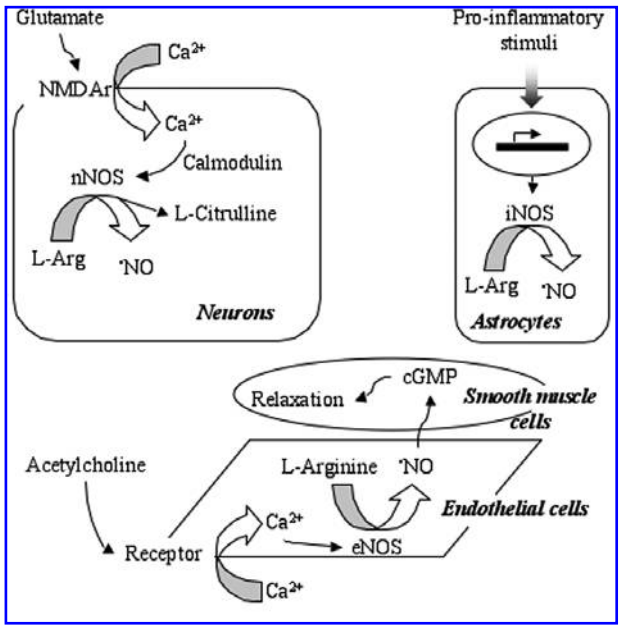

FIG. 1. Nitric oxide (NO) synthesis in brain cells. In neurons, glutamate-mediated *N*-methyl-D-aspartate receptor (NMDAr) stimulation triggers intracellular Ca^{2+} accumulation. Ca^{2+} binds to calmodulin and activates neuronal nitric oxide synthase (nNOS) isoform, which forms NO and citrulline from arginine. In astrocytes, certain pro-inflammatory stimuli promote the transcriptional induction of iNOS (inducible isoform), which forms large amounts of NO in Ca^{2+}-independent manner. A similar mechanism operates in microglial cells (not shown). In blood vessels, acetylcholine stimulates its receptor placed in the membrane of the endothelial cells, promoting intracellular Ca^{2+} accumulation and activation of endothelial NOS isoform (eNOS). eNOS-mediated NO is released to the neighboring smooth muscle cells, herein inducing cyclin GMP-mediated relaxation.

with certain cytokines, such as interferon-γ, tumor necrosis factor-α, or interleukin-1β, which act through their specific plasma membrane receptors (78, 83, 84, 86). In addition, neurons can respond to proinflammatory stimuli and take part in brain inflammation. Thus, neuronal iNOS expression has been described in different experimental settings, including cytokine stimulation of neuronal cell lines and primary neurons *in vitro,* as well as in animal models of stroke and neurodegeneration (56, 105), highlighting the impact on neurodegeneration. Endothelial cells from brain microvessels release NO after the interaction of an agonist (e.g., acetylcholine) with the plasma membrane receptor, which promotes Ca^{2+} entry and activation of the constitutive Ca^{2+}-dependent endothelial NOS isoform (eNOS or NOS3) (66) (Fig. 1). The NO formed by eNOS activation diffuses to the neighboring smooth muscle cells and activates soluble guanylyl cyclase, thereby triggering the cyclic GMP-dependent muscle relaxation that leads to vasodilatation (98).

FORMATION AND REACTIVITY OF PEROXYNITRITE

Following NO biosynthesis, there may be formation of peroxynitrite ($ONOO^-$), which is performed by the rapid and spontaneous reaction of NO with superoxide ($O_2^{\bullet -}$) (12) (Fig. 2). Thus, peroxynitrite formation is most favored under situations in which NO biosynthesis is associated with enhanced $O_2^{\bullet -}$ formation and/or impaired $O_2^{\bullet -}$ dismutation. Although peroxynitrite is only stable in alkaline solutions, it has a pK_a of ~6.8 and is thus rapidly protonated at physiological pH values to form peroxynitrous acid (ONOOH) (7). ONOOH gives rise to chemical species with hydroxyl radical (·OH)-like reactivity and nitrogen dioxide ($\cdot NO_2$) (7). The occurrence of such radical-mediated reactions confers peroxynitrite prooxidant properties that are thought to be responsible for the execution of the neurotoxic NO-mediated responses (7, 15, 20, 71). However, this is a controversial issue that has been recently revisited (16).

Radi *et al.* (95) reported that peroxynitrite, but not peroxynitrous acid, targets sulfhydryl (cysteine and the thiol group of albumin) oxidation, yielding the corresponding disulfide products (Fig. 2). A number of subsequent studies confirmed that the redox status of intracellular reduced glutathione (GSH)—the major antioxidant thiol in mammalian cells—would be a critical factor in dictating cellular susceptibility to the actions of peroxynitrite (17, 18). Since sulfhydryl groups are crucial for many active sites and the native conformation of many enzymes, peroxynitrite-mediated oxidation of these groups may change protein function. Peroxynitrite also reacts with glutathione to form *S*-nitrosoglutathione (GSNO) (81), and GSNO may act as a storage or transport form of NO possibly exerting physiological functions (3, 81, 107) or cytoprotection (34).

Another key reaction of peroxynitrite is that with tyrosine, an amino acid with regulatory functions in most proteins. Tyrosine can be nitrated by peroxynitrite to form 3-nitrotyrosine (62) (Fig. 2), a phenomenon that may regulate cellular functions. Low-molecular weight metal complex-containing proteins, such as superoxide dismutase (SOD), favor such reactions (8). In addition, peroxynitrite promotes the dimerization of tyrosine residues in proteins, forming 3,3′-dityrosine complexes (114), whose pathophysiological role still requires clarification.

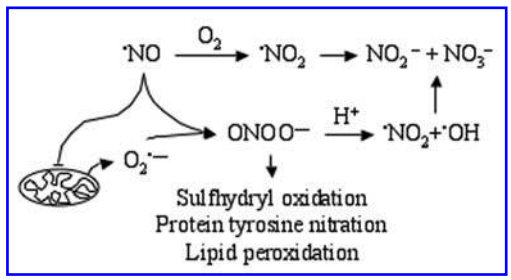

FIG. 2. Main pathways for nitric oxide degradation. After inhibition of cytochrome *c* oxidase, NO enhances superoxide ($O_2^{\bullet -}$) generation from mitochondria. Superoxide reacts with NO and forms peroxynitrite anion ($ONOO^-$). NO also reacts with O_2 forming nitrogen dioxide radical ($\cdot NO_2$), which rapidly decomposes to stable products nitrite (NO_2^-) and nitrate (NO_3^-). Peroxynitrite oxidizes sulfhydryl groups, nitrates tyrosine residues in proteins and causes lipid peroxidation, but at physiological pH rapidly protonates and forms nitrogen dioxide radical and hydroxyl radical before decomposing to nitrite and nitrate.

INHIBITION OF CYTOCHROME *C* OXIDASE BY NO AND PEROXYNITRITE

In intact astrocytes, endogenous NO reversibly modulates mitochondrial respiration (24) in a manner resembling the reversible inhibition of cytochrome *c* oxidase (complex IV of the mitochondrial respiratory chain) by exogenous NO that was observed in isolated muscle mitochondria (35) or in synaptosomes (27) (Fig. 3). The NO-dependent inhibition of mitochondrial function has been observed in a wide range of biological systems, including oligodendrocytes (80), neurons (17, 21), macrophages (41, 59, 110), synaptosomes (27, 103), isolated heart mitochondria (96), or brain submitochondrial particles (72). The mechanism responsible for the reversible inhibition of cytochrome *c* oxidase by NO involves the binding of NO with reduced cytochrome a_3 to form a nitrosyl–heme complex through the donation of one electron to ferric (Fe^{3+}) cytochrome a_3 (94, 108), then apparently interacting with the Cu^{2+}_B center (35, 108).

In addition, prolonged exposure of astrocytes to NO results in persistent damage to cytochrome *c* oxidase (19), possibly by the direct disruption of Cu^{2+}_A caused by peroxynitrite (104) (Fig. 3). The persistent inhibition of cytochrome *c* oxidase by peroxynitrite in the brain might have important pathophysiological consequences, whereas the reversible inhibition would be responsible for the rapid regulation of cellular functions (24, 25). Together, these works strongly suggest that the mitochondria from neural cells represent an important physiological or pathophysiological target of NO.

NO-mediated modulation of cytochrome *c* oxidase occurs in competition with O_2. Under most experimental conditions, cell and tissue preparations are exposed to atmospheric O_2 concentrations (~175–200 μ*M*), whereas *in vivo* they are subjected to O_2 concentrations that are one order of magnitude lower (~10–30 μ*M*). This implies that the NO concentrations needed to block cytochrome *c* oxidase activity *in vivo* are much lower than those needed to affect the activity of the enzyme in the test tube, and suggest that neuronal energy metabolism would be compromised under pathological conditions associated with reduced blood supply to the brain, such as stroke. However, it has recently been reported that the reduced status of cytochrome *c* oxidase in the brain tissue of adult rats subjected to transient ischemia is not prevented by exogenous administration of NOS inhibitors (40). Although this argues against a possible role for reversible cytochrome *c* oxidase inhibition by NO *in vivo*, these results have been questioned on the basis that it is not clear whether, in the adult brain, the NOS inhibitors used crossed the blood brain barrier (37). Accordingly, the role for NO in reversibly modulating brain cytochrome *c* oxidase activity *in vivo* remains to be fully elucidated.

INHIBITION OF NADH–UBIQUINONE OXIDORREDUCTASE BY NO AND PEROXYNITRITE

NO can promote the single-electron oxidation of ubiquinol to semiquinone, hence interfering with the transfer of the electron to cytochrome *b* (93). Since semiquinone is an effective source of $O_2^{\bullet -}$ in mitochondria, the presence of NO can thus enhance $O_2^{\bullet -}$, with which it reacts to form peroxynitrite. Consequently, persistent exposure of mitochondria to NO could produce peroxynitrite, which in turn might be responsible for the damage to NADH–ubiquinone oxidoreductase (complex I of the mitochondrial respiratory chain) previously reported in macrophages and heart mitochondria (36, 99) (Fig. 3). Thus, peroxynitrite could nitrate (and inactivate) tyrosine residues of the essential domains of complex I (99), although there is also evidence for the inactivation of complex I by NO-mediated *S*-nitrosylation of essential sulfhydryl groups of the protein (26, 36). In fact, prior studies obtained in isolated (heart and brain) mitochondria and intact neurons and astrocytes had shown that complex I activity was unaffected by peroxynitrite (17, 19, 29, 96) unless the intact nature of the mitochondria had previously been disrupted by sonication (96) or the cellular GSH status had previously been severely compromised (6). Together, these studies strongly support the notion that GSH protects mitochondria from NO and/or peroxynitrite-mediated complex I inhibition. Interestingly, in the substantia nigra of the brain of Parkinson's disease presymptomatic patients, GSH is severely depleted (63). Moreover, complex I activity is compromised in the same brain region (100). Accordingly, it could be specu-

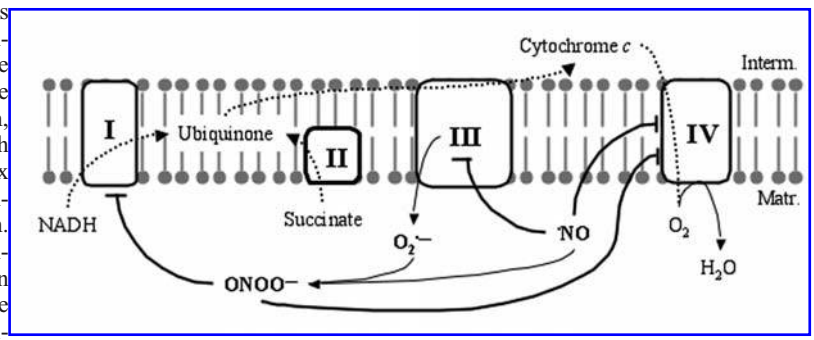

FIG. 3. Major targets of the mitochondrial respiratory chain by nitric oxide and peroxynitrite. NO rapidly and reversibly inhibits cytochrome *c* oxidase (complex IV) by competing with O_2. This causes an increase in the reduced state of upstream cytochromes of the mitochondrial respiratory chain. In addition, when persistently present, NO interferes with ubiquinone–cytochrome *c* reductase (complex III) activity and increases the rate of $O_2^{\bullet -}$ generation by the mitochondrial respiratory chain. By reaction of $O_2^{\bullet -}$ with NO, the mitochondria can thus form peroxynitrite, which can persistently inhibit both NADH–ubiquinone reductase (complex I) and cytochrome *c* oxidase activities. (interm, intermembrane space; matr, mitochondrial matrix)

lated that excessive NO production in the brain might contribute to the neuronal energy deficiency observed in this neurodegenerative disease (59).

INTERFERENCE OF NO AND PEROXYNITRITE WITH KREBS CYCLE ENZYMES

In purified preparations, peroxynitrite, but not NO itself, inhibits aconitase activity, apparently through inactivation of the iron–sulfur cluster prosthetic group (30, 55). In intact microglial cells, α-ketoglutarate dehydrogenase activity is impaired by NO formed endogenously from iNOS activity (89). It remains to be established whether the inhibition of aconitase and α-ketoglutarate dehydrogenase activities reach the threshold of Krebs cycle impairment. If so, these observations add support to the notion that NO metabolism may be an important target to combat the brain energy deficiency associated with neurodegenerative diseases.

CELL SIGNALING CONSEQUENCES OF NO-MEDIATED INHIBITION OF CYTOCHROME *C* OXIDASE

Recent evidence from our laboratory has shown that treatment of astrocytes with either exogenous or endogenous NO promotes the phosphorylation of Thr-172 of α_1-subunit of AMP-activated protein kinase (AMPK) (4). AMPK is a cell energy sensor that is activated in response to high AMP:ATP ratios (54) that triggers the phosphorylation of metabolic substrates to maintain the energy balance (28). On our hands, AMPK activation occurs secondary to the inhibition of cytochrome *c* oxidase by NO, which increases the AMP:ATP ratio by ~fivefold (32). However, the possible upregulation of AMPK by the subsequent changes in the redox status cannot be ruled out (97). In any case, these results directly show that NO-dependent regulation of mitochondrial respiration may have important cell signaling implications (Fig. 4). This notion is supported by recent observation that the transcription factor nuclear factor-κB is activated by $O_2^{\bullet -}$ after the single-electron donation to O_2, initially triggered by the NO-mediated reduction of mitochondrial electron transport chain cytochromes (87). Moreover, during hypoxia, cytochrome *c* oxidase inhibition by NO is stronger, and hence O_2 can be redistributed to other nonrespiratory oxygen-dependent targets (113), such as prolyl hydroxylases (53). In the presence of O_2, prolyl hydroxylases are needed to destabilize hypoxia-inducible factor (HIF) 1 alpha (HIF1-α), a transcription factor that, when stabilized, upregulates genes associated with glycolysis. Thus, during the NO-mediated inhibition of cytochrome *c* oxidase in hypoxia, prolyl hydroxylases do not register hypoxia, and hence HIF1-α continues to be destabilized (53). Accordingly, NO downregulates hypoxia-dependent HIF1-α transcription factor activation in response to hypoxia. Finally, NO-mediated interference with the mitochondrial respiratory chain induces changes in mitochondrial Ca^{2+} flux (22, 23), which induces the activation of endoplasmic reticulum stress-regulated transcription factor (119). Together, these observations strongly support the notion that the interaction of NO with cytochrome *c* oxidase is a molecular switch involved in the regulation of cell signaling pathways (Fig. 5).

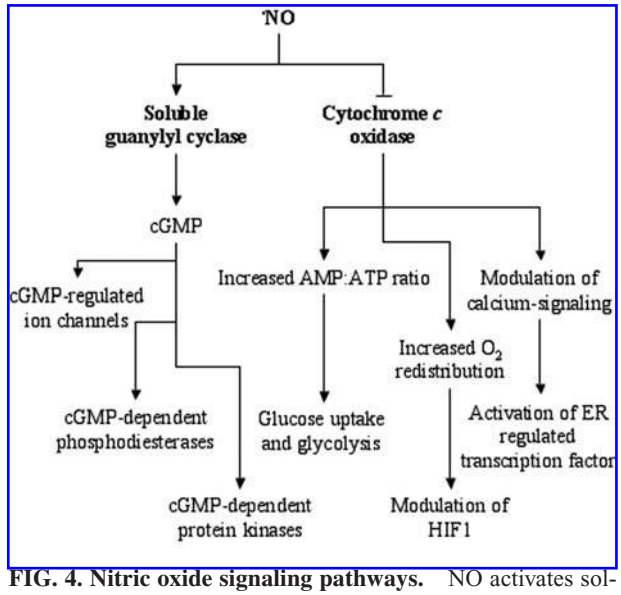

FIG. 4. Nitric oxide signaling pathways. NO activates soluble guanylyl cyclase to form cyclic GMP. Cyclic GMP thus mediates a number of cell signaling cascades, such as regulation of ion channels, cyclic GMP-dependent phosphodiesterases, or cyclic GMP-dependent protein kinases. On the other hand, NO reversibly inhibits cytochrome *c* oxidase in a competitive way with O_2. This interaction can be amplified by, at least, three ways. Thus, the inhibition of ATP synthesis transiently increases the AMP:ATP ratio, which is the trigger of AMP-activated protein kinase (AMPK) stimulation leading to several effects, including activation of glucose uptake and glycolysis. At low O_2 concentrations, endogenous NO allows O_2 redistribution to other O_2 nonrespiratory targets, such as prolyl hydroxylases. These do not register hypoxia and hence continue degrading hypoxia inducible factor 1-alpha (HIF1). Finally, mitochondrial calcium signaling is modulated by the energetic effect of NO-mediated inhibition of respiration.

THE ROLE OF GLIAL INOS IN NO- AND PEROXYNITRITE-MEDIATED NEUROTOXICITY

Different cell types respond differentially to NO. Glial cells are more resistant than neurons to NO excess (17, 80). Possible factors dictating this differential susceptibility are glutathione concentrations and the specific activity of antioxidant enzymes such as SOD, catalase, or glutathione peroxidase (17, 76). Thus, GSH is oxidized much more in neurons and in astrocytes when these cells are incubated with peroxynitrite (17) or with H_2O_2 (45). In fact, astroglial toxicity is only found when these cells are depleted of GSH before incubation with peroxynitrite (6). Together, these results strongly suggest that the cellular GSH status is a contributing factor that dictates the differential responses of neural cells to NO-mediated toxicity (18). However, additional factors may also contribute to such differences.

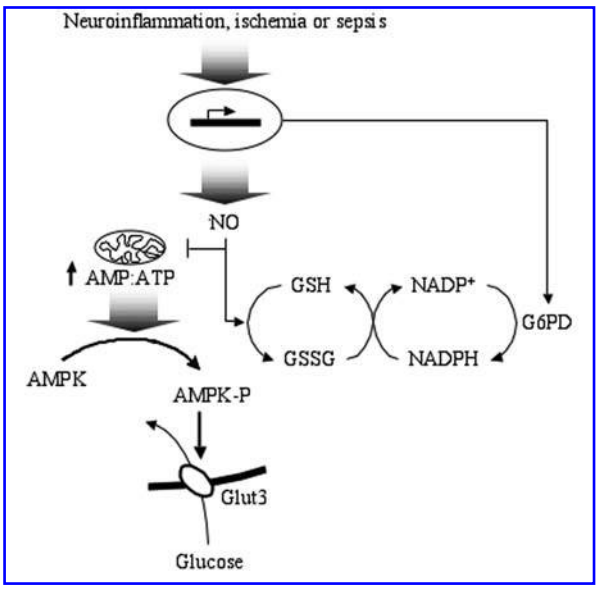

FIG. 5. Activation of glucose utilization by nitric oxide. Pro-inflammatory situations promote the transcriptional induction of iNOS. NO-dependent inhibition of mitochondrial respiration increases the AMP:ATP ratio, which is the trigger for AMPK phosphorylation (AMPK-P) and activation. As a result of this, Glut3-mediated glucose uptake is rapidly increased. On the other hand, the same pro-inflammatory situations promote the transcriptional induction of glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme of the pentose-phosphate pathway. iNOS-dependent NO formation oxidizes glutathione (GSH), but the induction of G6PD forms sufficient NADPH to regenerate GSH from oxidized glutathione (GSSG) and thus prevent further GSH depletion.

Incubation of astrocytes and neurons with identical amounts of NO triggers mitochondrial depolarization ($\Delta\psi_m$ decrease) in neurons, but no changes in $\Delta\psi_m$ in astrocytes (2) (see also Ref. 111). Interestingly, the maintenance of $\Delta\psi_m$ in astrocytes appears to be associated with NO-afforded cellular protection against apoptotic cell death (2, 111). NO could exert such antiapoptotic effect after the inhibition of cytochrome *c* oxidase (2, 9), which in turn switches on glucose uptake and glycolysis (see below), hence reactivating the cell energy metabolism and survival. However, others suggest that the changes in $\Delta\psi_m$ are mediated through a cyclic GMP-dependent mechanism (111).

Soon after the observation that NO plays a role in neurotransmission (49, 67), it was reported that NO accounts for the neurotoxicity of excessive NMDA receptor activation (38, 39). However, other authors failed to reproduce such an effect (65), although the considerable differences in the systems and protocols used might explain such discrepancies. For instance, Dawson *et al.* (38) pointed out that the age of the neuronal cultures used is an essential issue, since only old cultures (i.e., 14–21 days *in vitro*) showing prominent NMDA receptor expression are vulnerable to NO-dependent neurotoxicity. Keynes *et al.* (64) recently reported that the neurons of hippocampal organotypic slices fully expressing NMDA receptors are resistant to NMDA receptor-mediated nNOS activation. Furthermore, these authors found that exogenous NO is nontoxic to neurons in this experimental system, at least up to concentrations of 4.5 μ*M*. It should be mentioned that organotypic cultures of brain sections conserve intact astrocytes. Since astrocytes have a robust antioxidant system (see above), it is possible that these cells could help neurons to scavenge part of the added NO. This would lead to an overestimation of the concentration of NO that is neurotoxic. In fact, similar concentration values of exogenous NO (5 μ*M*) strongly inhibit mitochondrial ATP synthesis in hippocampal neurons in cultures without astrocytes (21). These results suggest that the discrepancy among the results of the different groups regarding the susceptibility of neurons to NO could be due to the experimental protocol used.

ROLE OF GLIAL-DERIVED NO IN HYPOXIA/ISCHEMIA

Brain ischemia is one of the most generally accepted pathogenic situation associated with iNOS-dependent glial NO formation. Thus, episodes of ischemia followed by reoxygenation promote the transcriptional induction of iNOS in astrocytes by interleukin-1 (73). *In vivo*, there is considerable body of evidence suggesting that such astrocyte-derived NO plays a neurotoxic role in ischemia/reoxygenation (14, 46, 60). The intracellular constituents of necrotic neurons, which are released after an ischemic episode, can also promote glial activation and NO production. Such glial-derived NO may in turn be deleterious to still live neighboring neurons. The mechanism of this neurotoxicity possibly involves mitochondrial respiratory chain inhibition (18, 109). It should be noted that in addition to excess NO, cytoprotective factors can also be transferred from glia to neurons (18, 42). Accordingly, neuronal damage is apparent only when the defense mechanisms of the brain are seriously compromised.

REGULATION OF GLUCOSE UPTAKE BY NO

Astrocytes are the only neural cell type having a reservoir of glycogen (116). This allows a transient supply of energy substrate during ischemia and during physiologic neurotransmission. In fact, the conversion of glycogen to glucose-6-phosphate is stimulated by synaptic activity (92). Glucose-6-phosphate can be converted to lactate (43, 44) and further released to the interstitial space to be taken up by surrounding neurons, which use lactate as an alternative energy substrate (77). In fact, lactate preserves neuronal activity during hypoglycemic episodes and is neuroprotective under certain pathophysiological conditions (101, 102) (reviewed in Ref. 91). However, neuroprotection can only be maintained transiently because the ability of astrocytes to store glycogen is very limited (43, 44). Accordingly, astrocytes should express suitable systems for taking up glucose efficiently enough to support neuronal energy metabolism during such stressful conditions (Fig. 5).

Astrocytes (33, 48), as well as macrophages (1) and epithelial cells (69), respond to LPS and cytokine-mediated activation by increasing glucose consumption in an NO-dependent fashion. Both transcriptional and posttranslational mecha-

nisms appear to be responsible for the increased affinity of astrocytes for glucose. Glucose is taken up by astrocytes through Glut-dependent facilitative, energy-independent glucose transporters (51). Up to 14 Glut members have been identified in humans (11, 82, 117), but in the brain the major glucose transporters expressed are Glut1 and Glut3. All brain cell types appear to express Glut1 (74), although the expression of Glut1 in cultured neurons is believed to be an adaptive consequence to *in vitro* conditions (75). The expression of Glut3 is confined to neurons (31, 85) and, in light of the kinetic parameters, it appears that Glut3 transporter activity is responsible for the high affinity for glucose shown by cerebellar neurons (52, 75). The mRNA and protein levels of the high-affinity Glut3 glucose transporter increase shortly (as from ~4 h) after incubation of astrocytes with LPS, with a low-oxygen atmosphere or with a low-glucose medium (33). Pharmacological evidence suggests that this increase is transcriptional, possibly through the activation of nuclear factor κB (NF-κB) (33). Since NF-κB transcriptionally promotes iNOS expression by LPS (118), it might be that the acquired increased in affinity of astrocytes for glucose may serve to protect the brain against stressful conditions.

Besides the transcriptional induction of glucose transporter in astrocytes, endogenous NO also stimulates glucose uptake (33) (Fig. 5). This NO-dependent increase in glucose uptake seems to be a compensatory energetic effect that is initially triggered by inhibition of the mitochondrial respiratory chain. The mechanism responsible may involve activation of AMPK, secondary to the inhibition of cytochrome *c* oxidase by NO (32). Hence the modulation of mitochondrial function by NO may thus represent an indirect mechanism for the regulation of glucose metabolism in astrocytes.

REGULATION OF THE PENTOSE-PHOSPHATE PATHWAY BY NO AND PEROXYNITRITE

Unlike neurons, astrocytes can efficiently maintain GSH status in its reduced form (17, 19). It is possible that this maintenance would be due to an enhanced glucose oxidation through the pentose-phosphate pathway. For instance, hepatocytes are prone to H_2O_2-mediated activation of glucose-6-phosphate dehydrogenase (G6PD), the enzyme that catalyzes the first rate-limiting step in the oxidative branch of the pentose-phosphate pathway (106, 113). Furthermore, the stimulation of this pathway in neurons (10) and astrocytes (69) has been proposed to elicit a protective action against H_2O_2 toxicity through the regeneration of NADPH, a cofactor necessary for GSH recovery from GSSG (66) (Fig. 5).

A factor possibly involved in the protection of astrocytes against endogenous NO-mediated GSH oxidation would be glucose utilization through the pentose-phosphate pathway (48). Thus, the incubation of astrocytes with peroxynitrite triggers the rapid activation of pentose-phosphate pathway and NADPH accumulation (47). This effect, which is due to the rapid stimulation of G6PD activity (47), suggests that glucose oxidation through the pentose-phosphate pathway is cytoprotective against excess NO.

ACTIVATION OF GLYCOLYSIS BY NO PROTECTS ASTROCYTES FROM CELL DEATH

It is well known that, in contrast to astrocytes, neurons do not easily elicit glycolysis after inhibition of mitochondrial function (90, 115). In keeping with this, incubation of astrocytes and neurons with NO inhibits the rate of O_2 consumption by ~85%, whereas glycolysis is only enhanced in the former cells (2). Increased glycolysis allows astrocytes to maintain ATP concentrations and cell death, but neuronal ATP concentrations progressively decrease due to NO, leading to cell death. These correlations suggest that the inhibition of mitochondrial respiration by NO would be associated with an upregulation of the glycolytic flux in astrocytes to prevent the depletion of ATP (2). The mechanism responsible for such glycolytic activation would be activation of 6-phosphofructo-1-kinase (PFK1), a key rate-limiting step in the glycolytic pathway (70). Fructose-2,6-bisphosphate ($F2,6P_2$), the most powerful PFK1 allosteric activator (57), is rapidly accumulated in astrocytes by NO (4), suggesting that NO would first activate 6-phosphofructo-2-kinase (PFK2), the $F2,6P_2$-forming enzyme (Fig. 6).

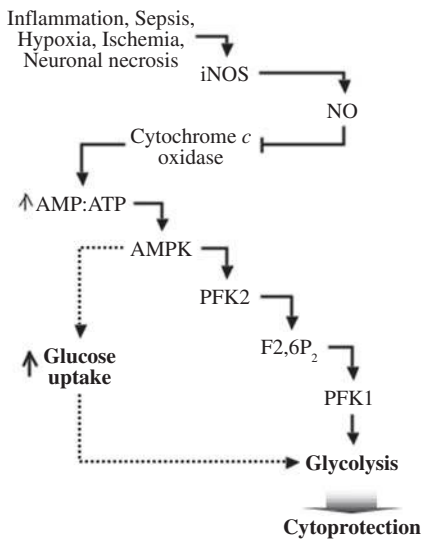

FIG. 6. Coordinated upregulation of glucose utilization by NO contributes to prevent cellular death. Astrocytes generate large amounts of NO that inhibits cytochrome *c* oxidase, increases the AMP:ATP ratio, and promotes AMPK phosphorylation and activation. Active AMPK thus activates 6-phosphofructo-2-kinase (PFK2), which forms fructose-2,6-bisphosphate ($F2,6P_2$), that is, the most potent positive effector of 6-phosphofructo-1-kinase (PFK1). The glycolytic pathway is this rapidly activated and ATP can be generated to maintain $\Delta\psi_m$ and prevent from apoptotic cell death. On the other hand, transcriptionally induced Glut3, and AMPK-mediated activation of Glut3 contributes to this sequence of events by supplying intracellular glucose for glycolysis.

This activation is cyclic GMP-independent and requires prior inhibition of mitochondrial respiration (4). Depletion of AMPK-α_1 by RNA interference renders astrocytes unable to form $F2,6P_2$, thereby preventing NO-dependent PFK1 and glycolysis activation (4). Together, these observations are compatible with the notion that the mechanism through which NO activates glycolysis first involves the inhibition of mitochondrial respiration, leading to enhancement of the AMP:ATP ratio. When transiently increased, the AMP:ATP ratio would activate AMPK, which phosphorylates and activates PFK2 to form $F2,6P_2$. Hence, elevations in $F2,6P_2$ would stimulate PFK1 activity, leading to the observed rapid increase in the glycolytic flux (4). The synthesis of ATP by the glycolytic pathway would hence be a transient response of certain cell types to compensate for the energy failure that would otherwise lead to cell death (Fig. 6).

CONCLUSION

The brain actively synthesizes nitric oxide. The binding of NO with soluble guanylyl cyclase allosterically activates the enzyme, which strongly increases cyclic GMP concentrations. The binding of NO with the a–a_3 binuclear center of cytochrome *c* oxidase rapidly decreases the affinity of the enzyme complex for O_2, hence reversibly inhibiting the mitochondrial electron flux and ATP synthesis. Despite promoting a profound degree of mitochondrial inhibition, astrocytes show remarkable resistance to NO and peroxynitrite; in contrast, neurons are highly vulnerable. Recent evidence suggests that these nitrogen-derived reactive species would modulate the key regulatory steps of glucose metabolism that would dictate cellular survival. Upregulation of the high-affinity glucose transporter, the stimulation of glycolysis at the level of PFK1, and activation of pentose-phosphate pathway at the level of G6PD may be important sites of action. The orchestrated stimulation of these glucose-metabolizing pathways by NO would represent a transient attempt by the glial cells to compensate for energy impairment and oxidative stress, and thus to emerge from an otherwise pathological outcome.

ACKNOWLEDGMENTS

JPB is funded by the Spanish Ministry of Science and Education (grant SAF2004-2038) and the Junta de Castilla-León (grant SA081/04). AAP is funded by the Fondo de Investigación Sanitaria (grant FIS03/1055) and the Junta de Castilla-León (grant SA082A05). The authors wish to thank Dr. María Delgado-Esteban, Dr. Paula García-Nogales, Dr. Victoria Vega-Agapito, Dr. Pilar Cidad, Dr. Emilio Fernández, and Professor Salvador Moncada for exciting collaborative efforts that led to part of the results discussed in this review.

ABBREVIATIONS

AMPK, AMP-activated protein kinase; $\Delta\psi_m$, mitochondrial membrane potential; Glut, sodium-independent glucose transporter; G6PD, glucose-6-phosphate dehydrogenase; GSH, reduced glutathione; GSSG, oxidized glutathione; GSNO, *S*-nitrosoglutathione; HIF1-α, hypoxia-inducible factor-1α; LPS, lipopolysaccharide; NMDA, *N*-methyl-D-aspartate; NOS1 (or nNOS), NOS2 (or iNOS), NOS3 (or eNOS), nitric oxide synthase, neuronal, inducible or endothelial isoforms, respectively; PFK1, 6-phosphofructo-1-kinase; PFK2, 6-phosphofructo-2-kinase; SOD, superoxide dismutase.

REFERENCES

1. Albina JE and Mastrofrancesco B. Modulation of glucose metabolism in macrophages by products of nitric oxide synthase. *Am J Physiol* 264: C1594–C1599, 1993.
2. Almeida A, Almeida J, Bolaños JP, and Moncada S. Different responses of astrocytes and neurons to nitric oxide: the role of glycolytically-generated ATP in astrocyte protection. *Proc Natl Acad Sci USA* 98: 15294–15299, 2001.
3. Almeida A and Bolaños JP. A transient inhibition of mitochondrial ATP synthesis by nitric oxide synthase activation triggered apoptosis in primary cortical neurons. *J Neurochem* 77: 676–690, 2001.
4. Almeida A, Moncada S, and Bolaños JP. Nitric oxide switches on glycolysis through the AMP protein kinase and 6-phosphofructo-2-kinase pathway. *Nat Cell Biol* 6: 45–51, 2004.
5. Barbeito LH, Pehar M, Cassina P, Vargas MR, Peluffo H, Viera L, Estevez AG, and Beckman JS. A role for astrocytes in motor neuron loss in amyotrophic lateral sclerosis. *Brain Res* 47: 263–274, 2004.
6. Barker JE, Bolaños JP, Land JM, Clark JB, and Heales SJR. Glutathione protects astrocytes from peroxynitrite-mediated mitochondrial damage: implications for neuronal/astrocytic trafficking and neurodegeneration. *Dev Neurosci* 18: 391–396, 1996.
7. Beckman JS, Beckman TW, Chen J, Marshall PA, and Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc Natl Acad Sci USA* 87: 1620–1624, 1990.
8. Beckman JS, Ischiropoulos H, Zhu J, van der Woerd M, Smith C, Chen J, Harrison J, Martin JC, and Tsai M. Kinetics of superoxide dismutase- and iron-catalyzed nitration of phenolics by peroxynitrite. *Arch Biochem Biophys* 298: 438–445, 1992.
9. Beltrán B, Mathur A, Duchen MR, Erusalimsky JD, and Moncada S. The effect of nitric oxide on cell respiration: a key to understanding its role in cell survival or death. *Proc Natl Acad Sci USA* 97: 14602–14607, 2000.
10. Ben-Yoseph O, Boxer PA, and Ross BD. Assessment of the role of the glutathione and pentose phosphate pathways in the protection of primary cerebrocortical cultures from oxidative stress. *J Neurochem* 66: 2329–2337, 1996.
11. Birnbaum MJ, Haspel HC, and Rosen OM. Cloning and characterization of a cDNA encoding the rat brain glucose-transporter protein. *Proc Natl Acad Sci USA* 83: 5784–5788, 1986.

12. Blough NV and Zafiriou OC. Reactions of superoxide with nitric oxide to form peroxonitrite in alkaline aqueous solution. *Inorg Chem* 24: 3502–3504, 1985.
13. Blumcke I, Eggli P, and Celio MR. Relationship between astrocytic processes and "perineuronal nets" in rat neocortex. *Glia* 15: 131–140, 1995.
14. Bolaños JP and Almeida A. Roles of nitric oxide in brain hypoxia-ischemia. *Biochim Biophys Acta* 1411: 415–436, 1999.
15. Bolaños JP, Almeida A, Stewart V, Peuchen S, Land JM, Clark JB, and Heales SJR. Nitric oxide-mediated mitochondrial damage in the brain: mechanisms and implications for neurodegenerative diseases. *J Neurochem* 68: 2227–2240, 1997.
16. Bolaños JP, García-Nogales P, and Almeida A. Provoking neuroprotection by peroxynitrite. *Curr Pharm Design* 10: 867–877, 2004.
17. Bolaños JP, Heales SJR, Land JM, and Clark JB. Effect of peroxynitrite on the mitochondrial respiratory chain: differential susceptibility of neurones and astrocytes in primary cultures. *J Neurochem* 64: 1965–1972, 1995.
18. Bolaños JP, Heales SJR, Peuchen S, Barker JE, Land JM, and Clark JB. Nitric oxide-mediated mitochondrial damage: a potential neuroprotective role for glutathione. *Free Radic Biol Med* 21: 995–1001, 1996.
19. Bolaños JP, Peuchen S, Heales SJR, Land JM, and Clark JB. Nitric oxide-mediated inhibition of the mitochondrial respiratory chain in cultured astrocytes. *J Neurochem* 63: 910–916, 1994.
20. Bonfoco E, Krainc C, Ankarcrona M, Nicotera P, and Lipton SA. Apoptosis and necrosis: two distinct events induced, respectively, by mild and intense insults with *N*-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures. *Proc Natl Acad Sci USA* 92: 7162–7166, 1995.
21. Brorson JR, Schumacker PT, and Zhang H. Nitric oxide acutely inhibits neuronal energy metabolism. *J Neurosci* 19: 147–158, 1999.
22. Brorson JR, Sulit RA, and Zhang H. Nitric oxide disrupts Ca^{2+} homeostasis in hippocampal neurons. *J Neurochem* 68: 95–105, 1997.
23. Brorson JR and Zhang H. Disrupted $[Ca^{2+}]_i$ homeostasis contributes to the toxicity of nitric oxide in cultured hippocampal neurons. *J Neurochem* 69: 1882–1889, 1997.
24. Brown GC. Nitric oxide regulates mitochondrial respiration and cell functions by inhibiting cytochrome oxidase. *FEBS Lett* 369: 136–139, 1995.
25. Brown GC, Bolaños JP, Heales SJR, and Clark JB. Nitric oxide produced by activated astrocytes rapidly and reversibly inhibits cellular respiration. *Neurosci Lett* 193: 201–204, 1995.
26. Brown GC and Borutaite V. Inhibition of mitochondrial respiratory complex I by nitric oxide, peroxynitrite and *S*-nitrosothiols. *Biochim Biophys Acta* 1658: 44–49, 2004.
27. Brown GC and Cooper CE. Nanomolar concentrations of nitric oxide reversibly inhibit synaptosomal respiration by competing with oxygen at cytochrome oxidase. *FEBS Lett* 356: 295–298, 1994.
28. Carling D. The AMP-activated protein kinase cascade–a unifying system for energy control. *Trends Biochem Sci* 29: 18–24, 2004.
29. Cassina A and Radi R. Differential inhibitory action of nitric oxide and peroxynitrite on mitochondrial electron transport. *Arch Biochem Biophys* 328: 309–316, 1996.
30. Castro L, Rodríguez M, and Radi R. Aconitase is readily inactivated by peroxynitrite, but not by its precursor, nitric oxide. *J Biol Chem* 269: 29409–29415, 1994.
31. Choeiri C, Staines W, and Messier C. Immunohistochemical localization and quantification of glucose transporters in the mouse brain. *Neuroscience* 111: 19–34, 2002.
32. Cidad P, Almeida A, and Bolaños JP. Inhibition of mitochondrial respiration by nitric oxide rapidly stimulates cytoprotective GLUT3-mediated glucose uptake through 5′-AMP-activated protein kinase. *Biochem J* 384: 629–636, 2004.
33. Cidad P, García-Nogales P, Almeida A, and Bolaños JP. Expression of glucose transporter GLUT3 by endotoxin in cultured rat astrocytes: the role of nitric oxide. *J Neurochem* 79: 17–24, 2001.
34. Clancy RM, Levartovsky D, Leszczynska-Piziak J, Yegudin J, and Abramson S. Nitric oxide reacts with intracellular glutathione and activates the hexose monophosphate shunt in human neutrophils: evidence for *S*-nitrosoglutathione as a bioactive intermediary. *Proc Natl Acad Sci USA* 91: 3680–3684, 1994.
35. Cleeter MWJ, Cooper JM, Darley-Usmar VM, Moncada S, and Schapira AH. Reversible inhibition of cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide. Implications for neurodegenerative diseases. *FEBS Lett* 345: 50–54, 1994.
36. Clementi E, Brown GC, Feelisch M, and Moncada S. Persistent inhibition of cell respiration by nitric oxide: crucial role of *S*-nitrosylation of mitochondrial complex I and protective action of glutathione. *Proc Natl Acad Sci USA* 95: 7631–7636, 1998.
37. Cooper CE. Competitive, reversible, physiological? inhibition of mitochondrial cytochrome oxidase by nitric oxide. *IUBMB Life* 55: 591–597, 2003.
38. Dawson VL, Dawson TM, Bartley DA, Uhl GR, and Snyder SH. Mechanisms of nitric oxide-mediated neurotoxicity in primary brain cultures. *J Neurosci* 13: 2651–2661, 1993.
39. Dawson VL, Dawson TM, London ED, Bredt DS, and Snyder SH. Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures. *Proc Natl Acad Sci USA* 88: 6368–6371, 1991.
40. De Visscher G, Springett R, Delpy DT, Van Reempts J, Borgers M, and Van Rossem K. Nitric oxide does not inhibit cerebral cytochrome oxidase in vivo or in the reactive hyperemic phase after brief anoxia in the adult rat. *J Cereb Blood Flow Metab* 22: 515–519, 2002.
41. Drapier J-C and Hibbs JB, Jr. Differentiation of murine macrophages to express nonspecific cytotoxicity for tumor cells results in L-arginine-dependent inhibition of mitochondrial iron-sulfur enzymes in the macrophage effector cells. *J Immunol* 140: 2829–2838, 1988.
42. Dringen R. Metabolism and functions of glutathione in brain. *Prog Neurobiol* 62: 649–671, 2000.
43. Dringen R, Gebhardt R, and Hamprecht B. Glycogen in astrocytes: possible function as lactate supply for neighboring cells. *Brain Res* 623: 208–214, 1993.
44. Dringen R and Hamprecht B. Glucose, insulin, and insulin-like growth factor I regulate the glycogen content of

astroglia-rich primary cultures. *J Neurochem* 58: 511–517, 1992.
45. Dringen R, Kussmaul L, Gutterer JM, Hirrlinger J, and Hamprecht B. The glutathione system of peroxide detoxification is less efficient in neurons than in astrocytes. *J Neurochem* 72: 2523–2530, 1999.
46. Faraci FM and Brian JE. Nitric-oxide and the cerebral circulation. *Stroke* 25: 692–703, 1994.
47. García-Nogales P, Almeida A, and Bolaños JP. Peroxynitrite protects neurons against nitric oxide-mediated apoptosis. A key role for glucose-6-phosphate dehydrogenase activity in neuroprotection. *J Biol Chem* 278: 864–874, 2003.
48. García-Nogales P, Almeida A, Fernández E, Medina JM, and Bolaños JP. Induction of glucose-6-phosphate dehydrogenase by lipopolysaccharide contributes to preventing nitric oxide-mediated glutathione depletion in cultured rat astrocytes. *J Neurochem* 72: 1750–1758, 1999.
49. Garthwaite J, Charles SL, and Chess-Williams R. Endothelium-derived relaxing factor release on activation of NMDA receptors suggests a role as intercellular messenger in the brain. *Nature* 336: 385–387, 1988.
50. Golgi C. Sulla fine anatomia degli organi centrali del sisterna nervoso, Milan, 1886.
51. Gorovits N, and Charron MJ. What we know about facilitative glucose transporters. Lessons from cultured cells, animal models and human studies. *Biochem Mol Biol Education* 13: 163–172, 2003.
52. Gould GW, Thomas HM, Jess TJ, and Bell GI. Expression of human glucose transporters in *Xenopus* oocytes: kinetic characterization and substrate specificities of the erythrocyte, liver, and brain isoforms. *Biochemistry* 30: 5139–5145, 1991.
53. Hagen T, Taylor CT, Lam F, and Moncada S. Redistribution of intracellular oxygen in hypoxia by nitric oxide: effect on HIF1alpha. *Science* 302: 1975–1978, 2003.
54. Hardie DG, Carling D, and Sim ATR. The AMP-activated protein kinase: a multisubstrate regulator of lipid metabolism. *Trends Biochem Sci* 14: 20–23, 1989.
55. Hausladen A and Fridovich I. Superoxide and peroxynitrite inactivate aconitases, but nitric oxide does not. *J Biol Chem* 269: 29405–29408, 1994.
56. Heneka MT and Feinstein DL. Expression and function of inducible nitric oxide synthase in neurons. *J Neuroimmunol* 114: 8–18, 2001.
57. Hers HG and Van Schaftingen E. Fructose 2,6-bisphosphate 2 years after its discovery. *Biochem J* 206: 1–12, 1982.
58. Hibbs JB, Jr., Taintor RR, Vavrin Z, and Rachlin EM. Nitric oxide: a cytotoxic activated macrophage effector molecule. *Biochem Biophys Res Commun* 157: 87–94, 1988.
59. Hunot S, Boissiere F, Faucheux B, Brugg B, Mouattprigent A, Agid Y, and Hirsch EC. Nitric oxide synthase and neuronal vulnerability in Parkinson's disease. *Neuroscience* 72: 355–363, 1996.
60. Iadecola C. Bright and dark sides of nitric oxide in ischemic brain injury. *Trends Neurosci* 20: 132–139, 1997.
61. Ignarro LJ, Buga GM, Wood KS, Byrns RE, and Chaudhuri G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci* 84: 9265–9269, 1987.
62. Ischiropoulos H, Zhu LC, Chen J, Tsai M, Martin JC, Smith CD, and Beckman JS. Peroxynitrite-mediated tyrosine nitration catalyzed by superoxide dismutase. *Arch Biochem Biophys* 298: 431–437, 1992.
63. Jenner P, Dexter DT, Sian J, Schapira AHV, and Marsden CD. Oxidative stress as a cause of nigral cell death in Parkinson's disease and incidental Lewy body disease. *Ann Neurol* 32: S82–S87, 1992.
64. Keynes RG, Duport S, and Garthwaite J. Hippocampal neurons in organotypic slice culture are highly resistant to damage by endogenous and exogenous nitric oxide. *Eur J Neurosci* 19: 1163–1173, 2004.
65. Kletzien RF, Harris PKW, and Foellmi LA. Glucose-6-phosphate dehydrogenase: a housekeeping enzyme subject to tissue-specific regulation by hormones, nutrients, and oxidant stress. *FASEB J* 8: 174–181, 1994.
66. Knowles RG and Moncada S. Nitric oxide synthases in mammals. *Biochem J* 298: 249–258, 1994.
67. Knowles RG, Palacios M, Palmer RMJ, and Moncada S. Formation of nitric oxide from L-arginine in the central nervous system: a transduction mechanism for stimulation of the soluble guanylate cyclase. *Proc Natl Acad Sci USA* 86: 5159–5162, 1989.
68. Kussmaul L, Hamprecht B, and Dringen R. The detoxification of cumene hydroperoxide by the glutathione system of cultured astroglial cells hinges on hexose availability for the regeneration of NADPH. *J Neurochem* 73: 1246–1253, 1999.
69. Le Goffe C, Vallette G, Charrier L, Candelon T, Bou-Hanna C, Bouhours JF, and Laboisse CL. Metabolic control of resistance of human epithelial cells to H2O2 and NO stresses. *Biochem J* 364: 349–359, 2002.
70. Nelson DL and Cox MM (Eds). *Lehninger Principles of Biochemistry*, Fourth Edition. New York: Freeman WH, 2004, p. 1100.
71. Lipton SA, Choi YB, Pan ZH, Lei SZ, Chen HSV, Sucher NJ, Loscalzo J, Singel DJ, and Stamler JS. A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. *Nature* 364: 626–632, 1993.
72. Lizasoain I, Moro MA, Knowles RG, Darley-Usmar V, and Moncada S. Nitric oxide and peroxynitrite exert distinct effects on mitochondrial respiration which are differentially blocked by glutathione or glucose. *Biochem J* 314: 877–880, 1996.
73. Maeda Y, Matsumoto M, Hori O, Kuwabara K, Ogawa S, Yan SD, Ohtsuki T, Kamada T, and Stern SD. Hypoxia/reoxygenation-mediated induction of astrocyte interleukin-6. A paracrine mechanism potentially enhancing neuron survival. *J Exp Med* 180: 2297–2308, 1994.
74. Maher F. Immunolocalization of GLUT1 and GLUT3 glucose transporters in primary cultured neurons and glia. *J Neurosci Res* 42: 459–469, 1995.
75. Maher F, Vannucci SJ, and Simpson IA. Glucose transporter proteins in brain. *FASEB J* 8: 1003–1011, 1994.
76. Makar TK, Nedergaard M, Preuss A, Gelbard AS, Perumal AS, and Cooper AJL. Vitamin E, ascorbate, glutathione, glutathione disulfide, and enzymes of glu-

tathione metabolism in cultures of chick astrocytes and neurones: evidence that astrocytes play an important role in antioxidative processes in the brain. *J Neurochem* 62: 45–53, 1994.
77. Medina JM, Fernández E, Bolaños JP, Vicario C, and Arizmendi C. In: *Endocrine and Biochemical Development of the Fetus and Neonate* (Cuezva JM, Pascual-Leone AM, and Patel MS, Eds.), Plenum Press, New York pp. 175–194, 1990.
78. Merrill JE, Murphy SP, Mitrovic B, Mackenzie-Graham A, Dopp JC, Ding M, Griscavage J, Ignarro LJ, and Lowenstein CJ. Inducible nitric oxide synthase and nitric oxide production by oligodendrocytes. *J Neurosci Res* 48: 372–384, 1997.
79. Mitrovic B, Ignarro LJ, Montestruque S, Smoll A, and Merrill JE. Nitric oxide as a potential pathological mechanism in demyelination: its differential effects on primary glial cells *in vitro*. *Neuroscience* 61: 575–585, 1994.
80. Morgello S, Uson RR, Schwartz EJ, and Haber RS. The human blood-brain barrier glucose transporter (GLUT1) is a glucose transporter of gray matter astrocytes. *Glia* 14: 43–54, 1995.
81. Moro MA, Darley-Usmar VM, Goodwin DA, Read NG, Zamora-Pino R, Feelish M, Radmski MW, and Moncada S. Paradoxical fate and biological action of peroxynitrite on human platelets. *Proc Natl Acad Sci USA* 91: 6702–6706, 1994.
82. Mueckler M, Caruso C, Baldwin SA, Panico M, Blench I, Morris HR, Allard WJ, Lienhard GE, and Lodish HF. Sequence and structure of a human glucose transporter. *Science* 229: 941–945, 1985.
83. Murphy S and Grzybicki D. Glial NO. Normal and pathological roles. *Neuroscientist* 2: 90–99, 1996.
84. Murphy S, Simmons ML, Agulló L, García A, Feinstein DL, Galea E, Reis DJ, Minc-Golomb D, and Schwartz JP. Synthesis of nitric oxide in CNS glial cells. *Trends Neurosci* 16: 323–328, 1993.
85. Nagamatsu S, Sawa H, Kamada K, Nakamichi Y, Yoshimoto K, and Hoshino T. Neuron-specific glucose transporter (NSGT): CNS distribution of GLUT3 rat glucose transporter (RGT3) in rat central neurons. *FEBS Lett* 334: 289–295, 1993.
86. Nomura Y and Kitamura Y. Inducible nitric oxide synthase in glial cells. *Neurosci Res* 18: 103–107, 1993.
87. Palacios-Callender M, Quintero M, Hollis VS, Springett RJ, and Moncada S. Endogenous NO regulates superoxide production at low oxygen concentrations by modifying the redox state of cytochrome c oxidase. *Proc Natl Acad Sci USA* 101: 7630–7635, 2004.
88. Palmer RMJ, Ferrige AG, and Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 327: 524–526, 1987.
89. Park LCH, Zhang H, Sheu KFR, Calingasan NY, Kristal BS, Lindsay JG, and Gibson GE. Metabolic impairment induces oxidative stress, compromises inflammatory responses, and inactivates a key mitochondrial enzyme in microglia. *J Neurochem* 72: 1948–1958, 1999.
90. Pauwels PJ, Opperdoes FR, and Trouet A. Effects of antimycin, glucose deprivation, and serum on cultures of neurons, astrocytes, and neuroblastoma cells. *J Neurochem* 44: 143–148, 1985.
91. Pellerin L. Lactate as a pivotal element in neuron-glia metabolic cooperation. *Neurochem Int* 43: 331–338, 2003.
92. Pellerin L and Magistretti PJ. Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. *Proc Natl Acad Sci USA* 91: 10625–10629, 1994.
93. Poderoso JJ, Carreras MC, Lisdero C, Riobó N, Schöper F, and Boveris A. Nitric oxide inhibits electron transfer and increases superoxide radical production in rat heart mitochondria and submitochondrial particles. *Arch Biochem Biophys* 328: 85–92, 1996.
94. Radi R. Reactions of nitric oxide with metalloproteins. *Chem Res Toxicol* 9: 828–835, 1996.
95. Radi R, Beckman JS, Bush KM, and Freeman BA. Peroxynitrite oxidation of sulfhydryls. The cytotoxic potential of superoxide and nitric oxide. *J Biol Chem* 266: 4244–4250, 1991.
96. Radi R, Rodríguez M, Castro L, and Telleri R. Inhibition of mitochondrial electron transport by peroxynitrite. *Arch Biochem Biophys* 308: 89–95, 1994.
97. Rafaeloff–Phail R, Ding L, Conner L, Yeh WK, McClure D, Guo H, Emerson K, and Brooks H. Biochemical regulation of mammalian AMP-activated protein kinase activity by NAD and NADH. *J Biol Chem* 279: 52934–52939, 2004.
98. Rees DD, Palmer RJM, and Moncada S. Role of endothelium-derived nitric oxide in the regulation of blood pressure. *Proc Natl Acad Sci USA* 86: 3375–3378, 1989.
99. Riobó NA, Clementi E, Melani M, Boveris A, Cadenas E, Moncada S, and Poderoso JJ. Nitric oxide inhibits mitochondrial NADH:ubiquinone reductase activity through peroxynitrite formation. *Biochem J* 359: 139–145, 2001.
100. Schapira AHV, Cooper JM, Dexter D, Clark JB, Jenner P, and Marsden CD. Mitochondrial complex I deficiency in Parkinson's disease. *J Neurochem* 54: 823–827, 1990.
101. Schurr A, Payne RS, Miller JJ, and Rigor BM. Brain lactate is an obligatory aerobic energy substrate for functional recovery after hypoxia: further in vitro validation. *J Neurochem* 69: 423–426, 1997.
102. Schurr A, West CA, and Rigor BM. Lactate-supported synaptic function in the rat hippocampal slice preparation. *Science* 240: 1326–1328, 1988.
103. Schweizer M and Richter C. Nitric oxide potently and reversibly deenergizes mitochondria at low oxygen tension. *Biochem Biophys Res Commun* 204: 169–175, 1994.
104. Sharpe MA and Cooper CE. Interaction of peroxynitrite with mitochondrial cytochrome oxidase. *J Biol Chem* 273: 30961–30972, 1998.
105. Small CI, Lyles GA, and Breen KC. Inducible form of nitric oxide synthase expression in rat cortical neuronal cells *in vitro*. *Neurobiol Dis* 17: 70–76, 2004.
106. Spolarics Z, Stein DS, and García ZC. Endotoxin stimulates hydrogen peroxide detoxifying activity in rat hepatic endothelial cells. *Hepatology* 24: 691–696, 1996.
107. Stamler JS, Simon DI, Jaraki O, Osborne JA, Francis S, Mullins M, Singel D, and Loscalzo J. S-nitrosylation of tissue-type plasminogen activator confers vasodilatory and antiplatelet properties on the enzyme. *Proc Natl Acad Sci USA* 89: 87–91, 1992.
108. Stevens TH, Bocian DF, and Chan SI. EPR studies of ^{15}NO-ferrocytochrome a_3 in cytochrome c oxidase. *FEBS Lett* 97: 314–316, 1979.

109. Stewart VC, Land JM, Clark JB, and Heales SJR. Pretreatment of astrocytes with interferon-a/ß prevents neuronal mitochondrial respiratory chain damage. *J Neurochem* 70: 432–434, 1998.
110. Szabó C, Day BJ, and Salzman AL. Evaluation of the relative contribution of nitric oxide and peroxynitrite to the suppression of mitochondrial respiration in immunostimulated macrophages using a manganese mesoprophyrin superoxide dismutase mimetic and peroxynitrite scavenger. *FEBS Lett* 381: 82–86, 1996.
111. Takuma K, Phuagphong P, Lee EK, Baba A, and Matsuda T. Anti-apoptotic effect of cGMP in cultured astrocytes. Inhibition by cGMP-dependent protein kinase of mitochondrial permeable transition pore. *J Biol Chem* 276: 48093–48099, 2001.
112. Trimmer BA, Aprille JR, Dudzinski DM, Lagace CJ, Lewis SM, Michel T, Qazi S, and Zayas RM. Nitric oxide and the control of firefly flashing. *Science* 292: 2486–2488, 2001.
113. Ursini MV, Parrella A, Rosa G, Salzano S, and Martini G. Enhanced expression of glucose-6-phosphate dehydrogenase in human cells sustaining oxidative stress. *Biochem J* 323: 801–806, 1997.
114. van der Vliet A, Eiserich JP, Kaur H, Cross CE, and Halliwell B. Nitrotyrosine as biomarker for reactive nitrogen species. *Methods Enzymol* 269: 175–184, 1996.
115. Walz W and Mukerji S. Lactate release from cultured astrocytes and neurons: a comparison. *Glia* 1: 366–370, 1988.
116. Wiesinger H, Hamprecht B, and Dringen R. Metabolic pathways for glucose in astrocytes. *Glia* 21: 22–34, 1997.
117. Wu X and Freeze HH. GLUT14, a duplicon of GLUT3, is specifically expressed in testis as alternative splice forms. *Genomics* 80: 553–557, 2002.
118. Xie Q-W, Kashiwabara Y, and Nathan C. Role of transcription factor NF-kB/rel in induction of nitric oxide synthase. *J Biol Chem* 269: 4705–4708, 1994.
119. Xu W, Liu L, Charles IG, and Moncada S. Nitric oxide induces coupling of mitochondrial signaling with the endoplasmic reticulum stress response. *Nat Cell Biol* 6: 1129–1134, 2004.

Address reprint requests to:
Professor Juan P. Bolaños
Departamento de Bioquímica y Biología Molecular
Universidad de Salamanca
Instituto de Neurociencias de Castilla y León
Centro Nacional de Investigaciones Cardiovasculares
Edificio Departamental, Campus Miguel de Unamuno
37007 Salamanca, Spain

E-mail: jbolanos@usal.es

Date of first submission to ARS Central, October 3, 2005; date of acceptance, December 2, 2005.

This article has been cited by:

1. Germaine Escames, Luis Carlos López, José Antonio García, Laura García-Corzo, Francisco Ortiz, Darío Acuña-Castroviejo. 2011. Mitochondrial DNA and inflammatory diseases. *Human Genetics* . [CrossRef]
2. K. J. Smith. 2011. Newly lesioned tissue in multiple sclerosis--a role for oxidative damage?. *Brain* **134**:7, 1877-1881. [CrossRef]
3. Xavier Fioramonti , Zhentao Song , Reema P. Vazirani , Annie Beuve , Vanessa H. Routh . 2011. Hypothalamic Nitric Oxide in Hypoglycemia Detection and Counterregulation: A Two-Edged Sword. *Antioxidants & Redox Signaling* **14**:3, 505-517. [Abstract] [Full Text] [PDF] [PDF Plus]
4. Fabrice Marcillac, Britta Brix, Cendrine Repond, Olaf Jöhren, Luc Pellerin. 2011. Nitric oxide induces the expression of the monocarboxylate transporter MCT4 in cultured astrocytes by a cGMP-independent transcriptional activation. *Glia* n/a-n/a. [CrossRef]
5. Ludmila A. Voloboueva, Rona G. Giffard. 2011. Inflammation, mitochondria, and the inhibition of adult neurogenesis. *Journal of Neuroscience Research* n/a-n/a. [CrossRef]
6. Li#Peng Yap, Jerome V. Garcia, Derick S. Han, Enrique Cadenas. 2010. Role of nitric oxide-mediated glutathionylation in neuronal function: potential regulation of energy utilization. *Biochemical Journal* **428**:1, 85-93. [CrossRef]
7. Rodrigo Mora, Ivana Dokic, Tim Kees, Christian M. Hüber, Denise Keitel, Renate Geibig, Britta Brügge, Hanswalter Zentgraf, Nathan R. Brady, Anne Régnier-Vigouroux. 2010. Sphingolipid rheostat alterations related to transformation can be exploited for specific induction of lysosomal cell death in murine and human glioma. *Glia* n/a-n/a. [CrossRef]
8. Frode FonnumNeurotoxicology . [CrossRef]
9. Letizia Palomba, Liana Cerioni, Orazio Cantoni. 2009. Arachidonic acid: A key molecule for astrocyte survival to peroxynitrite. *Glia* **57**:15, 1672-1679. [CrossRef]
10. N. Kyprianou, E. Murphy, P. Lee, I. Hargreaves. 2009. Assessment of mitochondrial respiratory chain function in hyperphenylalaninaemia. *Journal of Inherited Metabolic Disease* **32**:2, 289-296. [CrossRef]
11. S ARANEDA, L COMMIN, M ATLAGICH, K KITAHAMA, V PARRAGUEZ, J PEQUIGNOT, Y DALMAZ. 2008. VEGF overexpression in the astroglial cells of rat brainstem following ozone exposure. *NeuroToxicology* **29**:6, 920-927. [CrossRef]
12. M. R. Khazaei, M. Habibi-Rezaei, F. Karimzadeh, A. A. Moosavi-Movahedi, A. A. Sarrafnejhad, F. Sabouni, M. Bakhti. 2008. Microglial Cell Death Induced by Glycated Bovine Serum Albumin: Nitric Oxide Involvement. *Journal of Biochemistry* **144**:2, 197-206. [CrossRef]
13. Narayan R. Bhat , Douglas L. Feinstein . 2006. NO and Glial Cell Biology. *Antioxidants & Redox Signaling* **8**:5-6, 869-872. [Citation] [PDF] [PDF Plus]