Phycocyanobilin vs Excitotoxicity

Mark F. McCarty, NutriGuard Research, 1051 Hermes Ave., Encinitas, CA 92024

Abstract

Recent studies have demonstrated that intraneuronal activation of Nox2-dependent NADPH oxidase mediates the neuronal cell death triggered by excitotoxicity. Hence, the spirulina-derived chromophore phycocyanobilin (PhyCB), which can mimic the potent NADPH oxidase-inhibitory activity of its structural homologs biliverdin/bilirubin, may have utility for preserving neuronal integrity in multiple CNS disorders characterized by excitotoxicity, including stroke, trauma, epilepsy, and various neurodegenerative conditions. Concurrent inhibition of NADPH oxidase activity in activated microglia may also contribute to PhyCB’s utility in many of these syndromes. In light of recent evidence that IL-6-mediated activation of intraneuronal NADPH oxidase may play a role in the mild cognitive deficit associated with normal aging, long-term use of PhyCB might also aid preservation of cognitive function in the elderly. It would be logical to use PhyCB in concert with various other antioxidants which can ameliorate the downstream consequences of neuronal superoxide excess, such as lipoic acid, melatonin, N-acetylcysteine, coenzyme Q10, and the urate precursor inosine. Taurine may also be useful in this regard, as exerts anti-excitotoxic effects independent of its antioxidant activity.

Intraneuronal NADPH Mediates Excitotoxicity

Excitotoxicity – triggered by excessive activation of glutamate-triggered neuronal receptors (i.e. the ionotropic NMDA and AMPA/kainite receptors) that induce calcium influx – is a key mechanism of neuron death in stroke, cerebral trauma, and a broad range of neurodegenerative disorders.1,2 It has long been recognized that oxidative stress is an obligate mediator of excitotoxic neuronal apoptosis.3,4 Recently, Brennan and colleagues have presented cogent evidence that the primary source of superoxide in mouse cortical neurons exposed to NMDA is the NOX2 isoform of NADPH oxidase, which is activated via certain isoforms of PKC.5 Moreover, inhibition of NADPH oxidase with apocynin or with p47phox antisense prevents NMDA-triggered neuronal death in culture, and, in mice treated with apocynin prior to intra-hippocampal injection of NMDA, prevents hippocampal neurodegeneration. Likewise, p47phox knockout mice were resistant to NMDA-induced neurodegeneration. Superoxide is particularly toxic in the context of neuronal calcium overload owing to concurrent activation of nNOS; simultaneous production of superoxide and nitric oxide generates the potent neurotoxin peroxynitrite, which can induce neuron death via either apoptosis or necrosis. A further effect of peroxynitrite is to impair the function of glutamate transporters in astrocytes and neurons, slowing the clearance of extracellular glutamate and thereby potentiating the excitotoxic stimulus.6

These findings appear to rationalize previous findings: namely, that HO-1 inducers protect neurons from excitotoxicity,7,8 and that phycocyanin prefeeding markedly reduces the hippocampal cell loss triggered by kainite administration.9 The HO-1 product biliverdin, via conversion to bilirubin, is now known to inhibit NADPH oxidase, and the biliverdin derivative phycocyanobilin (PhyCB) – the chromophore of phycocyanin – can mimic this effect.10 Moreover, statins have been shown to protect neurons from
excitotoxicity$^{15-17}$ – presumably reflecting, at least in part, the ability of these drugs to suppress isoprenylation and membrane translocation of Rac, a key mediator of NADPH oxidase activation.$^{18}$

Brennan’s group has also shown that hyperglycemia, by providing increased reductive power for NADPH generation, potentiates the activation of NADPH oxidase associated with excitotoxicity, likely explaining why stroke tends to have a more devastating impact in diabetics.$^{19}$

The likely ability of PhyCB to suppress intraneuronal superoxide generation during excitotoxic episodes should often be complementary to its inhibitory impact on peroxynitrite generation by activated microglia;$^{20}$ microglia-derived peroxynitrite is believed to be an additional mediator of neuronal death in various neurodegenerative conditions.$^{21}$

It should be noted that activation of NADPH oxidase by ionotropic glutamate receptors actually plays a physiological role, contributing to the phenomenon of long-term potentiation that is crucial for learning. Thus, slight learning impairment has been noted in young Nox2 knockout mice – whereas these mice tend to have superior memory function as they age, presumably reflecting counterproductively high cerebral oxidative stress in aging mice.$^{22}$

**Interleukin-6 Stimulates Intraneuronal NADPH Oxidase during Aging**

It hardly seems likely that ionotropic glutamate receptors are the only neurons receptors that can trigger intraneuronal NADPH oxidase activation. Indeed, Dugan and colleagues have recently shown that IL-6 boosts Nox2 expression and activity in neurons of the prefrontal cortex and hippocampus of mice.$^{23, 24}$ Moreover, they find that expression of IL-6 in brain and serum rises as mice age, and that this results in progressive loss of certain GABAergic interneuron crucial for spatial learning. Thus, spatial learning is better preserved during aging in IL-6 knockout mice; moreover, in normal mice, chronic treatment with a superoxide dismutase-mimetic drug likewise aids preservation of spatial learning. Since plasma IL-6 has access to brain parenchyma,$^{23}$ this may rationalize previous clinical reports correlating increased plasma levels of IL-6 with poorer cognitive function and decreased hippocampal gray matter in middle-aged subjects$^{25-27}$ – implying that chronic systemic inflammation (as in metabolic syndrome$^{26}$ or periodontal disease$^{28-30}$) may accelerate the modest cognitive decline associated with normal aging. A further exciting implication of this work is that PhyCB or other effective cerebral antioxidant measures may be useful, not only for aiding survival of stressed neurons subjected to stroke, trauma, seizures, or neurodegenerative inflammation, but also in aiding preservation of cognitive function during healthy aging.

While a role for microglial NADPH oxidase activity in the mediation of various neurodegenerative disorders has long been appreciated, the pathogenic impact of excessive intraneuronal NADPH oxidase activity has only been recognized more recently. No doubt future research will establish that additional agonists can compromise neuron function or survival by activating intraneuronal NADPH oxidase.

**Adjuvant Antioxidants**

The utility of PhyCB for protecting oxidatively stressed cerebral neurons would likely be complemented by concurrent use of other antioxidants that can act to ameliorate the downstream consequences of excessive neuronal superoxide generation by boosting expression of antioxidant enzymes and/or glutathione, or by directly scavenging oxidants. Lipoic acid, melatonin, N-acetylcysteine, coenzyme Q10,
and inosine (which gives rise to the peroxynitrite scavenger urate) may be clinically useful in this regard, in light of the many studies demonstrating that these agents can exert potent neuroprotective effects in rodents.\textsuperscript{31-44}

Taurine, although classified as an antioxidant owing to its ability to detoxify myeloperoxidase-derived oxidants, has anti-excitotoxic activity unrelated to its antioxidant function. Excitotoxicity provokes release of both taurine and GABA from neurons; these agents then activate post-synaptic GABA(A) receptors which promote membrane hyperpolarization and thereby quell the excessive calcium influx triggered by glutamate.\textsuperscript{45-49} Theoretically, it should be feasible to up-regulate this natural protective mechanism by boosting brain levels of taurine. Indeed, taurine supplementation can increase brain levels of taurine in mice by up to 50%; conversely, it is known that vegetarians have relatively low systemic levels of taurine owing to lack of dietary taurine.\textsuperscript{50-52} Hence, it is not surprising that supplemental taurine has proved protective in rodent models of neurotoxicity in which excitotoxicity plays a prominent role.\textsuperscript{53-55} Moreover, there is recent evidence that supplemental taurine can up-regulate brain GABA synthesis and slow the age-related decline in memory and retention in aging mice, possibly by boosting the function of GABAergic neurons such as those shown by Dugan to be vulnerable to oxidative stress.\textsuperscript{56, 57} Thus, supplemental taurine may be a worthy component of a full-spectrum antioxidant regimen, centered on PhyCB, intended to preserve optimal brain function throughout life by controlling acute or chronic oxidative stress. Although such regimens may seem unduly complex for practical use, it should be feasible to devise specialty supplements and functional foods which could make such supplementation reasonably convenient for consumers.

References


(3) Sheldon AL, Robinson MB. The role of glutamate transporters in neurodegenerative diseases and potential opportunities for intervention. \textit{Neurochem Int} 2007 November;51(6-7):333-55.


(6) Sanganahalli BG, Joshi PG, Joshi NB. NMDA and non-NMDA receptors stimulation causes differential oxidative stress in rat cortical slices. \textit{Neurochem Int} 2006 October;49(5):475-80.


acid: implications for aging and age-related neurodegenerative disorders. *Neurochem Int* 2005 January;46(2):159-68.


