nNOS at a glance: implications for brain and brawn

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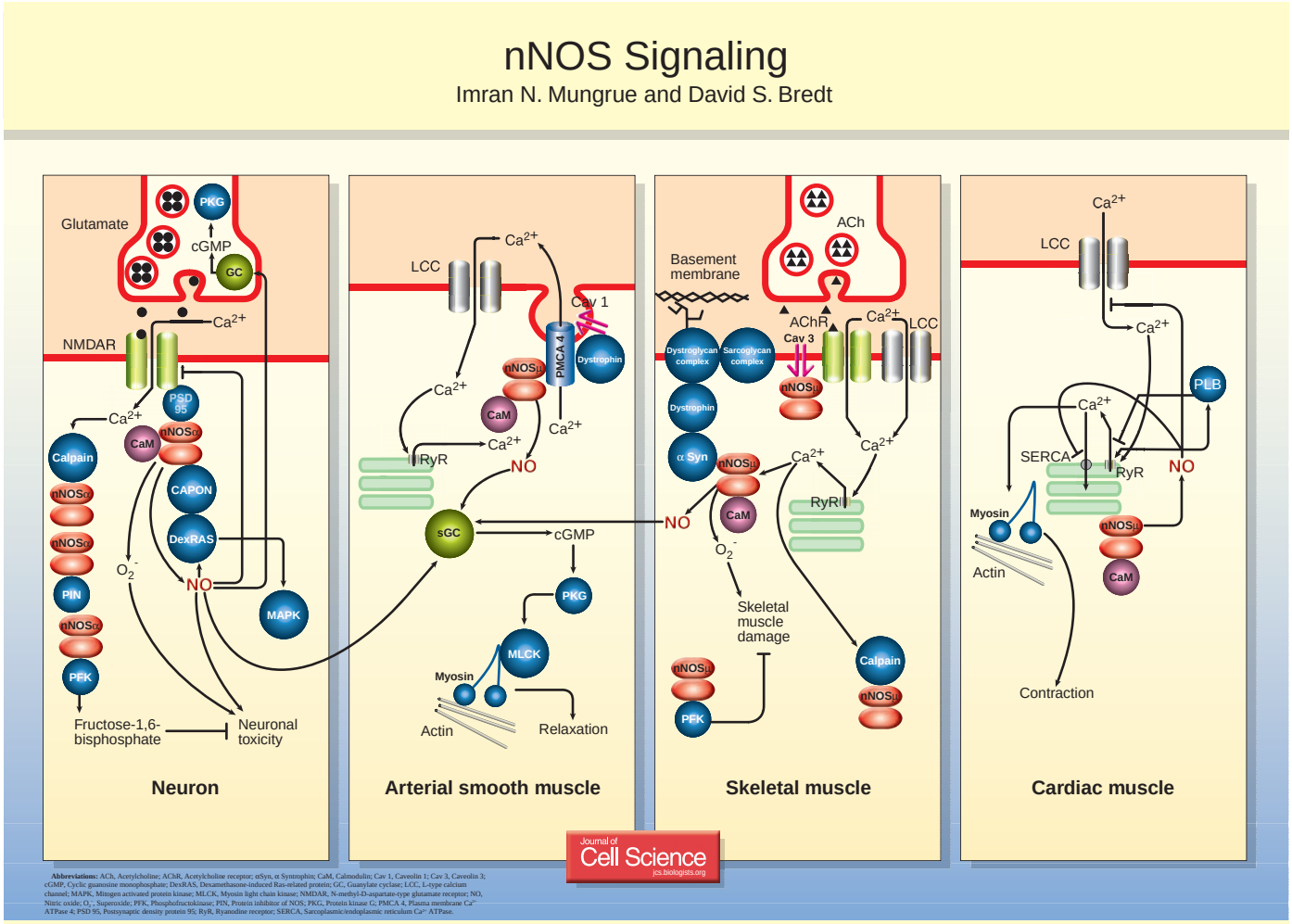
Nitric oxide (NO), formed enzymatically from L-arginine, functions as an endogenous signaling molecule in numerous organs and tissues throughout the animal and plant kingdoms. The first NO synthase (NOS) was isolated from mammalian brain and named neuronal NOS (nNOS, aka: NOS1) owing to its localization in neurons (Bredt et al., 1990; Bredt and Snyder, 1990). NO plays several important roles in the brain, including in regulation of synaptic signalling and plasticity. Additionally, high levels of nNOS protein are present in skeletal muscle (Brenman et al., 1995), where NO controls muscle contractility (Kobzik et al., 1994) and local blood flow (Thomas et al., 1998). nNOS activity is primarily regulated by increases in intracellular Ca^{2+}, which activate nNOS through calmodulin binding (Bredt and Snyder, 1990). NOS enzymes are homodimeric proteins. Recent studies show that NO actions in brain and muscle also rely crucially upon the association of nNOS with specific protein complexes in neurons and muscle cells, respectively. These physical interactions with nNOS allow for integration of NO signalling into distinct transduction cascades in specific cell types.

Neuronal nNOS

In the brain, the 160kDa nNOSa is the predominant splice variant, and contains an N-terminal PSD/Discs-large/ZO-1 homologous (PDZ)-binding domain, which anchors this complex to the postsynaptic density in the vicinity of the N-methyl-D-aspartate type-glutamate receptor (NMDAR). The PDZ domain of nNOS binds to a similar PDZ domain from the postsynaptic density protein, PSD-95, which in turn binds to the cytosolic tail of the NMDAR (Christopherson et al., 1999). These molecular interactions explain how Ca^{2+} influx through NMDA receptors is



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efficiently coupled to NO synthesis and activity (Sattler et al., 1999). Following its synthesis at postsynaptic sites, NO may diffuse back to the presynaptic terminal (Haley et al., 1992; Shibuki and Okada, 1991) and increase cGMP levels through activation of soluble guanylate cyclase (GC) (Boulton et al., 1994, 1995).

This membrane-localized nNOS complex is further linked to cytoplasmic signal transduction pathways via the physical interaction of nNOS with DexRas 1 and the adapter protein CAPON (Fang et al., 2000), which might activate a downstream MAP kinase cascade and modulate nuclear transcription. Functionally, nNOS might also represent a central component that regulates synaptic transmission and intercellular signaling, through negative regulation of the NMDAR by S-nitrosylation (Kim et al., 1999) and NO-dependent activation of DexRas (Fang et al., 2000). Additionally, the half-life of neuronal nNOSa protein is regulated by the Ca^{2+} sensitive protease calpain (Hajimohammadreza et al., 1997).

Whereas the small quantities of NO formed during synaptic transmission modulate neuronal signaling, excess NO mediates neurotoxicity in pathological situations, such as an ischemic stroke (Huang et al., 1994). This NO toxicity is accentuated in the presence of oxidative radicals such as O_2^-, which can also be generated by nNOS (Pou et al., 1992). Interestingly, nNOS-expressing neurons are spared from injury associated with elevated NO, which might partly be because of the physical association of nNOS with phosphofructokinase-M (PFK), the rate-limiting enzyme in glycolysis (Firestein and Bredt, 1999). Consequently, while therapeutic modulation of nNOS represents a potentially important approach in the setting of several clinically important neurological diseases, the balance between positive and negative effects of nNOS derived NO in the brain are complex and must be carefully weighed.

Skeletal muscle nNOSm

Skeletal muscle contains an alternatively spliced nNOSμ isoform that, when translated, results in the addition of a 34 amino acid segment within the reductase domain (Silvagno et al., 1996). NO is formed in contracting muscle, diffuses out of the muscle fibers and dilates adjacent blood vessels (Persson et al., 1990), by activating soluble guanylate cyclase (sGC) in arterial smooth muscle. This pathway helps to link skeletal muscle activity to increased local blood flow. Skeletal muscle nNOSμ is bound to the dystrophin associated protein complex through interaction of the nNOSμ PDZ domain and α-syntrophin (Brenman et al., 1996). Importantly, mutations of dystrophin (Brenman et al., 1995) or sarcoglycan (Crosbie et al., 2002) that underlie human muscular dystrophy cause a selective loss of nNOSμ from muscle membranes and thereby impair local blood flow (Grange et al., 2001). Furthermore, transgenic restoration of nNOSμ alleviates pathology in animal models of muscular dystrophy (Wehling et al., 2001), suggesting that NO augmentation represents a strategy to treat certain muscular dystrophies. Similar to nNOSα in brain, nNOSμ protein turnover in skeletal muscle is also regulated by Ca^{2+}-dependent calpain degradation (Laine and de Montellano, 1998).

Cardiac Muscle nNOSμ

An nNOS protein with the same electrophoretic mobility as nNOSμ localizes to the sarcoplasmic reticulum of cardiac muscle (Xu et al., 1999), and might be associated with the ryanodine receptor (Sears et al., 2003). The role of nNOS in the cardiac myocyte is complex and might regulate Ca^{2+} dynamics through activation of the ryanodine receptor (RyR), inhibition of sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) or the L-type Ca^{2+} channel, or through increasing phospholamban (PLB) protein levels (Sears et al., 2003). Interestingly, cardiac defects are common in muscular dystrophy (Emery, 2002) and are correlated with the down-regulation of cardiac nNOS expression (Bia et al., 1999). Future studies examining the roles of nNOS in the heart have important clinical implications. However, owing to the complex and crucial roles for nNOS and NO in cardiomyocyte signaling, and the potential for superoxide generation from excessive nNOS activity, therapeutic modulations must be performed with care to prevent adverse cardiac effects.

Smooth muscle nNOS

While endothelial NOS (eNOS)-derived NO is important in the regulation of arterial physiology and blood pressure, the identification of nNOS and nNOSμ in arterial smooth muscle (Boulanger et al., 1998; Schwarz et al., 1999) suggests that nNOS also participates in the regulation of vascular perfusion. Furthermore, neuron- (Hara et al., 1996) or skeletal-muscle-derived (Lau et al., 2000) NO generated from nNOS might also relax blood vessels, indicating that eNOS is not the sole modulator of NO-dependent arterial tone. Recent evidence also suggests that nNOS in smooth muscle is localized to caveoli in association with caveolin 1 and the plasma membrane Ca^{2+} efflux pump 4 (PMCA 4) (Schuh et al., 2001). By extruding Ca^{2+}, PMCA 4 might serve a role in the negative regulation of nNOS in the caveoli micro-domain and limit NO generation (Schuh et al., 2001).

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