Research progress on neurobiology of neuronal nitric oxide synthase

Chun-Xia LUO, Dong-Ya ZHU

Department of Pharmacology, School of Pharmacy, Laboratory of Cerebrovascular Disease, Nanjing Medical University, Nanjing 210029, China

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Abstract: Neuronal nitric oxide synthase (nNOS) is mainly expressed in neurons, to some extent in astrocytes and neuronal stem cells. The alternative splicing of *nNOS* mRNA generates 5 isoforms of nNOS, including nNOS- α , nNOS- β , nNOS- μ , nNOS- γ and nNOS-2. Monomer of nNOS is inactive, and dimer is the active form. Dimerization requires tetrahydrobiopterin (BH₄), heme and *L*-arginine binding. Regulation of nNOS expression relies largely on cAMP response element-binding protein (CREB) activity, and nNOS activity is regulated by heat shock protein 90 (HSP90)/HSP70, calmodulin (CaM), phosphorylation and dephosphorylation at Ser847 and Ser1412, and the protein inhibitor of nNOS (PIN). There are primarily 9 nNOS-interacting proteins, including post-synaptic density protein 95 (PSD95), clathrin assembly lymphoid leukemia (CALM), calcium/calmodulin-dependent protein kinase II alpha (CAMKIIA), Disks large homolog 4 (DLG4), DLG2, 6-phosphofructokinase, muscle type (PFK-M), carboxy-terminal PDZ ligand of nNOS (CAPON) protein, syntrophin and dynein light chain (LC). Among them, PSD95, CAPON and PFK-M are important nNOS adapter proteins in neurons. The interaction of PSD95 with nNOS controls synapse formation and is implicated in N-methyl-D-aspartic acid-induced neuronal death. nNOS-derived NO is implicated in synapse loss-mediated early cognitive/motor deficits in several neuropathological states, and negatively regulates neurogenesis under physiological and pathological conditions.

Keywords: neuronal nitric oxide synthase; cAMP response element-binding protein; post-synaptic density protein 95; synaptogenesis; neurogenesis

1 Introduction

Nitric oxide (NO) is an important biological messenger that plays a prominent role in the physiologic function of the central nervous system (CNS)^[1]. NO is generated from the catalytic combination of *L*-arginine with molecular oxygen by a family of 3 isoforms of nitric oxide synthase (NOS). There are 3 genetically different isoforms of NOS that account for NO production, including neuronal nitric oxide syn-

thase (nNOS) found in neuronal tissues, inducible nitric oxide synthase (iNOS) whose synthesis is induced by pro-inflammatory cytokines or endotoxin, and endothelial nitric oxide synthase (eNOS) expressed in endothelial cells^[2,3]. nNOS and eNOS are constitutively expressed. Their activities are calcium-dependent, while the activity of iNOS is calcium-independent, since it is fully activated at the basal level of intracellular calcium^[4]. nNOS activity was identified first in the cerebellum, and later in skeletal muscle, ileum, kidney, testis, skin, and neutrophils^[5,6]. Different members of the NOS family are known to regulate different functions. nNOS-derived NO constitutes the largest proportion of NO in the body^[2] and mediates numerous physiological and patho-

Corresponding author: Dong-Ya ZHU

Tel & Fax: +86-25-86862818

E-mail: dyzhu@njmu.edu.cn

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physiological functions. In the CNS, nNOS is co-localized with the N-methyl-D-aspartate receptors (NMDARs) and its activity is associated with NMDAR-mediated calcium influx^[7]. NO acts as a neuromodulator that regulates synaptic plasticity^[8], sleep, appetite, body temperature and neurosecretion^[9-11]. It is also involved in neurodevelopment^[12] and in the regulation of expressions of various genes^[13]. In several neurodegenerative disorders such as stroke^[14,15], Parkinson's disease (PD), Alzheimer's disease (AD), and amyotrophic lateral sclerosis (ALS)^[4], changes in nNOS expression have been detected. Excessive production of NO may exert neurotoxicity, particularly in the presence of oxidative stress, since NO may react with superoxide to form peroxynitrite^[16]. Thus, NO could contribute to the pathogenesis of neurodegenerative disorders^[4]. On the other hand, moderate amount of NO can exert neuroprotective effects^[17]. The present review aimed to discuss the research advances on nNOS over the last decade,

including its structure, expression, activity regulation and adapter proteins, as well as its role in synaptogenesis, synapse loss, neurogenesis and neuronal death (Fig. 1).

2 Chemical structure

Of the 3 isoforms of NOS described, nNOS was originally purified and cloned from the neuronal tissue and recognized as an isoform of NOS expressed in both immature and mature neurons^[18,19]. Recently, nNOS has been shown to be more widely distributed in other types of cells in brain, including astrocytes^[20] and neuronal stem cells^[21,22]. Additionally, other non-neuronal cell types including cardiac myocytes^[23], skeletal myocytes^[24], and the adventitia of a subset of neuronal blood vessels^[25] also express nNOS. The expression pattern of nNOS is further complicated by mRNA splicing^[3] that leads to the generation of 5 isoforms (Fig. 2). Among these isoforms, nNOS- α and nNOS- μ have

Fig. 1 An overview of the 5 nNOS isoforms, and their expression regulation, activity regulation, and implications in synaptic plasticity, neurogenesis and neuronal death.

Fig. 2 Splice variants of rat nNOS. nNOS-α: amino acids 1-1433; nNOS-β: amino acids 236-1433 of nNOS-α; nNOS-γ: amino acids 336-1433 of nNOS-α; nNOS-μ: amino acids 1-1433 of nNOS-α with a 34-amino-acid insert in the FMN-binding domain. nNOS-2 (detected in mouse): amino acids 1-1433 of nNOS-α with 504-608 amino acids deleted.

an N-terminal post-synaptic density (PSD)/Disc-large/ZO-1 homologous (PDZ)-binding domain that anchors them to specific subcellular structures, while nNOS-β and nNOS-γ are thought to be localized in the cytoplasm. nNOS-2 (detected in mouse nNOS) is a natural variant of nNOS, lacking amino acid residues 504-608 in nNOSα. Only nNOS-α and nNOS-β contribute significantly to NO production in the brain^[26]. nNOS-α, a protein that consists of 1 434 amino acids with a predicted molecular weight of 160.8 kDa^[1], is the full-length form of nNOS and is expressed in brain, skeletal muscle, cardiac muscle, and arterial smooth muscle. It is active as a homodimer and its monomerization leads to its ubiquitylation and degradation by the proteasome pathway^[27]. The PDZ domain at its amino terminal end mediates its interaction with various proteins^[28]. For example, the PDZ domain of nNOS-α binds to a similar domain from the post-synaptic density protein 95 (PSD95), localizing nNOS-α to the post-synaptic density region, which is important for synaptic plasticity^[26]. nNOS-β is an important source of NO in certain brain regions such as the cortex and the striatum^[29]. nNOS-β is produced by translation initiation within exon 1a, which contributes to the lack

of the first 236 N-terminal amino acids in nNOS-α. nNOS-β also lacks the PDZ domain in nNOS-α^[26]. The physiological roles of different splice variants in the brain remain still elusive. The catalytic activities of nNOS-β and nNOS-γ heterologously expressed in COS cells were reported to be 80% and 3% of nNOS-α activity, respectively^[29]. Heterologous expression of TnNOS, an mRNA transcript encoding a human equivalent of the mouse nNOS-γ, in Chinese hamster ovary cells gave a calcium-dependent NOS producing comparable amounts of NO as did the nNOS-α-transfected cell lines, which is contrast with the very low NOS activity of mouse nNOS-γ^[3]. nNOS-μ has an additional 34-amino acid insertion between the calmodulin (CaM)- and flavin-binding domains, and is the most extensively characterized nNOS splice variant. Its kinetic parameters $V_{\max}$ and K_m , and its regulation by Ca^{2+} -CaM are found to be essentially identical to those in nNOS-α^[30]. nNOS-2 has a deletion of a highly conserved region of nNOS-α that is critical for *L*-arginine binding, and this leads to the proposition that nNOS-2 may be catalytically inactive and therefore functions as a dominant negative regulator of nNOS activity^[3]. Recently, it has been demonstrated that nNOS-2

and nNOS-1 (nNOS- α , nNOS- β , nNOS- γ and nNOS- μ) play opposite roles in morphine actions. Downregulation of nNOS-1 can prevent the appearance of morphine tolerance. In contrast, downregulation of nNOS-2 by using an antisense probe targeting a unique splice junction can impair the analgesic activity of morphine^[31,32].

nNOS is inactive in the monomer form, and dimer is its active form. Dimerization requires tetrahydrobiopterin (BH₄), heme and *L*-arginine binding^[1]. nNOS monomer exhibits a bidomain structure containing an oxygenase domain (N-terminal) and a reductase domain (C-terminal) which can be separated by a CaM-binding motif^[3]. The oxygenase domain which binds the substrate *L*-arginine contains a BH₄-binding site and a cytochrome P-450-type heme active site. Also, there is a binding site for Zn which facilitates nNOS dimerization. The reductase domain which binds the substrate NADPH contains a binding site for flavin mononucleotide (FMN) and flavin-adenine dinucleotide (FAD)^[33-35]. Within the FMN-binding domain, there is an autoinhibitory loop that controls nNOS activity. Electrons (e⁻) donated by NADPH can transfer from the reductase domain to the oxygenase domain via FMN and FAD, and this electron flow process (NADPH→FAD→FMN→heme) can be facilitated by Ca²⁺/CaM binding^[1].

3 Regulation of nNOS expression

The human *nNOS* gene displays the highest degree of transcriptional complexity due to the alternative usage of 12 exon 1 variants scattered over 50 kb^[6]. *nNOS* expression is potentially regulated by more than 9 separate alternative first exons, which splice to a common exon 2 that contains a large 5' untranslated region (UTR) before the start methionine^[5]. Human *nNOS* transcripts start with exon 2 UTR. Exon 2 UTR itself constitutes a promoter, which enables self-expression, while the exon 2 upstream flanking genomic region displays inhibitory activity in a cell type-specific manner^[36]. Mutational analysis has revealed that promoter activity of the human exon 2 UTR relies largely on 2 conserved cAMP response element-binding protein (CREB)/activator protein 1 (AP-1)-binding sites which mask activity of other cis-acting elements located within the first 345 bp of the exon 2 UTR. In

mouse, one of these additional cis-acting elements has been identified as a steroidogenic factor 1-binding site, which is not well conserved in human^[37]. Several *nNOS* exon 1 variants as well as the exon 2 UTR itself contain numerous ATG base triplets that may serve as start codons for μ ORFs, being able to confer posttranscriptional inhibition of gene expression^[38]. Expression of the human *nNOS* gene can be stimulated by various cAMP-activating agents due to increased transcription of either exon 1f or exon 1g^[6,39]. Several compounds that activate members of the CREB and AP-1 families of transcription factors have been identified. In human neuronal A673 cells, *nNOS* mRNA expression could be stimulated by dibutyl-*c*-AMP (db-*c*-AMP) (4.3 fold), basic fibroblast growth factor (bFGF) (2.5 fold), thapsigargin (4.3 fold), and valproic acid (2.5 fold), and the *nNOS* mRNA expression by db-*c*-AMP is protein kinase A (PKA)-dependent^[36]. In cortical neurons, *nNOS* exon 2 contains the regulatory sequences that respond to Ca²⁺. The Ca²⁺ entry through voltage-sensitive Ca²⁺ channels regulates nNOS expression through alternate promoter usage. Deletion and mutational analysis of the *nNOS* exon 2 promoter has revealed 2 critical cAMP/Ca²⁺ response elements (CREs), both of which are immediate upstream of the transcription start site^[40]. Elevated cytosolic Ca²⁺ may induce calmodulin-dependent kinase II (CaMKII) which in turn mediates phosphorylation of CREB family members^[41]. nNOS-derived NO may amplify CREB/AP-1-dependent responses due to amplification of calcium-induced gene stimulation in neuronal cells via PKA-dependent phosphorylation of CREB^[42].

4 Regulation of nNOS activity

4.1 Heat shock protein 90 (HSP90)/HSP70 At the post-transcriptional level, nNOS- α is regulated through phosphorylation, degradation, sub-cellular localization, and dimerization^[28]. HSP90 is important for nNOS- α stabilization, and it regulates nNOS dimerization through the facilitation of heme insertion in the monomer^[43]. However, nNOS- β may be another client protein for HSP90 since nNOS- β also aggregates after inhibition of HSP90^[28]. HSP90 can be inhibited physiologically by NO-mediated S-nitrosylation^[44]. Thus, a feedback regulatory mechanism may exist between nNOS and

HSP90, to regulate nNOS degradation and aggregation. HSP70 is another chaperone of nNOS. The 2 chaperones have opposite actions on nNOS ubiquitination, with HSP70 stimulating and HSP90 inhibiting. As nNOS undergoes toxic damage, the heme/substrate-binding cleft opens, exposing hydrophobic residues as an initial step of unfolding. As long as HSP90 forms even transient complexes with the opening cleft, ubiquitination by HSP70-dependent ubiquitin E3 ligases is inhibited. When unfolding of the cleft progresses to a state that cannot cycle with HSP90, HSP70-dependent ubiquitination is unopposed. In this way, the HSP70/HSP90 machinery makes the quality control decision for stabilization versus degradation of nNOS (Fig. 3)^[43,45].

4.2 CaM CaM has been reported to function as an allosteric activator of nNOS. In the absence of CaM and Ca^{2+} , electron flow from FAD to FMN will slow down^[46]. Binding of CaM to nNOS facilitates electron flow from NADPH to the reductase domain flavins and from the reductase domain to the haem centre^[1]. nNOS is inactive at the basal level of intracellular Ca^{2+} . When the intracellular Ca^{2+} level is increased due to some stimulating factors, CaM will bind to nNOS, leading to the activation of nNOS. In contrast, when intracellular Ca^{2+} concentration decreases to the basal level, CaM will dissociate from nNOS and nNOS returns to the inactive state^[47]. Despite the structural similarity among the 3 NOS isoforms, each isoform has a unique catalytic profile^[48]. The

differences between eNOS and nNOS are the greatest. eNOS has a 6-fold lower NO synthesis activity and a 6-16-fold lower cytochrome *c* reductase activity than nNOS^[49]. This can be explained by the fact that regulation of the FMN conformational equilibrium differs markedly between eNOS and nNOS^[50].

4.3 Phosphorylation Phosphorylation of nNOS has an effect on its activity^[3]. Protein phosphorylation is regulated by some kinases and phosphatases, such as PKA, CaMKs, PKC, and phosphatase 1. CaMKII phosphorylates nNOS at Ser847, which reduces nNOS activity by inhibiting Ca^{2+} -CaM binding. By contrast, protein phosphatase 1 decreases the level of nNOS phosphorylation at Ser847, leading to an increase in nNOS activity^[51]. Another phosphorylation site of nNOS is Ser1412. Phosphorylation at this site increases nNOS activity, whereas dephosphorylation by phosphatases decreases nNOS activity^[1,46].

4.4 Protein inhibitor of nNOS (PIN) The N-terminal extension of nNOS also contains a binding site for the 89-amino acid protein PIN. A previous study showed that PIN was only coimmunoprecipitated with nNOS but not with eNOS or iNOS^[52]. Nuclear Magnetic Resonance spectroscopy has been used to map the PIN-binding region of rat nNOS to Met228-His244^[53] and to determine that PIN binds to nNOS with a 1:2 stoichiometry^[3]. In fact, PIN neither inhibits nNOS nor promotes monomerization. Instead, it may function as a light chain of myosin and dynein participating in nNOS axonal transport rather than as an inhibitor of nNOS^[1].

4.5 Adapter proteins of nNOS nNOS has 9 primary interacting proteins, including post-synaptic density protein 95 (PSD95), clathrin assembly lymphoid leukemia (CALM), CAMKIIA, Disks large homolog 4 (DLG4), DLG2, 6-phosphofructokinase, muscle type (PFK-M), carboxy-terminal PDZ ligand of nNOS (CAPON) protein, syntrophin and dynein light chain (LC)^[54]. PSD95, CAPON and PFK-M are important adapter proteins of nNOS in neurons. The NH2 terminus of nNOS- α contains a PDZ domain that interacts with other proteins^[55-57]. PSD95, a multivalent synaptic scaffolding protein and a core component of the post-synaptic density, can link nNOS to NMDARs through PDZ-PDZ coupling, and accounts for the efficient activation of nNOS by NMDARs stimulation^[58].

Fig. 3 A schematic illustration of the HSP70/HSP90 machinery that makes the quality control decision for stabilization or degradation of nNOS.

NMDARs have attracted particular attention due to their crucial roles in excitatory synaptic transmission, plasticity, and neurodegeneration^[59]. NMDARs are tetramers composed of NR1 and NR2 (A-D), and rarely NR3 (A-B) subunits^[60]. nNOS is a critical signaling protein at the downstream of NMDARs and is implicated in apoptosis^[61]. PSD95 tethers nNOS to the mouths of NMDAR channels, and this selective colocalization is believed to underlie the source specificity hypothesis, which states that calcium influx through NMDARs is especially neurotoxic^[62]. Thus, complete ablation of PSD95 with antisense and dissociation of the entire PSD95 molecule from the NMDAR with PDZ₁₋₂ decoy constructs are neuroprotective in ischemia models^[58,63]. The PDZ₁ domain of PSD95 can interact with the COOH-terminus of NMDAR, while PDZ₂ is free to bind the NH₂-terminal region of nNOS. Both the nNOS PDZ domain and the adjacent β finger sequence are implicated in this interaction. Interaction between nNOS and PSD95 is important for glutamate-induced activation of p38 α , a stress-activated protein kinase, and the ensuing cell death, and the nNOS-PDZ₂ interface is a target for designing neuroprotective drugs^[64]. The interaction among NMDARs, PSD95 and nNOS is critical for nNOS activation and NO generation, leading to neuron and astrocyte apoptosis^[61].

CAPON is another important adapter protein of nNOS. It is a 55-kDa protein containing a C-terminal domain that binds to the PDZ domain of nNOS, and an N-terminal phosphotyrosine-binding (PTB) domain^[65]. CAPON interacts with Dexas1, a brain-enriched member of the Ras family of small G proteins and is selectively induced by dexamethasone. The interaction of nNOS with CAPON is implicated in NO delivery to Dexas1, leading to S-nitrosylation of Dexas1 on Cys11^[66]. Dexas1 binds to the peripheral benzodiazepine receptor-associated protein (PAP7), and PAP7 in turn binds to the divalent metal transporter (DMT1), an iron import channel mediating iron uptake in neurons^[67]. Dexas1 might participate in CNS neuronal cell death through regulating NO signaling by binding to nNOS and CAPON^[68]. CAPON is also associated with tumor suppressor protein Scribble, a protein that is found both presynaptically and postsynaptically. The Scribble-CAPON association is direct,

mediated through the PTB domain of CAPON and the fourth PDZ domain of Scribble. Overexpression of CAPON leads to an increase in dendritic protrusion, which depends on the PTB domain of CAPON, suggesting an important role of CAPON in mammalian synapses^[69].

Another adapter protein of nNOS is PFK-M. PFK-M binds to nNOS in skeletal muscle and in neurons through the interaction with the PDZ domain of nNOS. In brain, nNOS and PFK-M are both concentrated in synaptosomes, specifically in the synaptic vesicle fraction. The interaction of nNOS with PFK might have a neuroprotective effect due to the product of PFK activity, fructose-1,6-bisphosphate^[70].

5 nNOS with synaptogenesis and synapse loss

Postsynaptic scaffolding proteins of the membrane-associated guanylate kinase family, including PSD95, SAP97, and SAP102, play major roles in synapse organization and function^[71]. Among these proteins, PSD95 has been particularly studied because it is one of the major constituents of excitatory PSD in the CNS^[72], and is directly involved in synaptic plasticity^[73]. Overexpression of PSD95 in neurons is associated with modifications of the properties of synaptic transmission, which mimic and occlude long-term potentiation (LTP)^[74]. This effect appears to be related to the role of PSD95 in the synaptic targeting of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors^[75] and in the regulated expression of PSD95 during plasticity^[76]. More recently, it has been demonstrated that expression of PSD95 may induce changes in spine shape, a marked increase in the spine volume and an enlargement of the PSD, resulting in formation of synapses with complex and perforated or segmented PSDs. Interestingly, PSD95 overexpression is associated with an increase in nNOS expression in PSDs and the formation of multiinnervated spines (MISs), while deletion of the PDZ₂ domain of PSD95 would specifically prevent the increase of nNOS expression and MIS formation^[77]. In addition, PSD95 overexpression combined with siRNA-mediated down-regulation of nNOS may markedly reduce nNOS expression at the synapse and prevent MIS formation. The pharmacological blockade of nNOS also prevents MIS formation. Conversely, exogenous application of the NO

donor DETA-NONOate or enhancement of cGMP signaling, a classical downstream target of NO, could reproduce MIS formation in the entire slice culture without alterations of PSD size. Moreover, DETA-NONOate treatment could rescue the absence of MISs in PDZ₂-transfected cells, and the pharmacological blockade of nNOS interfered with spine and synapse development under control conditions. These findings suggest that PSD95 controls synapse formation through regulation of nNOS expression at the synapse, the release of NO, and most likely the activation of cGMP signaling in presynaptic axons^[77]. nNOS also regulates dendritic spine development through its interaction with CAPON^[69]. Neurotrophic factors promote dendritic growth by stimulating NO production and S-nitrosylation of histone deacetylase 2 (HDAC2)^[78].

Synapse loss, rather than cell death, is a main factor for cognitive decline in AD^[79,80], PD^[81], Huntington's disease (HD)^[82], multiple sclerosis (MS)^[83], and HIV dementia^[84] in patients and/or in animal models. Synaptic stripping from the motoneuron surface also occurs in the progression of several motoneuron pathologies, such as ALS, progressive muscular atrophy, and traumatical damage of adult motor axons^[85]. nNOS has been considered as a key molecule for detrimental synaptic changes. For instance, upregulation of nNOS is a common hallmark, occurring in motoneurons and reactive astrocytes in ALS^[86-88], in neurons and/or glial cells in PD and AD^[89-92], and in the striatum of HD models at early stages^[93,94]. Additionally, NO participates in projection refinement during development^[95] and in synapse loss suffered by motoneurons after motor nerve injury^[85,96]. nNOS-derived NO will be produced in response to beta-amyloid protein, and is indicated to be a key mediator of AD, since it may trigger mitochondrial fission, synaptic loss and neuronal damage, partly via S-nitrosylation of dynamin-related protein 1 (forming SNO-Drp1)^[97]. It has been revealed that *de novo* nNOS expression is sufficient to induce synapse loss from motoneurons at adult and neonatal stages. In brainstem slices from neonatal animals, this effect requires prolonged activation of the soluble guanylyl cyclase (sGC)/protein kinase G (PKG) pathway and RhoA/Rho kinase (ROCK) signaling. Synapse elimination involves paracrine/retrograde action of NO.

Furthermore, before bouton detachment, NO increases synapse myosin light chain (MLC) phosphorylation, which is known to trigger actomyosin contraction and neurite retraction. NO-induced MLC phosphorylation is dependent on cGMP/PKG-ROCK signaling. In adulthood, motor nerve injury may induce NO/cGMP-dependent synaptic stripping which strongly affects ROCK-expressing synapses, and increases the percentage of p-MLC-expressing inputs before synapse destabilization. This cascade could trigger synapse loss that underlies early cognitive/motor deficits in several neuropathological states^[98].

6 nNOS and neurogenesis

The dogma that new nerve cells are not generated in adult mammal brain has been challenged recently. Actually, more and more studies report that new cells are generated throughout the CNS well into adulthood in all mammals, including humans^[99-103]. Neurogenesis in adult brain, convincingly demonstrated within the subventricular zone of the forebrain (SVZ) and the subgranular layer of hippocampal dentate gyrus (DG)^[104-106], is a dynamic process regulated by environmental, endocrine, and pharmacological stimuli^[107-109]. Cerebral ischemia insults have been shown to trigger neurogenesis from neural stem cells or progenitor cells located in SVZ and DG^[110-112]. Newborn immature neurons then migrate into the ischemic lesion and differentiate into mature neurons, where they are functionally recruited in the CA1 region, striatum or the granule cell layer circuitry^[113-116], which raises the reparative potential of endogenous progenitors for neurodegenerative disorders, including stroke.

NO has been demonstrated to be a physiological inhibitor of neurogenesis in adult SVZ and the olfactory bulb^[117]. Mice treated with 7-nitroindazole (7-NI), an inhibitor of nNOS, exhibit an increase in the number of mitotic cells in the SVZ, the rostral migratory stream (RMS) and the olfactory bulb, but not in the DG. However, recently, inhibition of nNOS has been shown to be capable of enhancing progenitor cell proliferation in the DG in normal^[118] and in ischemic brain^[15]. Consistently, it is found that the number of cells generated in the olfactory subependyma and DG is augmented either by intracerebroventricular infusion of an NOS inhibitor, or by

null mutation of nNOS^[104]. Also, the anti-proliferative effect of nNOS-derived NO on SVZ and DG has been demonstrated in a rat model of cerebral ischemia^[119]. In addition, nNOS inhibition can reverse CMS-induced impairment in neurogenesis^[120]. All the above experimental results suggest a negative regulation on SVZ neurogenesis by endogenous NO derived from nNOS. Nevertheless, the role of nNOS in hippocampal neurogenesis remains controversial. In sharp contrast to the anti-proliferative effect of nNOS-derived NO in adult animals, NO donor administration has been reported to promote neurogenesis^[121,122]. Two factors may explain this inconsistency: the difference in experimental protocols, and the way of drug administration, since intravenous or hippocampal administration of a NO donor that elevates NO level may increase cerebral blood flow, indirectly affecting neurogenesis.

7 nNOS and neuronal death

NMDARs are heterotetramers consisting of NR1 and NR2 (A–D), and rarely, NR3 subunits^[123,124]. The synaptic NMDARs form large and dynamic signaling complexes in the postsynaptic membrane, mainly through the interaction of their intracellular NR2 C-terminus with PSD95^[125], a scaffolding protein that binds both NMDARs and nNOS at excitatory synapses and assembles them into a macromolecular signaling complex^[63,64]. PSD95 is an important signaling enzyme in the CNS. Activation of nNOS depends on its association with PSD95 and NMDARs-mediated calcium influx^[58]. These findings provide a framework for understanding NMDAR-mediated excitotoxicity. Evidence from knockout mice and other models demonstrates the contribution of nNOS to glutamate-induced neuronal death^[126]. In brain, nNOS exists in particulate and soluble forms^[127] and is mainly distributed in cytosol far from membranes in a patch-like form^[128]. nNOS is targeted to membranes by binding to syntrophin, PSD95, PSD93 or SAP9026. Only neurons lacking PSD95 or nNOS but not other proteins exhibit reduced excitotoxic vulnerability^[56]. Interestingly, solubility of nNOS is decreased following ischemia^[129]. Recently, it has been demonstrated that ischemia-induced neuronal death is caused by NMDAR-dependent nNOS translocation from cytosol to membrane

via nNOS-PSD95 interaction, and that disrupting nNOS-PSD95 interaction can prevent against NMDA-induced excitotoxicity and cerebral ischemic damage^[130].

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神经元型一氧化氮合酶的神经生物学研究进展

罗春霞, 朱东亚

南京医科大学药学院药理教研室, 南京医科大学脑血管病研究室, 南京 210029

摘要: 神经元型一氧化氮合酶(neuronal nitric oxide synthase, nNOS)主要表达于神经元, 在星形胶质细胞和神经干细胞中也有一定水平的表达。不同的mRNA 拼接形式产生了nNOS 蛋白的5种亚型, 包括nNOS- α 、nNOS- β 、nNOS- μ 、nNOS- γ 和nNOS-2。nNOS 单体不具催化活性, 二聚体是其活性形式。nNOS 单体发生二聚化需要四氢生物蝶呤、血红素以及L-精氨酸的结合。nNOS的表达在很大程度上依赖于cAMP反应元件结合蛋白的活化, 其催化活性的调节与热休克蛋白90/热休克蛋白70、钙调节蛋白、PIN 蛋白, 以及自身Ser847和Ser1412位点的磷酸化和脱磷酸化相关。能与nNOS相互作用的蛋白主要有9种, 包括突触后密度蛋白95(post-synaptic density protein 95, PSD95)、CALM、CAMKIIA、DLG4、DLG2、PFK-M、CAPON、syntrophin和dynein 轻链。其中PSD95、CAPON和PFK-M是神经元中最重要的nNOS 调节蛋白。PSD95与nNOS的相互作用能介导突触形成, 并参与N-甲基-D-天冬氨酸诱导的神经元死亡。此外, nNOS来源的一氧化氮还与多种神经病理状态早期突触丢失导致的认知、运动功能缺陷相关, 并对生理病理的成年神经发生起负调控作用。

关键词: 神经元型一氧化氮合酶; cAMP反应元件结合蛋白; 突触后密度蛋白95; 突触发生; 神经发生