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Regulation of NMDA receptor Ca^{2+} signalling and synaptic plasticity

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Abstract

NMDARs (*N*-methyl-D-aspartate receptors) are critical for synaptic function throughout the CNS (central nervous system). NMDAR-mediated Ca^{2+} influx is implicated in neuronal differentiation, neuronal migration, synaptogenesis, structural remodelling, long-lasting forms of synaptic plasticity and higher cognitive functions. NMDAR-mediated Ca^{2+} signalling in dendritic spines is not static, but can be remodelled in a cell- and synapse-specific manner by NMDAR subunit composition, protein kinases and neuronal activity during development and in response to sensory experience. Recent evidence indicates that Ca^{2+} permeability of neuronal NMDARs, NMDAR-mediated Ca^{2+} signalling in spines and induction of NMDAR-dependent LTP (long-term potentiation) at hippocampal Schaffer collateral-CA1 synapses are under control of the cAMP/PKA (protein kinase A) signalling cascade. Thus, by enhancing Ca^{2+} influx through NMDARs in spines, PKA can regulate the induction of LTP. An emerging concept is that activity-dependent regulation of NMDAR-mediated Ca^{2+} signalling by PKA and by extracellular signals that modulate cAMP or protein phosphatases at synaptic sites provides a dynamic and potentially powerful mechanism for bi-directional regulation of synaptic efficacy and remodelling.

Keywords

calcium; *N*-methyl-D-aspartate receptor (NMDAR); protein kinase; signalling; synaptic plasticity

Introduction

NMDARs [NMDA (*N*-methyl-D-aspartate) receptors] are glutamate-gated ion channels that are pivotal to the regulation of synaptic function. A striking feature of NMDARs is their high permeability to Ca^{2+} . NMDAR-mediated Ca^{2+} influx is essential for neuronal differentiation, neuronal migration, synaptogenesis, synaptic remodelling, long-lasting changes in synaptic efficacy, such as LTP (long-term potentiation) and LTD (long-term depression), and cognitive functions such as learning and memory [1–6]. Dysregulation of NMDARs is implicated in schizophrenia and the excitotoxic neuronal death associated with a number of brain disorders, including stroke, epilepsy, head trauma, Huntington's disease, Alzheimer's disease and AIDS dementia [4].

NMDARs are heteromeric assemblies of NR1, NR2 and NR3 subunits, which co-translationally assemble in the ER (endoplasmic reticulum) to form functional channels with

differing physiological and pharmacological properties and distinct patterns of synaptic targeting at excitatory synapses throughout the CNS (central nervous system) [1–3]. Additional molecular diversity arises by alternative RNA splicing of the NR1 subunit [7]. Studies involving mice deficient in NR1 demonstrate that it is a subunit essential for neurogenesis and survival [8]. Moreover, deletion of the NR2B gene results in mice with no synaptic NMDA responses; mice die soon after birth [9].

Targeting of NMDARs to synaptic sites is dynamically regulated in an activity-dependent manner and is thought to play a role in normal synaptic transmission and in some forms of NMDAR-dependent synaptic plasticity. Normal NMDAR activity requires accurate delivery and targeting to the synapse. Assembled NMDARs are targeted selectively to the postsynaptic side of glutamatergic synapses [10] and appear [together with AMPARs (α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors)] at nascent synapses within 1–2 h of initial axodendritic contact [11]. At mature synapses, NMDARs are delivered within hours of experience-dependent synaptic activation [11] and are reciprocally regulated by changes in synaptic activity [12–15]. The mechanisms governing these processes are largely unknown. Whereas the NR1 subunit is abundantly expressed in virtually all regions of brain, NR2A–D subunits are differentially expressed in developing and adult rat brain [16,17]. At the time of birth, NMDARs at synapses of the hippocampus and visual cortex contain primarily NR1 and NR2B subunits [1–4]. Over the course of postnatal development, there is a progressive inclusion of the NR2A subunit [17–20]. As NR1/NR2A channels display the fastest decay kinetics [21], this conversion in synaptic NMDAR subunit composition results in shortening of NMDAR-mediated synaptic currents in the visual cortex [20,22]. The change in subunit composition coincides with the closing of the critical period [21]. Studies by Bear and colleagues show that regulation of synaptic NMDAR subunit composition is not static, but rather dynamically and bi-directionally regulated by sensory experience, whereas dark-rearing delays the NR2A/B subunit switch, exposure to light rapidly increases levels of NR2A (in <2 h) [23,24]. Although the subunit switch was postulated to require local protein synthesis, a role for transcription of mRNA encoding NR2A was not addressed.

Synaptic NMDARs are localized to PSDs (postsynaptic densities), where they are structurally organized (and spatially restricted) in a large macromolecular signalling complex comprising scaffolding and adaptor proteins, which physically link the receptors to kinases and phosphoprotein phosphatases and other downstream signalling proteins [4,25]. PSD-95/SAP (synapse-associated protein)-100 and SAP-102 are synaptic scaffolding proteins and members of the large PSD-95 family of modular PDZ-containing proteins that anchor NMDARs in the PSD [26]. The NMDAR-mediated rise in postsynaptic Ca^{2+} activates a network of kinases and phosphatases that promote persistent changes in synaptic strength. Coupling of PKA (protein kinase A) and protein phosphatase-1 to synaptic NMDARs by the AKAPs (A-kinase-anchoring proteins) such as AKAP-9 (also known as yotiao) and AKAP-150 enables bi-directional regulation of NMDAR channel activity by PKA [27,28]. The present article reviews mechanisms underlying the regulation of NMDAR Ca^{2+} permeability, NMDAR Ca^{2+} signalling in spines and NMDAR-dependent synaptic plasticity by PKA.

Synaptic plasticity of NMDAR currents

The prevailing view is that NMDARs function as the trigger of LTP and LTD and that the primary expression mechanism of synaptic plasticity involves alterations in the number, phosphorylation state [5,29,30] and/or subunit composition [31] of synaptic AMPARs. Recent studies indicate that synaptic NMDAR number and/or subunit composition are also regulated in response to neuronal activity and sensory experience [4,32,33], and that

NMDARs not only serve as the trigger of synaptic plasticity, but also may contribute to the expression of LTP and LTD [34–39]. In adult rats, HFS (high-frequency stimulation) of the Schaffer collateral–CA1 synapse elicits LTP involving PKC (protein kinase C)-/Src-dependent synaptic incorporation of NMDARs [35]. At mossy fibre–CA3 pyramidal cell synapses, HFS potentiates NMDA synaptic currents in a PKC- and SNARE-(soluble *N*-ethylmaleimide-sensitive fusion protein-attachment protein receptor) dependent manner (LTP_{NMDA}) [38,39]. The LTP_{NMDA} requires activation of group I metabotropic glutamate receptors (mGluR1 and mGluR5). Low-frequency trains of glutamate uncaging over individual dendritic spines of CA1 neurons induce long-term depression (spine LTD_{NMDA}) of NMDAR-mediated synaptic currents that is Ca²⁺-dependent and requires activation of NR2B-containing NMDARs [40]. This stimulation paradigm reduces the Ca²⁺ rise per unit of NMDAR current in spines (also known as fractional Ca²⁺ of the NMDA synaptic currents). Such adaptive changes in NMDAR function might play an important role in metaplasticity and in stabilizing activity of neuronal networks.

NMDAR-mediated Ca²⁺ signalling in spines

Dendritic spines are morphological specializations that protrude from the main shaft of neuronal dendrites (for reviews, see [41–44]). Most excitatory synapses of the mature mammalian brain occur on spines, and a typical mature spine has a single synapse located at its head. The spine head contains the PSD, an electron-dense structure that comprises receptors, signalling proteins, adaptors and scaffolding proteins (for reviews, see [41–44]). Whereas some spine heads also have smooth ER, others are filled with ribosomes. The spine neck is thought to restrict diffusional exchange of signalling molecules between the spine head and the parent dendrite. Thus the spine functions to segregate Ca²⁺ and to integrate postsynaptic signals from different sources of Ca²⁺.

A small, but significant, fraction of the inward current through NMDARs is carried by Ca²⁺ ions (fractional Ca²⁺ current ~10%) [45–48]. Owing to the relatively small volume of the spine head, activation of synaptic NMDARs can trigger large rises in spine Ca²⁺ [44,49,50]. Considerable evidence indicates that NMDARs are the predominant channel that mediates subthreshold Ca²⁺ signals in spines of hippocampal neurons ([51–53], but see [58]). In the hippocampal CA1, NMDARs in individual spines exhibit heterogeneity in subunit composition and differing fractional Ca²⁺ currents dependent on NMDAR subunit composition [54]. Because NMDAR subunit composition in the hippocampus is developmentally regulated and exhibits a switch from being primarily NR2B-containing at early ages postnatal to primarily NR2A-containing spine Ca²⁺ would also be expected to be developmentally regulated. In addition NMDARs carry a substantial fraction of the total synaptic charge [55] and may be important for recurrent excitation in cortical networks [56].

Modulation of NMDARs by PKA

The role of cAMP/PKA signalling in the induction and late, protein synthesis-dependent, phase of NMDAR-dependent LTP at Schaffer collateral–CA1 synapses has attracted considerable attention. Pharmacological blockade of PKA reduces induction of NMDAR-dependent LTP at Schaffer collateral–CA1 synapses, suggesting a ‘gating’ role for PKA in LTP [57]. NMDARs are molecular targets of PKA phosphorylation [58,59]; however, the effects of PKA on NMDAR function are less clear. Activation of PKA or inhibition of PPI (protein phosphatase I) potentiates NMDAR-mediated currents [60–65]. Moreover, targeted mutation or disruption of AKAP-150, which links PKA to NMDARs and AMPARs, impairs protein synthesis-dependent [66] and-independent LTP [67].

Zukin and colleagues recently found that Ca²⁺ permeability of neuronal NMDARs, activity-dependent NMDAR-mediated Ca²⁺ signalling in dendritic spines and induction of NMDAR-

dependent LTP at hippocampal Schaffer collateral–CA1 synapses are under the control of the cAMP/PKA signalling cascade [62]. PKA blockers reduced the relative fractional Ca^{2+} influx through NMDARs as assessed by Ca^{2+} -reversal potential shift analysis (and the Goldman–Hodgkin–Katz equation) and by simultaneous recording and Ca^{2+} imaging of hippocampal neurons. PKA blockers shifted the E_{rev} of NMDA currents in the negative direction in a Ca^{2+} -dependent manner. PKA blockers produced little no impact on permeation of univalent ions through NMDARs. These findings are consistent with selective modulation by PKA of NMDAR Ca^{2+} permeability. Studies involving two-photon laser-scanning microscopy reveal that PKA blockers profoundly inhibit activity-dependent NMDAR-mediated Ca^{2+} rises in dendritic spines, with no significant effect on synaptic current [62]. Consistent with this, PKA blockers markedly attenuated induction of NMDAR-dependent LTP at Schaffer collateral–CA1 synapses, an effect reversed by a brief pulse of high extracellular Ca^{2+} to hippocampal slices before the tetanus [62]. These findings indicate that NMDAR-dependent synaptic plasticity is also under the control of the cAMP/PKA signalling cascade. Thus, by enhancing Ca^{2+} influx through NMDARs in spines, PKA can regulate the induction of LTP.

The effect of PKA on NMDAR Ca^{2+} permeation is developmentally regulated [62]. The impact of PKA blockers on NMDAR-mediated currents in immature neurons in culture is greater than that at synapses of hippocampal slices from mature animals. Similarly, the impact of PKA blockers was greater on NMDAR-mediated currents in immature [DIV (days *in vitro*) 7–10] neurons in culture. A possible scenario is that PKA exclusively modulates Ca^{2+} permeation through NMDARs and that the fractional Ca^{2+} current is substantially greater for NR2B-containing NMDARs than for NR2A-containing NMDARs [54]. However, NR1/NR2A and NR1/NR2B NMDARs expressed in HEK (human embryonic kidney)-293 cells show comparable fractional Ca^{2+} currents, as assessed by shifts in reversal potential [68]. In this case, our findings would predict that, whereas PKA selectively increases Ca^{2+} flux through NR2A-containing receptors, it modulates bivalent and (to a lesser extent) univalent ionic flux through NR2B-containing receptors. Future studies are warranted to distinguish between these possibilities.

PKA isoforms in NMDAR-dependent synaptic plasticity

Whereas the role of cAMP/PKA signalling in the induction of NMDAR-dependent LTP at Schaffer collateral–CA1 synapses is established [62,69], the identity of the PKA isoform involved in LTP was, until recently, unclear. To identify the PKA isoform involved in induction of NMDAR-dependent LTP at CA1 synapses, Zukin and colleagues examined hippocampal synaptic plasticity in mice lacking specific PKA regulatory subunits [70]. PKA RII β is the major regulatory subunit that links PKA to NMDARs at synapses. Stimulation by cAMP promotes dissociation of PKA catalytic subunits from RII regulatory subunits within dendritic shafts and trafficking subunits into spines [71]. In young [P (postnatal day) 10–P14] mice lacking the PKA RII β subunit, protein synthesis-independent NMDAR-dependent LTP at the Schaffer collateral–CA1 synapse in the hippocampus was deficient, but NMDAR-dependent LTD was normal [70]. This finding was of interest in that the RII β isoform is not required for normal NMDAR-mediated responses, excitatory synaptic transmission, presynaptic short-term plasticity or NMDAR-dependent LTD. This phenotype is unlikely to be caused by structural abnormalities, in that light and electron microscopy both showed that the brain has an overall normal structure and organization in PKA RII β -knockout mice [72]. In contrast, in young adult (P21–P28) mice lacking PKA RII β , presynaptic function (assessed by paired-pulse facilitation), excitatory synaptic transmission (assessed by input–output relationships) and NMDAR-dependent LTP was normal, but NMDAR-dependent LTD was abolished. These findings indicate that distinct PKA isoforms

may subserve distinct forms of synaptic plasticity and are consistent with a developmental switch in the signalling cascades required for LTP induction.

The finding that LTP is impaired at developing CA1 synapses of PKA RII β -knockout mice is consistent with findings by Crair and colleagues that LTP is impaired at developing thalamocortical synapses in the barrel cortex [73]. Thus the role of RII β in NMDAR-dependent LTP at developing synapses is not limited to Schaffer collateral-CA1 synapses. Our finding that LTP is normal at Schaffer collateral-CA1 synapses of PKA RII β -knockout mice at 3–4 weeks of age is consistent with those of Hell and colleagues, who show that mice expressing mutant AKAP-150 that cannot bind to PKA exhibit impaired LTP at 7–12 weeks but normal LTP at 4 weeks of age [67]. The finding that LTD is impaired at mature Schaffer collateral-CA1 synapses are consistent with findings by Daw and colleagues that PKA RII β is critical to NMDAR-dependent LTD at synapses of mature visual cortex [74]. This finding extends studies by Daw and colleagues in that it shows that PKA RII β subserves distinct forms of plasticity at CA1 synapses of the hippocampus at different developmental stages. Abel and colleagues found normal LTD and impaired protein synthesis-dependent LTP at CA1 synapses of 8–12-week-old mice deficient in PKA anchoring [66], suggesting a possible second ‘developmental switch’ in LTP signalling at even later ages. Targeted deletion of RII β severely reduces LTP at mossy fibre-CA3 synapses [75] and homosynaptic LTD and depotentiation (but not LTP) at Schaffer collateral-CA1 synapses of adult mice at 4–6 weeks of age [76]. Findings of Yang et al. [70] are consistent with these studies and extend them in that they identify a role not only for RII β , but also for RII β in LTD at CA1 synapses of adult mice.

Recent studies indicate that the signalling cascades involved in the induction phase of LTP at CA1 synapses are developmentally regulated. Whereas LTP is dependent upon CaMKII after P20, it is dependent on PKA at P7–P8 [77]. Consistent with this notion, findings by Zukin and colleagues showed that PKA RII β is critical to LTP at young ages (P10–P14), but not at more mature ages (P21–P28) [70]. These findings extend the work of Malenka and colleagues in that they identify for the first time a critical role for the PKA RII β subunit in LTP signalling in young, but not mature, rodent hippocampus [77]. The PKA RII β subunit binds directly to AKAP-150 [78,79], which in turn binds via PSD-95 and/or SAP-97 and tethers the PKA holoenzyme to the NMDAR NR2B subunit. At the time of birth, NMDARs in the hippocampus contain primarily NR1 and NR2B subunits. Over the course of postnatal development, there is a progressive inclusion of the NR2A subunit [1,3,4]. A possible scenario is that, at young ages, synaptic NMDARs are NR2B-containing and are more sensitive to regulation by PKA. At later ages, at a time when the number of spines has increased and synaptic connections have formed, NMDARs are primarily NR2A- or NR2A/NR2B-containing and become relatively less sensitive to regulation by PKA [62].

The finding of a critical role for the PKA RII β subunit in LTP signalling in young, but not mature, rodent hippocampus [70] is also consistent with findings of others that, early in development, PKA phosphorylation of GluR4-long is a primary mediator of activity-dependent synaptic incorporation of AMPARs, a mechanism thought to underlie enhanced synaptic strength [80,81]. GluR4 delivery requires PKA-dependent phosphorylation at Ser⁸⁴² in its C-terminal tail [80,81]. Thus a deficit in RII β , which is required for correct targeting of PKA to AMPARs, could account for impaired LTP in the CA1 cells of RII β -knockout mice. The finding that LTP is not completely abolished even at young ages [70] could be explained by a role for CaMKII-dependent, PKA-independent delivery of GluR2-long. In contrast, in adult hippocampus, activity drives PKA-independent insertion of GluR2-long, as well as PKA-dependent delivery of GluR1 [82]. These findings predict a slight impairment of LTP at CA1 synapses of adult RII β -knockout mice. Thus PKA phosphorylation of AMPARs mediates plasticity through synaptic incorporation of

AMPA early in development; in mature hippocampus, PKA is thought to play a gating role through phosphorylation and delivery of GluR1 [81]. An alternative scenario is that the RII β subunit contributes to PKA regulation of NMDAR Ca²⁺ permeability and thus modulates the induction of LTP in young mice. In addition to LTP, PKA RII β knockout is also involved in the regulation of LTD induction in young adulthood, but not early in development. Consistent with these findings, Type I and Type II regulatory subunits of PKA differentially regulate synaptic plasticity in the visual cortex. Whereas the PKA RI subunit is required for LTP and LTD at synapses on to layer II/III visual cortex cells, but not ocular dominance plasticity [83], the PKA RII β subunit is required for ocular dominance plasticity and LTD, but not LTP [74]. Pharmacological blockade by PKA abolishes all three forms of plasticity [84,85]. These studies establish PKA subunit-specific roles in different forms of plasticity within a given region. They do not preclude a possible developmental switch in LTP signalling as reported here and by others [77].

In summary, NMDAR-mediated Ca³⁺ signalling in dendritic spines is not static, but can be remodelled in a cell- and synapse-specific manner by NMDAR subunit composition, protein kinases and neuronal activity during development and in response to sensory experience. Recent studies show that NMDAR-mediated rises in spine Ca²⁺ and NMDAR-dependent synaptic plasticity are under the control of the cAMP/PKA signalling cascade and external signals that regulate cAMP. Targeted deletion of the RII β subunit or disruption of the PKA-binding site on AKAPs disrupts this regulation. Future studies are warranted to external identify the site(s) on NMDARs or receptor-associated proteins that serve as the molecular target of this functional interaction between PKA and synaptic NMDARs.

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Abbreviations used

AKAP	A-kinase-anchoring protein
AMPA	α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor
CNS	central nervous system
DIV	days <i>in vitro</i>
ER	endoplasmic reticulum
HFS	high-frequency stimulation
LTD	long-term depression
LTP	long-term potentiation
mGluR	metabotropic glutamate receptor
NMDA	<i>N</i> -methyl-D-aspartate
NMDAR	NMDA receptor
P	postnatal day
PKA	protein kinase A
PKC	protein kinase C
PSD	postsynaptic density

SAP synapse-associated protein

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