Translational Regulation of the N-Methyl-D-Aspartate Receptor Subunit NR1

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Key Words

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Abstract

Formation of functional N-methyl-D-aspartate (NMDA) receptor channels requires the essential NMDA receptor subunit NR1 and one or more of the modulatory subunits NR2A–D and in some cases an additional subunit NR3A or NR3B. Recent studies indicate that NR1 expression is regulated at translation under both physiological and pathological conditions. The rat pheochromocytoma cell line (PC12) has been used as a model system for NR1 gene expression studies. Characterization of the posttranscriptional regulatory mechanisms suggested the posttranslational degradation and translational regulation of NR1 protein in PC12 cells. In addition a recent study on the translational regulation of NR1 mRNA in intact brain identified two translationally distinct pools of NR1 mRNA. In this review we summarize the evidence for translational regulation of NR1 expression in PC12 cells and the brain.

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Introduction

N-Methyl-D-aspartate receptors (NMDARs) are a subset of ionotropic glutamate receptors that require the binding of both glutamate and glycine for activation [1]. In addition to their traditional role in mediating fast excitatory neurotransmission, NMDARs contribute to the regulation of neuronal gene expression and play key roles in the formation of neural networks during brain development and the formation of long-lasting memory in the adult brain. On the other hand, both NMDAR hypo- and hyperfunction are thought to contribute to the pathophysiology of a variety of neurological and psychiatric diseases [2, 3].

The formation of functional NMDARs requires an essential NR1 subunit, one or more NR2 subunits (NR2A–D) and in some cases additional NR3 subunits (NR3A, B) [1]. NMDARs in turn associate with additional proteins to form a large, dynamic, postsynaptic receptor complex [4]. Interestingly, the NR1 subunit contains the binding site for the coagonist glycine, while the NR2 subunits provide the glutamate binding sites. Recent studies indicate that association of NR1 with the two additional subunits NR3A or NR3B form glutamate-insensitive excitatory glycine receptors in recombinant expression systems [5].

The assembly of functional NMDARs, their subcellular location and the formation of the large NMDAR-signaling complex are highly regulated [6–12]. It has recently been shown that solitary NMDAR subunits are retained in the endoplasmic reticulum and rapidly degraded [13,

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14]. These recent results highlight the importance of posttranscriptional mechanisms in the regulation of NMDARs.

The lack of functional NMDARs in the rat pheochromocytoma cell line PC12 and the fact that these cells expressed only little NR1 protein despite the presence of NR1 and NR2 mRNAs presented the first evidence of posttranscriptional regulation of the expression of NMDARs [15, 16]. A discrepancy between the expression of NR1 mRNA and protein has now been observed in primary neuronal cultures and a number of physiological and pathophysiological states in the brain [17–21]. The finding that the transcription of NR1 mRNA does not appear to be subject to stringent transcriptional regulation and that the NR1 promoter appears to possess the features of a housekeeping gene is consistent with the notion that the discordance between NR1 mRNA and protein expression is under posttranscriptional control [22, 23].

In this review we summarize the evidence for translational regulation of NR1 expression in PC12 cells and the brain. Potential molecular mechanisms underlying this type of control of NMDA receptor expression and evidence of translational regulation of NR2 subunits are reviewed in the paper by VanDongen and VanDongen [24] in this volume. Intracellular trafficking of NMDA receptors is reviewed by Perez-Otaño and Ehlers [25]. The transcriptional regulation of NMDARs was reviewed previously by Dingledine et al. [1].

Posttranscriptional Regulation of NR1 Protein Expression in PC12 Cells

PC12 cells contain on average about 230 copies of NR1 mRNA molecules but only 50 molecules of NR1 protein [16, 26]. Comparing the NR1 mRNA and protein levels in PC12 cells with those in the brain, we found that the developing rat brain contained an average of 2- to 6-fold more NR1 protein per 100 pg of NR1 mRNA than PC12 cells [26].

In order to investigate whether the low levels of NR1 protein in PC12 cells were due to its rapid degradation, we performed an analysis of the turnover of NR1 protein in this cell line. We observed indeed that almost 80% of the total NR1 protein was rapidly degraded with a half-life of 1.6 h [26]. In addition, we found that most of the NR1 protein in PC12 cells was an immature form that was retained in the endoplasmic reticulum [26]. Adenovirus-mediated transfer of NR2 subunits resulted in the formation of functional heteromeric NMDA receptors in

PC12 cells and an increase in endogenous NR1 protein with no detectable change in NR1 mRNA levels [27]. Together, these data indicate that the lack of sufficient NR2 subunits leads to the posttranslational degradation of unassembled NR1 protein in PC12 cells.

A number of studies in primary neuronal cultures, such as hippocampal neurons [28] and cerebellar granule cells [29], indicate that posttranslational regulation of NR1 expression does occur in neurons as well as in PC12 cells. In both hippocampal neurons and cerebellar granule cells, NR1 protein is present in intracellular and surface compartments [28, 29]. In cerebellar granule cells the intracellular pool of NR1 was found to have a shorter half-life than the surface pool [29]. Furthermore, the intracellular pool consisting of unassembled NR1 was retained in the endoplasmic reticulum and destined for degradation [29]. Unlike the intracellular pool, the surface pool of NR1 consisted of NR1 subunits that were coassembled with NR2 subunits [29].

Degradation of unassembled NR1 subunits, however, proved not to be the only reason for the very low steady state level of NR1 protein in PC12 cells.

Investigations of the translational activity of NR1 mRNA in PC12 cells revealed that its translation appears to be inhibited at initiation [30]. Reporter gene expression studies further revealed that the 3'UTR of NR1 exerts a significant inhibitory effect on the translation in PC12 cells [30]. The comparison of the 3'UTR sequences of NR1 in rat, mouse and human showed high sequence conservation of a 420-bp segment [30]. This conserved stretch of sequence is capable of forming a secondary structure with a free energy of -16.5 kcal/mol. In PC12 cells this stretch of conserved 3'UTR sequence significantly inhibited translation in PC12 but not human embryonic kidney (HEK) 293 cells [30]. These data suggest that as yet unidentified cellular factors may regulate NR1 mRNA translation by binding to this conserved stretch of secondary structure in the NR1 3'UTR sequence. As the 3'UTR conserved sequence that caused the inhibition of NR1 mRNA translation is common to all splice variants, all NR1 isoforms may be subject to this form of translational regulation [30].

The NR1 mRNAs from rat and human also share a 46-bp sequence at the 5'UTR that is capable of forming a stem loop structure with a free energy of -9.0 kcal/mol [31, 32]. The NR1 mRNA also contains 2 transcription start sites at -276 and at -238 bp upstream of the translation start site. In PC12 cells, transcription starts at the upstream -276 site and the resulting NR1 mRNA therefore includes the conserved 46-bp sequence at the 5'UTR

[23]. Reporter gene expression studies, however, failed to show an inhibitory effect of the 5'UTR on NR1 mRNA translation in PC12 cells.

In contrast, unexpectedly, the presence of the NR1 5'UTR sequence appeared to reduce the inhibition produced by the 3'UTR sequence. In accordance with our results, the NR1 5'UTR did not inhibit the mRNA translation in the *Xenopus* oocyte and rabbit reticulocyte translation systems [33].

Translational Regulation of NR1 Protein Expression in the Brain

Motivated by the discovery of evidence for translational regulation of NR1 expression in PC12 cells, we evaluated the translational control of NR1 mRNA in the developing and mature rat brain. A comparison of the steady-state levels of NR1 mRNA and protein during brain development revealed a progressive increase in NR1 protein expression during rat brain development [26]. Therefore, we performed an analysis of the association of mRNA with ribosomes in order to assess directly the translational activity of NR1 mRNA [34].

The results of this analysis revealed the presence of two, translationally distinct pools of NR1 mRNA in the rat brain. One pool of NR1 mRNA was associated with large polyribosomes (polysomes) at all stages of postnatal brain development. A second NR1 mRNA pool corresponding to approximately half of the NR1 mRNA at postnatal day 4 (P4) was translationally blocked during early brain development. At P4, both NR1 mRNA pools were distributed throughout the brain. After P8, the translationally blocked NR1 mRNA pool became progressively active although brain translational activity decreased overall [35].

It is possible that the translationally blocked pool of NR1 mRNA corresponds to a 'rapid response' pool that can be activated for translation depending on the physiological requirements of the cell. Thus, NR1 protein expression may be subject to two levels of control: (1) posttranslational degradation of unassembled NR1 protein and (2) regulation of NR1 mRNA translation. Both of these regulatory mechanisms may in turn be controlled by the regulation of expression or the synthesis of NR2 and/or NR3 subunits. In fact, NR2 subunit expression is subject to both temporal and spatial regulation during brain development [33, 36–39].

Alternatively, the translationally blocked pool of NR1 mRNA in brain may represent a pool that is in transit to

dendrites. Localized protein synthesis in dendrites as a requirement for synaptic plasticity has been reported [40]. The involvement of NMDARs in activity-dependent synaptic plasticity is well established [41]. Translocation of NR1 mRNA and the regulation of the NR1 protein expression in dendrites have been demonstrated [42]. Regulation of their translation may be necessary at the early developmental period for the intense synaptic reorganization [35].

The underlying mechanisms involved in the translational regulation of the blocked pool of NR1 mRNA in brain have not been established. Translational inhibition of NR1 mRNA by its 3'UTR and its association with the cytoskeleton has been demonstrated in PC12 cells. Similarly, in brain, NR1 mRNA has been found to be associated with the cytoskeleton [35]. Cytoskeletal proteins have been reported to bind specifically to 3'UTR [43, 44]. Therefore, the conserved secondary structure of the NR1 3'UTR may serve dual functions such as stabilization and translational regulation of the NR1 mRNA in brain.

Conclusions

Both the translational regulation of NR1 mRNA and posttranslational degradation of NR1 protein contribute to the regulation of NR1 subunit expression during brain development. Characterization of molecular mechanisms underlying the NMDAR expression and function will be helpful to determine how neurons respond to developmental, environmental and pathological stimuli. The translational regulation of NR1, the essential subunit of NMDARs, may play a role in NMDAR-associated physiological functions such as the establishment of cortical circuits and synaptic plasticity underlying learning and memory as well as pathophysiological processes underlying cognitive decline and neurodegenerative and psychiatric disorders. A deficit in NMDA receptor activity is believed to be involved in the pathophysiology of psychotic symptoms in schizophrenia [45]. Unraveling the translational and posttranslational regulatory mechanisms of NR1 expression may identify novel targets for the development of drugs for the treatment of NMDAR-related diseases such as schizophrenia and depression.

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