BMC Pharmacology

Poster presentation

NMDA treatment stabilizes the mRNA encoding for α_2 subunit of the NO-sensitive guanylyl cyclase by decreasing AUF1 protein level through a NO-cGMP-dependent mechanism

Magdalena Torres*[1], Sandra Jurado[1], Elena López[1], Francisco M Reimunde[2], Santiago Lamas[2] and Fernando Rodríguez-Pascual[2]

Address: [1]Departamento de Bioquímica, Facultad de Veterinaria, Universidad Complutense, E-28040 Madrid, Spain and [2]Centro de Investigaciones Biológicas (CIB), Consejo Superior de Investigaciones Científicas (CSIC), Ramiro de Maeztu 9, E-28040 Madrid, Spain

Email: Magdalena Torres* - mitorres@vet.ucm.es

* Corresponding author

from 2nd International Conference of cGMP Generators, Effectors and Therapeutic Implications
Potsdam, Germany, 10–12 June, 2005

Published: 16 June 2005

BMC Pharmacology 2005, **5**(Suppl 1):P55 doi:10.1186/1471-2210-5-S1-P55

Background

Posttranscriptional mechanisms of gene regulation, particularly those affecting mRNA stability, are emerging as critical effectors of gene expression changes. Although the mechanisms determining mRNA turnover are poorly understood, they are generally believed to involve RNA-binding proteins recognizing specific RNA sequences. Best characterized among the RNA sequences that influence mRNA stability are AU-rich elements (AREs), usually found in the 3'untraslated regions (UTR). The stability of a particular mRNA is controlled by specific interactions between structural elements of the mRNA and RNA-binding proteins. Of these proteins the best studied are AUF1 proteins that destabilize and ELAV-like proteins that stabilizes different mRNAs.

The objective of the present investigation was to characterize the 3'UTR of the α_2 mRNA and to study the molecular mechanism accounting for the NMDA-dependent up-regulation of this mRNA in cerebellar granule cells in culture.

Results

As we have previously shown NMDA-treatment caused an increase of the NO-sensitive guanylyl cyclase α_2 mRNA. This effect is transcriptional and translational independent but depends on the mRNA turnover. We have cloned and sequenced the 3'flanking region of NO-sensitive guanylyl cyclase α_2 mRNA, using two oligonucleotide pairs (one of them designed inside the coding region), which amplify two overlapping products of 853 pb and 1167 pb. The length of the complete fragment is 1873 pb (GenBank accession number AY795577) and bears many AU-rich motifs, which may be targets for the binding of different proteins that may stabilize or destabilize the mRNA. These fragments were employed to synthesize radiolabeled RNA probes to perform RNA-protein binding assays and protein-RNA complexes were observed in the assay where the 853 pb fragment was incubated with nuclear extracts obtained from cerebellar granule cells. The mRNA encoding for α_2 was immunoprecipitated by using specific antibodies against AUF1, indicating that these proteins bind to this particular mRNA "in vivo" and may affect its stability. NMDA treatment effectively decreases the AUF1 protein levels in nuclear protein extracts and increases α_2 mRNA, both effects were prevented by ODQ and KT5823, inhibitors of guanylyl cyclase and cGMP-dependent protein kinase, which became activated by NMDA stimulation. Thus, NMDA increases α_2 mRNA stability in cerebellar granule cells by decreasing the level of AUF1 proteins through a mechanism that implies cGMP synthesis and activation of cGMP-dependent protein kinase.