IDENTIFICATION OF TRANSCRIPTION FACTORS ACTIVATING Na+/Ca2+ EXCHANGER ISOFORM 3 GENE IN BRAIN

1)Mascolo L. 2)Formisano L. 3)Guida N. 4)Laudati G. 5)Serani A. 6)Molinaro P. 7)Di renzo G. 8)Annunziato L.

University of Neaples Federico II Department of Neuroscience

Na+/Ca2+ exchanger 3 (NCX3), one of the three isoforms of the NCX family, is highly expressed in the brain and is involved in the maintenance of intracellular Na+ and Ca2+ homeostasis. NCX3 plays a fundamental role in the pathogenesis of ischaemic stroke as demonstrated by the fact that its ablation worsens the experimentally-induced ischemic damage. Until now, the transcription factors activating ncx3 gene expression in brain are still unknown. By a bioinformatic analysis we found that on ncx3 minimal promoter sequence (ncx3-br) there are putative binding sites for cAMP response element binding protein (CREB), Specificity protein 1 family (Sp1-4), Early Growth Response 1 (EGR1), activating enhancer binding protein 2 alpha (AP2) and GATA binding proteins (GATA1, GATA2 and GATA3); here we investigated the role of all these transcription factors in modulating ncx3 gene in rat cortical neurons. To this aim, luciferase experiments were performed in neurons (DIV7) co-transfected with constructs containing the cDNA of the above mentioned transcription factors and a vector containing ncx3-br sequence (pGL3-ncx3). Interestingly, we found that only GATA3 transfection significantly increased ncx3 promoter activity. Next, to confirm GATA3 involvement in modulating NCX3, we performed luciferase assay, qRT-PCR and Western Blotting analysis for NCX3 in cortical neurons transfected for 24 hours with the construct overexpressing GATA3 or with a specific siRNA for GATA3 (siGATA3), and we found that GATA3 overexpression and GATA3 silencing were able to significantly increase or reduce ncx3 luciferase activity, gene and protein expression, respectively. Finally, we demonstrated that site-direct mutagenesis of GATA sequence on ncx3-br was able to significantly reduce ncx3 promoter activity in cortical neurons, demonstrating that GATA3 acts in a sequence specific manner on ncx3-br promoter sequence. Collectively these results identify GATA3 as a new transcriptional activator of NCX3 in brain.