MicroRNAs (miRNAs) play a key role in post-transcriptional regulation of gene expression in almost every biological process, by interacting with complementary regions mainly within the 3’-UTR of target mRNAs. miRNAs interfere with translation and/or stability of the mRNA, thus leading to inhibition of protein synthesis. A single mRNA may be regulated by multiple miRNAs and, on the other hand, a single miRNA can regulate several mRNAs, thus modulating protein expression of several different genes simultaneously (3).

miRNAs have a fundamental role in nervous system development and function, with major involvement in neurogenesis, neuronal differentiation and survival, as well as in neuroplasticity (2). Recent studies suggest a possible contribution of miRNAs in the pathophysiology of neuropsychiatric disorders, including major depression (3, 4). Studies also suggested a possible involvement of miRNAs into the action of psychotropic drugs, such as the mood stabilizers lithium and valproate and antidepressants (AD) (5-9).

Aim of our study was to analyze whether treatment with two different AdS, fluoxetine (FLX), a selective serotonin reuptake inhibitor (SSRI), and desipramine (DMI), a tricyclic AD with predominant action on the noradrenergic reuptake, modulate rat hippocampal miRNome expression. Moreover, in order to assess the time course of AD treatments on the miRNome expression profile, treatments were performed for different time lengths: 3, 7, and 14 days.

RESULTS

8 miRNAs were similarly modulated by both FLX and DMI after 7 days of treatment

METHODS

9 rats for each experimental group were treated by i.p. injections with 10 mg/kg of drugs or vehicle for 3, 7 or 14 days. miRNome expression analysis was carried out by Quantitative Real Time PCR (qRT-PCR) reaction by using TaqMan Array rodent MicroRNA A+B Cards Set v3.0, according to the manufacturer’s protocol (Life Technologies). Briefly, total RNA including miRNAs was isolated from each hemi-hippocampus (randomly right or left) using mirVana miRNA Isolation Kit (Life Technologies) and then 100 ng of total RNA was retrotranscribed by means of MegaplexTM RT Primers and TaqMan MicroRNA Reverse Transcription Kit. The cDNA was then preamplified by using Megaplex Predesigned Primers (Life Technologies).

qRT-PCR was carried out by using the comparative CT (ΔΔCT) method. Raw Ct values were extracted from HiSeq SDX files using the Applied Biosystems SDS 2.3 software, with a threshold value of 0.1 and automatic baseline. Ct values were normalized by the ΔCT method on endogenous controls U6, U65, Y1 and snoRNA51. Statistical analysis was carried out with SAM (Significance Analysis of Microarrays software, version 4.0, Stanford University, http://www.stat.stanford.edu/tibs/SAM/false Discovery Rate <0.05). Bioinformatic analyses were performed in order to identify miRNA putative target genes and molecular pathways potentially involved by means of MiRMir (10). For each miRNA the top most significant targets were selected and included in the annotation analysis performed with Gene Ontology subcategories (Biological Processes, Molecular Function and Cellular Component) andKEGG pathways (exact Fisher test, p<0.05).