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Hippocampal subregion-specific microRNA expression during epileptogenesis in experimental temporal lobe epilepsy

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ABSTRACT

Since aberrant miRNA expression has been implicated in numerous brain diseases, we studied miRNA expression and miRNA regulation of important signaling pathways during temporal lobe epileptogenesis in order to identify possible targets for epilepsy therapy.

The temporal profile of miRNA expression was analyzed in three brain regions (CA1; dentate gyrus, DG; parahippocampal cortex, PHC) associated with epileptogenesis in a rat model for temporal lobe epilepsy. Tissue was obtained after electrically-induced status epilepticus (SE) at 1 day ($n = 5$), 1 week ($n = 5$) and 3-4 months ($n = 5$), and compared with control tissue ($n = 10$) using the Exiqon microRNA arrays which contain capture probes targeting all miRNAs for rat ($p < 0.01$, and a 1.5 fold up- or downregulation). Expression of three blood plasma miRNAs from the same group of rats was also investigated in rats in order to determine whether plasma miRNAs could serve as potential biomarkers of the epileptogenic process. Molecular pathways potentially altered by the expression of multiple miRNAs were identified using a web-based algorithm, DIANA.

In CA1 and DG, more upregulated than downregulated miRNAs were present during each stage after SE. The highest numbers of upregulated miRNAs were encountered during the chronic stage in the DG. In PHC, a high number of downregulated miRNAs were detected. Key pathways involved, based upon quantitatively altered miRNA expression were: axon guidance, MAPK signaling pathway, focal adhesion, TGFβ, ErbB-, Wnt- and mTOR signaling, and regulation of actin skeleton. Expression of plasma miRNAs was differentially regulated after induction of SE.

This study identified several signaling pathways possibly involved in temporal lobe epileptogenesis, not previously indicated by RNA microarray studies. These include miRNAs that regulate the ErbB and Wnt pathways and focal adhesion, which may represent interesting new targets for therapeutic interventions.

Introduction

Several large-scale genomic studies in both human and experimental temporal lobe epilepsy (TLE) have shown altered expression of genes associated with key biological processes. Such work has led to the development of new therapeutic strategies, some in preclinical trials (for review see Aronica and Crino, 2011; Pitkanen and Lukasiuk, 2011; Vezzani et al., 2010). More recently, microRNAs (miRNAs) have also been identified as a new class of post-transcriptional regulators of numerous biological processes within the central nervous system (Gantier, 2010; Quinn and O'Neill, 2011; Sonkoly et al., 2008). MiRNAs bind to mRNA targets leading to degradation of the transcript or repression of its translation. MiRNAs introduce a new level of regulatory complexity to pathogenic processes underlying different neurological disorders, including epilepsy (Bian and Sun, 2011). Differential expression of several miRNAs has been shown in animal TLE models (Aronica et al., 2010; Hu et al., 2012; Jimenez-Mateos et al., 2011, 2012; Liu et al., 2010; Omran et al., 2012; Risbud et al., 2011; Song et al., 2011), and human TLE (Kan et al., 2012; Omran et al., 2012). For example, specific miRNAs, such as miR-146a (Aronica et al., 2010; Iyer

Abbreviations: miRNAs, microRNAs; DG, dentate gyrus; PHC, parahippocampal cortex; MAPK, mitogen-activated protein kinase; TGFβ, transforming growth factor beta; mTOR, mammalian target of rapamycin; TLE, temporal lobe epilepsy; SE, status epilepticus; RIN, RNA Integrity Number; MEF2C, myocyte enhancer factor 2C; SV2A, synaptic vesicle protein 2A; Limk1, LIM domain kinase 1; ERK, extracellular signal-regulated kinase; JNK, c-JUN N-terminal kinase; MEK1, mitogen-activated protein kinase kinase.

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et al., 2012; Omran et al., 2012) and miR-134 (Jimenez-Mateos et al., 2012) are upregulated in both experimental and human epilepsy. The use of exogenous miRNA analogs or related RNAi (interference) approaches represents a promising new strategy for epilepsy therapy (antiepileptogenic therapies) (Boison, 2010). Interestingly, silencing miR-134 after status epilepticus (SE) in mice reduces the subsequent occurrence of spontaneous seizures and exerts neuroprotective actions (Jimenez-Mateos et al., 2012) and reduction of hippocampal miR-132 levels has been shown to reduce seizure-induced neuronal death (Jimenez-Mateos et al., 2011). Conversely, silencing of miR-146a induces a pro-inflammatory response exerting pro-ictogenic effects (Iori et al., 2013). These recent studies suggest that it might be possible to modify epileptogenesis using strategies that target miRNAs.

SE leads to altered expression of a large number of genes (Becker et al., 2003; Gorter et al., 2006; Lukasiuk et al., 2006): a concomitant qualitative and quantitative change in the expression of the associated miRNAs might also be expected. However, although several published studies have used miRNA arrays, a large-scale miRNA expression study at different stages of epileptogenesis has not been reported so far. The present study was designed to identify the spatiotemporal dynamics of a large number of miRNAs potentially involved in the epileptogenic process. We analyzed samples both at sequential time points (including the early stages of epileptogenesis) and in distinct brain areas considered to have different sensitivities to, and roles in, epileptogenesis. Moreover, we analyzed blood plasma expression levels of three miRNAs (known to be associated with the immune response (Danger et al., 2013; Davidson-Moncada et al., 2010; Liu et al., 2010; Xu et al., 2013) and found to be strongly upregulated in our array analysis), in the same groups of rats in order to investigate whether plasma miRNAs could serve as potential biomarkers of the epileptogenic process.

We used an established rat model for TLE in which SE is induced by electrical stimulation (Gorter et al., 2001). This model is characterized by extensive neuronal death, glial activation and mossy fiber sprouting during the first months after SE and spontaneous seizures that start after a short latent period of approximately 1 week (Gorter et al., 2001, 2002, 2006). The comparison of miRNA expression patterns between different brain regions within the same animal offers the unique possibility to detect the most relevant miRNAs and their associated pathways that are involved in TLE epileptogenesis, and may direct future strategies aimed at preventing or modifying the epileptogenic process.

Materials and methods

Experimental animals

Adult male Sprague Dawley rats (Harlan Laboratories, Horst, The Netherlands) weighing 300–500 g were used in this study, which was approved by the University Animal Welfare committee. The rats were housed individually in a controlled environment (21 ± 1 °C; humidity, 60%; lights on from 8:00 A.M. to 8:00 P.M.; food and water available ad libitum).

Electrode implantation and seizure induction

At 2–3 months of age, rats were anesthetized with an intramuscular injection of ketamine (57 mg/kg; Alfasan, Woerden, The Netherlands) and xylazine (9 mg/kg; Bayer, Leverkusen, Germany), and placed in a stereotactic apparatus. To record hippocampal EEG, a pair of insulated stainless-steel electrodes (70 μm wire diameter; tips were 80 μm apart) were implanted into the left dentate gyrus under electrophysiological control as described previously (Gorter et al., 2001). A bipolar stimulation electrode (distance between tips 500 μm) was implanted in the angular bundle. Several weeks after electrode implantation, rats underwent tetanic stimulations (50 Hz) of the hippocampus in the form of a succession of trains of pulses every 13 s. Each train had a duration of 10 s and consisted of biphasic pulses (pulse duration, 0.5 ms; maximal intensity, 500 μA). Stimulation was stopped when the rats displayed sustained forelimb clonus and salivation for minutes, which usually occurred within 1 h. Stimulation never lasted longer than 90 min. EEG signals were amplified via a field effect transistor on the headstage and then led to a differential amplifier (CyberAmp; Molecular Devices, Burlingame, CA), amplified (20×), filtered (1–60 Hz), and sampled by a seizure detection program at a frequency of 200 Hz per channel (Harmonie; Stellate Systems, Montreal, Quebec, Canada). EEG recordings were visually monitored and screened for seizure activity. Behavior was observed during electrical stimulation and several hours thereafter. Immediately after termination of the stimulation, periodic epileptiform discharges (PEDs) occurred at a frequency of 1–2 Hz and lasted several hours (SE). During this period rats had frequent seizures as observed by both their behavior and electroencephalography (EEG). The end of SE could be clearly defined by the disappearance of 1–2 Hz PEDs. In previous studies we have noticed that rats that exhibit SE for at least 4 h all develop seizures in later life (Gorter et al., 2001; van Vliet et al., 2004).

Seizure groups

Rats were killed at three successive time points: (1) at 1 day after SE (group D; acute stage; $n = 5$); (2) at 1 week after SE (group W; $n = 6$) (the rats in this group did not exhibit spontaneous seizures during the first week, i.e., they were in the latent period); and (3) at 3–4 months after SE (group M; $n = 5$); for this latter group, we selected rats that exhibited daily seizures; age-matched controls (3 months, $n = 5$ and 4–7 months of age, $n = 5$) that were implanted but not stimulated except for field potential recordings, were also included (group C; $n = 10$). The scheme is presented in Fig. 1a.

The EEG of all chronic epileptic rats was monitored for at least one week, to ensure that daily seizures occurred. When they exhibited an increasing number of seizures during the first 4 weeks after SE, they were disconnected and reconnected during the last week before killing, for quantification of their daily seizures. They were sacrificed around three to four months after SE.

Tissue collection

After decapitation, the hippocampus and the parahippocampal region [(PHC), which includes mainly the entorhinal cortex and parts

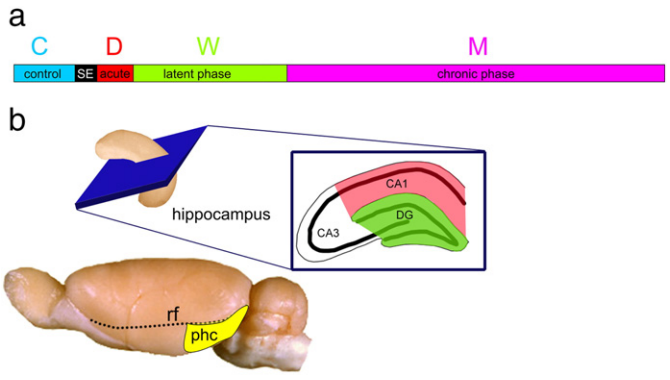

Fig. 1. a) Timeline representing the time points after status epilepticus (SE) when rats were sacrificed in order to determine the miRNA profiles. During the acute stage (1 day after SE; D) rats can still have occasional seizures in the aftermath of the SE. During the latent stage which on average lasts 1 week (W) rats do not exhibit seizures. Rats that were sacrificed during the chronic stage (3 months after SE; M) exhibited daily seizures. b) Schematic representations of the dissected areas from the hippocampus (CA1, CA3, DG) and the parahippocampal cortex (in yellow) that had been removed by dissection at the ventrocaudal part underneath the rhinal fissure.