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Research Paper

Myeloid differentiation factor 88 is up-regulated in epileptic brain and contributes to experimental seizures in rats

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ABSTRACT

Accumulating evidence supports that activation of inflammatory pathways is a crucial factor contributing to the pathogenesis of seizures. In particular, the activation of interleukin-1 beta (IL-1β) system exerts proconvulsant effects in a large variety of seizure models. Myeloid differentiation factor 88 (MyD88) is a critical adaptor protein in the signaling cascade elicited by IL-1β. The present study aimed to investigate the expression pattern of MyD88 in rat models of seizures and in patients with refractory temporal lobe epilepsy (TLE), and to study the role of MyD88 in epileptic seizures. Our results revealed that MyD88 was up-regulated in the hippocampus of rats in the lithium-pilocarpine model of acute seizures. Importantly, MyD88 overexpression was also significantly present in the brain from chronic epileptic rats and the temporal neocortex specimens from drug-resistant TLE patients. In the acute seizure model, both the behavioral and electrographic seizure activities were record and analyzed in rats for 90 min, starting immediately after pilocarpine injection. ST2825, a synthetic MyD88 inhibitor, was administered intracerebroventricularly (2.5–5.0–10 μg in 2 μl) 20 min before pilocarpine injection. We found that ST2825 at doses of 5 μg and 10 μg significantly inhibited the pilocarpine-induced behavioral and electrographic seizures. Moreover, 10 μg ST2825 prevented the proconvulsant actions of IL-1β. As previous evidence suggested that IL-1β proconvulsant effects was mediated by enhancing the phosphorylation level of the NR2B subunit of *N*-methyl-D-aspartate (NMDA) receptor, we then probed whether this molecular was involved in the effect of the pharmacological inhibition. Our results revealed that 10 μg ST2825 markedly reversed the increased Tyr^{1472}-phosphorylation of the NR2B subunit of NMDA receptor observed in the proconvulsant conditions of IL-1β and in seizures induced by pilocarpine alone. These findings indicate that altered expression of MyD88 might contribute to the pathogenesis of seizures and targeting of this adaptor protein might represent a novel therapeutic strategy to suppress seizure activities.

1. Introduction

Accumulating experimental and clinical evidence supports that activation of inflammatory pathways is a common factor contributing to the pathogenesis of seizures (Maroso et al., 2011b). Inflammatory responses, which are induced in the brain by infection, febrile seizures, stroke, and neurotrauma, are associated with acute symptomatic seizures and an increased risk of developing epilepsy (Pitkanen et al., 2015; Vezzani et al., 2013; Vezzani et al., 2015). Pronounced inflammatory processes have been described in the epileptic brain tissue in various experimental models of seizures and in patients affected by drug-resistant epilepsy. Experimental studies have shown that induction of specific inflammatory pathways contributes to seizures, and their pharmacological inhibition represents a potential strategy to reduce seizure activities (Vezzani et al., 2013; Vezzani et al., 2008).

Abbreviations: ANOVA, analysis of variance; Con, control; CPS, complex partial seizures; DD, death domain; EEG, electroencephalography; GFAP, glial fibrillary acidic protein; Iba1, ionized calcium-binding adaptor molecule 1; i.c.v., intracerebroventricular; IL-1, interleukin-1; rrIL-1β, rat recombinant IL-1β; IL-1R1, interleukin-1 receptor type 1; IL-1Ra, IL-1 receptor antagonist; i.p., intraperitoneally; KA, kainic acid; MAP2, microtubule-associated protein-2; MyD88, myeloid differentiation factor 88; NA, not applicable; NMDA, *N*-methyl-D-aspartate; PBS, phosphate-buffered saline; Pilo, pilocarpine; SD, Sprague-Dawley; SE, status epilepticus; SGS, secondary generalized seizures; SRS, spontaneous recurrent seizures; ST, ST2825; TIR, Toll/IL-1 receptor; TLE, temporal lobe epilepsy.

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Among the various inflammatory pathways that may be involved in the pathogenesis of seizures, the activation of the interleukin-1 beta (IL-1β) system attracts much interest (Maroso et al., 2011b; Vezzani et al., 2011). Studies in experimental models have demonstrated that IL-1β played a crucial role in neuronal network hyperexcitability underlying seizures via its functional receptor interleukin-1 receptor type 1 (IL-1R1) (Vezzani et al., 1999; Vezzani et al., 2000). Both IL-1β and L-1R1 were up-regulated in the experimental and human chronic epileptic tissue in brain areas involved in seizure generation and propagation (Ravizza et al., 2008; Vezzani et al., 1999). Pharmacological studies in experimental models of seizures demonstrated that the intracerebral application of IL-1β exacerbated seizures (Balosso et al., 2008; Vezzani et al., 1999). Blockade of IL-1β-mediated signaling using IL-1 receptor antagonist (IL-1Ra) had powerful anticonvulsant effects (Vezzani et al., 2000). Promisingly, Kenney-Jung et al. reported that the recombinant version of IL-1Ra (anakinra) has antiseizure effects in a child with super-refractory status epilepticus secondary to febrile infection-related epilepsy syndrome (Kenney-Jung et al., 2016). In addition, inhibition of IL-1β production strongly reduced seizure activities (Maroso et al., 2011a; Ravizza et al., 2006). These data indicate that elevated levels of IL-1β could result in proconvulsant effects.

Myeloid differentiation factor 88 (MyD88), originally isolated as a myeloid differentiation primary gene, is known to act as an essential adaptor molecule in the IL-1-IL-1R1 signaling (Burns et al., 1998; Muzio et al., 2013; Wesche et al., 1997). MyD88 is composed of an N-terminal death domain (DD) and a C-terminal Toll/IL-1 receptor (TIR) domain, separated by a short linker region (Hardiman et al., 1996). MyD88 forms homodimers in vivo through DD-DD and TIR-TIR domain interactions (Burns et al., 1998). The TIR domain of MyD88 is responsible for binding the IL-1 receptor complex, thus favoring the recruitment of downstream effectors to propagate the signal inside in the cells (Dunne et al., 2003). MyD88-deficient mice were defective in the production of interleukin-6 and tumor necrosis factor-α in response to IL-1β stimulation (Adachi et al., 1998). In addition, overexpression of MyD88 dominant negative mutant proteins blocked IL-1β signaling in vitro (Dupraz et al., 2000). Further, it has been demonstrated that loss of MyD88 expression and/or function had anti-inflammatory effects in experimental models of several pathological situations, such as arthritis (Joosten et al., 2003), early atherosclerosis (Bjorkbacka et al., 2004), cardiac hypertrophy (Zhu et al., 2011), and multiple sclerosis (Prinz et al., 2006). Taken together, MyD88 is a critical component in the signaling cascade elicited by IL-1β. Although IL-1β exerts proconvulsant actions in large variety of seizure models, up to date, the role of MyD88 in the onset and recurrence of seizures has not been explored.

In this study, the expression pattern of MyD88 was examined in two rat models and in patients with temporal lobe epilepsy (TLE). We then investigated the effect of MyD88 pharmacological inhibition on pilocarpine-induced acute seizure activities using a small synthetic MyD88 inhibitor ST2825 (Loiarro et al., 2007). In addition, the effect of MyD88 inhibition on IL-1β proconvulsant actions was also evaluated. Notably, accumulating evidence has demonstrated that the phosphorylation of the NR2B subunit of *N*-methyl-D-aspartate (NMDA) receptor is the final molecular targets by which IL-1β signaling exerts proconvulsant properties (Balosso et al., 2014; Balosso et al., 2008; Viviani et al., 2003). Therefore, we probed whether this molecular was involved in the effect of the pharmacological treatments on seizures.

2. Materials and methods

2.1. Human brain specimens and clinical data

Twenty-five patients undergoing surgery for medically intractable TLE and fifteen nonepileptic control subjects were included for Western blotting analysis and immunohistochemistry. All of the temporal neocortex specimens were chosen randomly from our established brain tissue bank, which has been reported in our previous publications (Han et al., 2012; Pan et al., 2010; Zhang et al., 2013). The procedures were carried out with the formal written consent of the patients or their legal next of kin. The experimental protocol was conducted in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans and the Ethics Committee on human research at Chongqing Medical University, Chongqing, China.

Presurgical evaluation included a detailed history, neurological examination, interictal and ictal electroencephalographic studies, neuropsychological testing, and neuroradiological studies. The diagnosis of each patient complied with the criteria established by the International League Against Epilepsy (Kwan et al., 2010). All TLE patients were refractory to maximal dose of three or more antiepileptic drugs, including carbamazepine, clonazepam, lamotrigine, phenobarbital, phenytoin, topiramate, and valproic acid. All tissue blocks were resected for strictly therapeutic purposes. After lesion resection, electrodes for intraoperative electroencephalography (EEG) were positioned on the remaining edges of the tissue to ensure that the lesion had been completely resected. The postoperative pathological examination confirmed the diagnosis of hippocampal sclerosis according to the international consensus classification (Blumcke et al., 2013).

For comparison, we obtained fifteen histologically normal anterior temporal neocortex specimens from patients treated for posttrauma intracranial hypertension. The specimens were taken only for treatment purpose. These patients had no history of epilepsy or exposure to antiepileptic drugs. A conventional neuropathological examination revealed no signs of central nervous system disease.

Table 1 summarized the clinical features of the patients with TLE and the nonepileptic controls involved in this study. There were no significant difference in age, sex, or topography of the studied tissues between the TLE group and the nonepileptic control group.

2.2. Animals

Adult male Sprague-Dawley (SD) rats (200–250 g, aged 8–10 weeks) were obtained from Chongqing Medical University. Rats were housed under specific pathogen-free environment on a fixed 12 h light/dark cycle (light on at 7:00 A.M.) at a constant temperature (24–25 °C) with free access to food and water. All animal experiments complied with the ARRIVE guidelines and were carried out in accordance with EU Directive 2010/63/EU for animal experiments as well as the Ethics Committee of Chongqing Medical University. All efforts were made to minimize the number of animals used and their suffering.

Table 1
Clinical features of the patients with refractory TLE and the nonepileptic controls.

Clinical variable	TLE group (n = 25)	Control group (n = 15)	*p* value*
Age (years)			
Mean ± SD	25.12 ± 8.15	23.33 ± 8.07	0.505
Range	16–54	17–48	
Epilepsy onset (years)			
Mean ± SD	14.4 ± 7.16	NA	NA
Range	2–25	NA	
Epilepsy duration[a] (years)			
Mean ± SD	10.92 ± 6.72	NA	NA
Range	3–30	NA	
Seizure type			
CPS	6	NA	NA
SGS	14	NA	
CPS and SGS	5	NA	
Male/female ratio	14:11	9:6	0.804

CPS, complex partial seizures; NA, not applicable; SGS, secondary generalized seizures.
* *p* values were calculated using Student's *t*-test (age) or Chi-square test (male/female ratio). $p < 0.05$ was considered statistically significant.
[a] Epilepsy duration was determined as the time between epilepsy onset (onset of habitual seizures) and surgery.