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Endogenous cannabinoid system alterations and their role in epileptogenesis after brain injury in rat

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ABSTRACT

Post-traumatic epilepsy (PTE) is one of the most common complications resulting from brain injury, however, antiepileptic drugs usually fail to prevent it. Several lines of evidence have demonstrated that the endogenous cannabinoid system (ECS) plays a pivotal role during epileptogenesis in several animal models. A recent study has shown that a cannabinoid type 1 (CB_1) receptor antagonist could suppress long-term neuron hyperexcitability after brain injury, but the underlying mechanisms remain largely unknown. In this study, we first analyzed the dynamic expression of different components of the ECS at various time points after brain injury in rats. Then, we conducted a 12-month-long session of behavioral monitoring after the brain injury, and based on the results, the rats were divided into a PTE group and a non-PTE group. Finally, the changes in the ECS between the two groups were compared. We found that the ECS exhibited a biphasic alteration after brain injury; the expression of the CB_1 receptor and 2-arachidonoylglycerol (2-AG) in the PTE group was significantly higher than that of the non-PTE group 12 months after traumatic brain injury. Our preliminary results indicated that the ECS might be involved in post-traumatic epileptogenesis.

1. Introduction

Traumatic brain injury (TBI) is a major cause of death and disability worldwide, especially in children and young adults. The relative risk of developing post-traumatic epilepsy (PTE) for those suffering from mild and severe TBI was two and seven times higher, respectively, than their healthy counterparts (Christensen et al., 2009). Unfortunately, there are currently no antiepileptic drugs (AEDs) that can prevent the pathogenesis of or cure PTE (Temkin, 2001).

Recently, endocannabinoids (eCBs), which have been used for the treatment of seizures since ancient times, have been highlighted again for their anticonvulsant function (Piomelli, 2003). The endogenous cannabinoid system (ECS)is a group of endogenous cannabinoid receptors located in the central and peripheral nervous systems consisting of two G protein-coupled receptors (cannabinoid type 1 receptor, CB_1, and cannabinoid type 2 receptor, CB_2), the ligand anandamide (AEA) and 2-arachidonoylglycerol (2-AG) (Ahn et al., 2008; Di Marzo, 2008). The primary biosynthetic route for 2-AG is mediated by two enzymes, sn-1-diacylglycerol lipase-α and sn-1-diacylglycerol lipase-β (DAGL-α and DAGL-β). The 2-AG-degrading enzyme is monoacylglycerol lipase (MAGL) (Bisogno et al., 2003; Serrano et al., 2012). *N*-acylphosphatidylethanolamine-specific phospholipase D (NAPE-PLD) and fatty acid amide hydrolase (FAAH) play key roles in the synthesis and degradation of AEA, respectively (Cravatt et al., 1996; Dinh et al., 2002). CB_1 receptors, distributed mainly in the central nervous system, are located at the excitatory and inhibitory

Abbreviations: ECS, endogenous cannabinoid system; CB_1 receptor, cannabinoid receptor type 1; TBI, traumatic brain injury; AEDs, antiepileptic drugs; eCBs, endocannabinoids; PTE, post-traumatic epilepsy; FPI, fluid percussion injury; AEA, anandamide; 2-AG, 2-arachidonoylglycerol; DAGL, sn-1-diacylglycerol lipase; MAGL, monoacylglycerol lipase; NAPE-PLD, *N*-acylphosphatidylethanolamine-specific phospholipase D; FAAH, fatty acid amide hydrolase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

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nerve terminals, and they are able to inhibit the release of glutamate or GABA (Christensen, 2012; Christensen et al., 2009). The eCBs, which are synthesized at the post-synaptic membrane, suppress neurotransmitter release when they bind to CB_1 receptors (Christensen, 2012). The biphasic effect of the ECS on epilepsy, which is exerted through the CB_1 receptor, has been demonstrated in several animal epilepsy models, such as the electroshock-induced epilepsy model, pilocarpine-induced epilepsy model, fluid percussion injury (FPI)-induced epilepsy model, Mg^{2+}-induced epilepsy model, and pentylenetetrazole-induced epilepsy model (Blair et al., 2006; Chen et al., 2007; Echegoyen et al., 2009; Falenski et al., 2009; Gholizadeh et al., 2007; Kozan et al., 2009; Marsicano et al., 2003; Vinogradova et al., 2011; Wallace et al., 2003, 2002). The use of a single CB_1 receptor antagonist could increase seizure duration and shorten latency in the acute stage after modeling (Wallace et al., 2003, 2002), but it played an anticonvulsant role in the chronic stage (Chen et al., 2007; Vinogradova et al., 2011). This phenomenon also exists in the PTE model. Regarding the application of a CB_1 receptor antagonist early after TBI, the anticonvulsant role emerged 6 weeks after TBI (Echegoyen et al., 2009).

Researchers have observed changes in the ECS after TBI in the acute (Lopez-Rodriguez et al., 2015; Panikashvili et al., 2001) and chronic stages, however, the relationship between these changes and the development of PTE was not elucidated. In this study, to elucidate the changes in the ECS after TBI in both the acute and chronic stages, we first measured the expression level of the CB_1 receptor and its ligand-related enzymes at different time points after TBI. Then, we recorded the occurrence of PTE through behavioral monitoring after establishing the TBI model. Finally, the changes in the ECS between PTE rats and non-PTE rats were compared.

2. Materials and methods

2.1. FPI model establishment

Adult male Sprague-Dawley rats (280–300 g, Vital River Laboratories, Beijing, China) were housed in groups under a 12 h light/dark cycle. The ambient temperature was maintained at 20–23 °C. All animal experiments were performed in accordance with the Guidance for Animal Experimentation of the Capital Medical University and the Beijing Guidelines for the Care and Use of Laboratory Animals.

Animals were anesthetized with intraperitoneal injection (i.p.) of chloral hydrate (60 mg/kg) and placed in a stereotactic frame (Kopf, Germany). A burr hole (5 mm diameter) was drilled, and the center was 6 mm posterior to the bregma and 6 mm left of the midline. The dura remained intact, imitating the closed brain injury of humans. The TBI model was established with a fluid-percussion device (Amscien Instruments, Richmond, VA, USA). A brief (21–23 ms) pulse of pressurized fluid was applied against the exposed dura (Kharatishvili et al., 2006). Pressure pulses were measured and recorded by a transducer built into the fluid percussion device. The force of impact was adjusted to 2.8–3.3 atm to establish the severe brain injury model. After FPI, the incision was sutured and gentamicin was injected i.p. to avoid infection.

2.2. Seizure monitoring and analysis

Behavioral and electrophysiological seizures of forty rats were monitored intermittently for 12 months after FPI. In detail, each rat was monitored once every 2 weeks. The duration of monitoring was 24 h. The rats were monitored in cages with sufficient food and water. After a 12-month monitoring period, the rats were divided into 2 groups: a PTE group and a non-PTE group. Relative risk factors of PTE, including body weight, impact force, time of transient apnea and the onset of immediate seizure, were analyzed.

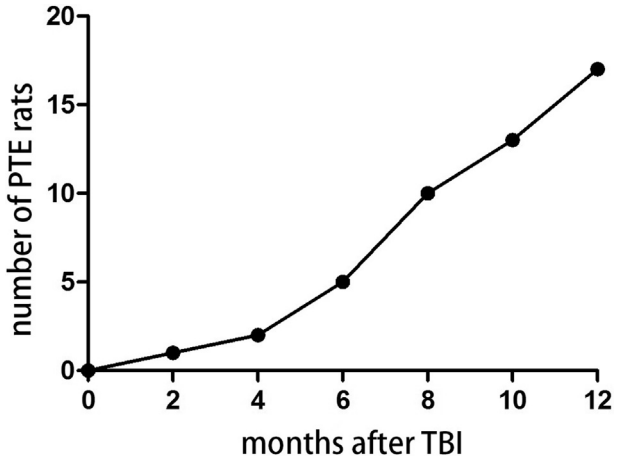

Fig. 1. The number of rats with epilepsy after lateral FPI. Twelve months after FPI, 17 rats developed PTE.

Two microelectrodes were implanted bilaterally into the hippocampi 2 weeks after FPI to detect electrographic seizures. The coordinates of the hippocampus were 4.5 mm lateral to the midline, 5.6 mm posterior to the bregma and 5.5 mm below the skull surface. Continuous EEG monitoring was performed every 2 weeks for each rat along with seizure monitoring via a 64-channel video EEG system (NicoletOne Sleep, Viasys Healthcare Inc., U.S.A). A Nicolet C64 brain electricity amplifier was used to amplify the signal and convert it into a digital format using the supporting software (V5.50.0.1564, Nicolet sleep). The sampling rate was 200 Hz, the resolution was 0.5 microvolts, and 50-Hz noise was suppressed. Ictal electroencephalography (EEG) was defined as repetitive spikes, spike-wave charges in the ictal phase lasting at least 5 s (Kharatishvili et al., 2006).

The severity of seizures was evaluated manually by two researchers independently via video footage according to Racine's scale (Racine, 1972): score 0: electroencephalographic seizure without any detectable motor manifestation; score 1: mouth and face clonus, head nodding; score 2: clonic jerks of one forelimb; score 3: bilateral forelimb clonus; score 4: forelimb clonus and rearing; and score 5: forelimb clonus with rearing and falling.

2.3. Changes in the ECS after FPI

The ipsilateral (left) hippocampus was acquired from acute and chronic FPI rats. Real-time PCR and Western blot were used to quantify the expression of the CB_1 receptor and ligand-related enzymes from different levels.

2.3.1. Hippocampus preparation in an acute FPI rat model

Rats receiving FPI (n = 32) were divided into 4 groups: a half-hour group (n = 8), a 24-h group (n = 8), a one-week group (n = 8) and a four-week group (n = 8). Rats in the four groups were sacrificed under anesthesia after a half hour, 24 h, 1 week and 4 weeks after FPI, respectively. The left hippocampus of each rat was separated and stored in liquid nitrogen for further examination. Eight rats with only a skull burr hole were regarded as the sham control group. Their hippocampi were taken half an hour after modeling and stored in liquid nitrogen.

2.3.2. Hippocampus preparation in a chronic FPI rat model

Behavioral monitoring and EEG recording of rats receiving FPI (n = 40) were conducted as described above. After 12 months of observation, the rats were divided into 2 groups: a PTE group and