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Interleukin-1β enhances susceptibility to hyperthermia-induced seizures in developing rats

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ABSTRACT

Cytokines have been shown to influence susceptibility to febrile seizures and epilepsy. In this study, the role of interleukin-1β (IL-1β) was examined in developing rats. IL-1β and interleukin-1 receptor antagonist (IL-1ra) were administered to developing rats, and seizures were induced by moist warm air. Twenty male Lewis rats (21–23 days old) were divided into two groups (IL-1β and saline control groups) and two holes were made in the skull for EEG electrodes. We applied human recombinant IL-1β intra-nasally 1 h before seizures induced by moist warm air. The brain temperature at the appearance of seizure discharges on EEG, and the latency time from the hyperthermia onset until the appearance of seizure discharges on EEG were measured. And the same study using IL-1ra was performed. The median brain temperature for the IL-1β group, 42.6 °C (range: 41.8–43.0), was significantly lower than that for the control, 42.9 (42.3–43.4) ($P = 0.043$). The brain temperature for the IL-1ra group, 43.3 (42.8–43.7), was significantly higher than that for the control, 42.9 (42.2–43.5) ($P = 0.011$), and the latency time for the IL-1ra group, 398 s (270–561), was significantly longer than that for the control, 325 (252–462) ($P = 0.035$). These results demonstrate that IL-1β promotes hyperthermia-induced seizures in developing rats.

1. Introduction

Febrile seizure (FS) is the most common convulsive disorder in children. More than 5% of the normal population have experienced at least one febrile seizure, with as much as 7–14% estimated for Japan and the Pacific islands.[1] Although FS does not generally induce long-term brain damage or cognitive deficits, several retrospective studies have demonstrated a significant relationship between a history of prolonged FS during early childhood and mesial temporal sclerosis, which is responsible for intractable temporal lobe epilepsy.[2]

The pathogenesis of FS remains unknown. Several previous studies have reported that various inflammatory cytokines play an important role in host defense responses during infections. In addition, they can act as endogenous pyrogens and their levels are elevated in plasma or cerebrospinal fluid (CSF) of patients with FS or epileptic seizures.[3–6] Among them, interleukin-1β (IL-1β) is thought to be an important cytokine in FS, because high levels of IL-1β have been observed in the CSF of FS patients.[7] However, it could not be determined whether increased or decreased cytokine levels were a result or cause of central nervous system disease. Blood and CSF samples are usually collected once disease symptoms are detected, and the levels of some inflammatory cytokine mRNAs, which are associated with cytokine production, become elevated with electrical stimulated experimental seizures.[8]

Recently, the role of genetic polymorphism of cytokines in the pathogenesis of FS has been analyzed. A previous case–control study of Japanese FS patients demonstrated that the −511C/T polymorphism of the *IL1B* gene was associated with development of simple FS with sporadic occurrence.[9] However, an additional study reported no significant relationship between the production of IL-1β and the development of FS.[10]

Hyperthermia is a strong, precipitating factor for the induction of seizures. Seizures are often induced by febrile episodes in patients with a positive history of severe, febrile seizure, severe myoclonic epilepsy in infancy, or specific subtypes of childhood epilepsy.[11] Experimental hyperthermia-induced seizures (HS) have been used in animal models to clarify the mechanisms involved in FS.[12–15] A previous study, using immature, transgenic rodents, demonstrated that IL-1β receptor-deficient mice are resistant to experimental HS.[16] On the contrary, IL-1β exhibited an anti-epileptic role in a rat seizure model.[17] The present study

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demonstrates the role of IL-1β and IL-1 receptor antagonist (IL-1ra) in HS of developing rats.

2. Materials and methods

2.1. Animals

Male, Lewis rat pups (21–23 days old) were kept with their mothers in standard, housing conditions, including a 12-h light/12-h dark cycle, controlled temperature, and free access to food and water during the entire experimental period. All experimental procedures conformed to guidelines from the Ministry of Education of Japan, and were approved by the animal experimental committee of Ehime University Graduate School of Medicine (No. TE-17-2).

2.2. Preparation for recording electroencephalography and brain temperature

A stereotaxic holder (Narishige Co., Ltd., Tokyo, Japan) was used to fix the head in place. Two holes were made in the skull over the right frontal and occipital cortex for placement of silver electroencephalography (EEG) electrodes and plastic receptacles (Unique Medical Co., Ltd., Tokyo, Japan). An additional hole was made over the left, central cortex for placement of the needle brain temperature thermometer (Unique Medical Co., Ltd.). These manipulations were performed under anesthesia with an intraperitoneal injection of pentobarbital sodium (Dainippon Pharma Co., Ltd., Osaka, Japan, 30 mg/kg). Cefotaxime sodium (Aventis Pharma Co., Ltd., Tokyo, Japan), 500 mg/kg, was intraperitoneally injected to prevent bacterial infections.

2.3. Drug application and induction of hyperthermia-induced seizures

Twenty male, Lewis rats were divided into two groups 72 h after surgery: IL-1β ($n = 10$) and control ($n = 10$) groups. Recombinant human IL-1β (500 ng; Bender MedSystems, Vienna, Austria) was dissolved in 0.9% saline for a total volume of 20 μl and administered intranasally to rats in the IL-1β group. Rats in the control group received only 0.9% saline. The intranasal administration method was based on previous studies that demonstrated the effectiveness of this method.[18] One hour after intranasal administration of recombinant human IL-1β or saline, each rat was placed in a special, plastic cage. HS was induced by moist warm air (45–50 °C), followed by EEG monitoring. Brain temperature was measured at the onset of seizure discharge, based on EEG, and latency time was recorded from the onset of hyperthermia until the appearance of seizure discharges on EEG.

In the second experimental design, an additional 20 male, Lewis rats were divided into two groups: IL-1ra ($n = 10$) and control ($n = 10$). Recombinant human IL-1ra (500 ng: Bender MedSystems) or saline was intranasally applied 1 h prior to moist, warm air-induced seizures. Brain temperature and latency time were measured, according to the above-described methods.

2.4. Measurement of IL-1β concentration

A third experiment was designed to measure IL-1β concentrations in the central nervous system, which consisted of male, Lewis rats divided into two groups: IL-1β ($n = 5$) and control ($n = 5$). Recombinant human IL-1β (500 ng) or saline was intranasally administered. One hour later, each rat was anesthetized with diethyl ether (Kanto Chemical Co., Inc., Tokyo, Japan), and the brain was quickly removed. The frontal lobes (anterior to the optic chiasma) were isolated and stored at −80 °C until further use. Samples were weighed and homogenized in 50 mM PBS (pH 7.4). Thereafter, they were centrifuged for 15 min (5000 rpm), and the supernatants were harvested and stored. IL-1β Human Easy ELISA (Amersham Biosciences UK Ltd., Buckinghamshire, England) was performed to determine human IL-1β levels in the frontal lobe. The IL-1β detection level of this ELISA was determined to be 1.1 pg/ml.

2.5. Statistics

Mann-Whitney *U*-test was performed for statistical analysis in this study. Values were expressed median (range), and a *P*-value of <0.05 was considered to be significant.

3. Results

3.1. Brain temperature at seizure onset and latency times

All experimental rats suffered from stereotypical seizures during hyperthermia, and completely recovered following the seizures. During a typical seizure, all movements abruptly stopped, and the rat displayed a tonic posture. Later in the course of seizures, facial clonus appeared and spread to generalized, clonic movement. Background EEG activity, prior to HS, consisted of moderate, sustained, irregular, theta rhythm (Fig. 1A). Spikes and spike-wave bursts, which were consistent with clinical seizures, were induced for several minutes after warming by moist, warm air (Fig. 1B).

As shown in Fig. 2A, the median brain temperature at seizure onset of the IL-1β group, 42.6 °C (range: 41.8–43.0), was significantly less than the control group, 42.9 (42.3–43.4) ($P = 0.043$). However, in terms of latency time, there was no difference between the two groups, 296 s (244–447) vs. 366 s (298–440) ($P = 0.052$) (Fig. 2B). Following administration of IL-1ra, however, significantly elevated the brain temperature in the IL-1ra

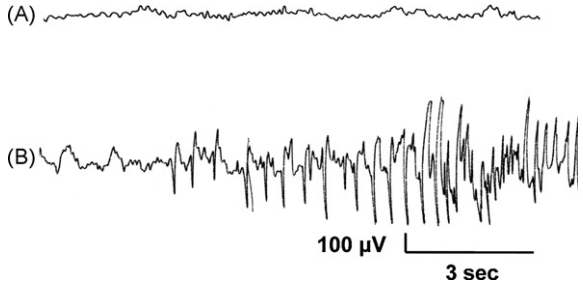

Fig. 1. EEG findings in rats after warming. Moderate sustained irregular 5–8 Hz activities were measured prior to warming (A). Spikes and spike-wave bursts were induced several minutes after warming with moist, warm air (B).

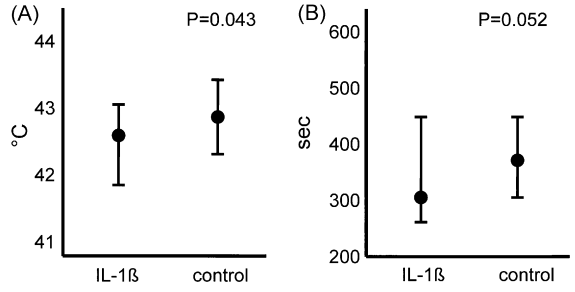

Fig. 2. Brain temperature at seizure onset, as well as seizure latency, after administration of IL-1β. The data are presented as the median and range. The brain temperature of the IL-1β group, 42.6 °C (range: 41.8–43.0), was significantly less than the control group, 42.9 (42.3–43.3) ($P = 0.043$) (A). There was no significant difference between latency time of the IL-1β group, 296 s (244–447), and the control group, 366 (298–440) ($P = 0.052$) (B).