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Research report

Electroconvulsive stimulation transiently enhances the permeability of the rat blood-brain barrier and induces astrocytic changes

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ABSTRACT

The blood-brain barrier (BBB) plays important roles in both the physiological and pharmacological state of the brain. Transiently enhancing the permeability of the BBB may allow use of more types of medications for neuropsychiatric diseases. Several studies have demonstrated that seizures cause a transient decrease in BBB integrity. We studied the timing of BBB changes following seizures and the role of astrocytes in this process. Rats received 10 applications of electroconvulsive stimulation (ECS). They were then infused with sodium fluorescein, a fluorescent substance that rarely passes the BBB, via the inferior vena cava. After 120 min of circulation, the amount of sodium fluorescein in the brain was measured by two methods in vivo fluorescence imaging (total radiant efficiency) and the brain concentration of sodium fluorescein. To assess any changes to the BBB, we measured S100B in serum, which is a standard marker of BBB breakdown that is expressed by astrocytes. We also examined ultrastructural changes following ECS. Total radiant efficiency and the brain concentration of sodium fluorescein were significantly increased in treated rats compared to controls when sodium fluorescein was injected immediately after ECS but not when the injection was performed more than 15 min after ECS. Astrocytic endfeet showed swelling around brain capillaries following ECS. In conclusion, ECS transiently enhances the permeability of the BBB, which may be accompanied by changes in astrocytic endfeet.

1. Introduction

The blood-brain barrier (BBB) plays important roles in both the physiological and pharmacological state of the brain. The "neurovascular unit" is composed of brain capillary endothelial cells, astrocytes, pericytes, neurons, and microglia (Lo et al., 2003). The BBB protects the central nervous system against harmful situations, such as toxins and infections, by regulating the passage of substances into the brain. However, this barrier also prevents entry of many types of medications into the brain. Transiently enhancing permeability of the BBB may allow the use of more types of medications to treat neuropsychiatric diseases by allowing access of medications to the brain. Jordão et al. (2010) showed that transcranial focused ultrasound transiently enhances permeability of the BBB, enabling removal of more amyloid-β peptide plaques with anti-amyloid-β antibodies compared to the intact BBB (Jordão et al., 2010). Several studies have also demonstrated that seizures may cause a transient decrease in BBB integrity (Bolwig, 1988; Bolwig et al., 1977c; Gurses et al., 2009; Zimmermann et al., 2012), and electron microscopy revealed increased vesicular transport of horseradish peroxidase across endothelial cells after electroconvulsive stimulation (ECS) in rats (Bolwig et al., 1977a; Bolwig et al., 1977b).

However, the length of time that ECS enhances BBB permeability and how this affects substances smaller than horseradish peroxidase (MW > 40,000) are unclear. To address these questions, we used an in vivo imaging system (IVIS) to quantify how long sodium fluorescein (NaFlu; MW = 376) takes to distribute in the

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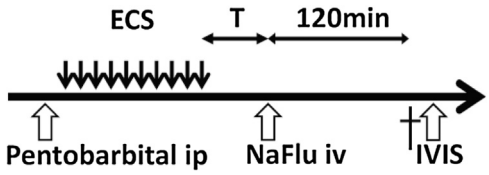

Fig. 1. Time course of the study. After induction of anaesthesia with intraperitoneal sodium pentobarbital, electroconvulsive stimulation (ECS) was delivered 10 times, once per minute. Sodium fluorescein (NaFlu) was injected intravenously after completion of the course of ECS (T = 0, 15, 30, or 60 min) and allowed to circulate for 2 h. Animals were sacrificed after that.

brain, and we measured ECS-associated alterations to BBB permeability. The amount of NaFlu was measured two ways. Fluorescence strength was detected by in vivo fluorescence imaging, and the concentration of NaFlu was measured following extraction from brain. Additionally, we used electron microscopy to identify any changes to astrocytic endfeet, which envelope the BBB and may mediate changes in BBB permeability following ECS.

2. Material and methods

2.1. Animals

Experimental procedures were conducted in 6-week-old Wistar female rats weighing 100–150 g. Three animals were housed per cage and were given ad libitum access to ordinary lab chow and water. They were housed on a 12-h light-dark cycle at constant temperature (22–26 °C). All procedures performed on animals were approved by the Institutional Animal Care and Use Committee of Juntendo University Faculty of Medicine (approval no. 1148). Animals were randomly chosen from cages and allocated to one of five ECS groups (T = 0 (n = 11), 15 (n = 5), 30 (n = 5), 60 (n = 5), control (n = 19)) or ECS × 10 without NaFlu (n = 4).

2.2. ECS procedure

After induction of anaesthesia with intraperitoneal sodium pentobarbital (3.0 mg/100 g body weight), ECS was delivered through ear clip electrodes using a pulse generator (Ugo Basile, Comerio, Italy; 80 mA, 1-s stimulus duration, 100-Hz unidirectional square wave pulses, and 0.5-msec pulse width). This consistently resulted in tonic-clonic seizure activity. To sufficiently stimulate the entire brain, seizures were induced once per minute for 10 times. The durations of the tonic-clonic phases of seizures were 10–20 s. Sham-treated rats were handled identically and injected intraperitoneally with sodium pentobarbital but received no electrical stimuli. The study design is summarised in Fig. 1.

2.3. Behavioural tests

Rats underwent some behavioural tests to reveal whether ECS influenced behaviour. Animals were randomly chosen from cages and allocated to two groups [ECS (n = 4) and Control (n = 4)]. Behavioural assessments were performed on rats to confirm spontaneous alteration behaviour using a Y-maze test and locomotor activities using the open-field test a day after ECS. Details of methods have been previously described (Nakamura et al., 2013).

2.4. In vivo fluorescence imaging

Rats were randomly divided into five ECS groups and one sham group. Immediately after ECS or sham treatment, we infused NaFlu (MW: 376, 2%, 5 ml/kg) at various intervals post-ECS (groups 1–4; Time (T) = 0, 15, 30, or 60 min) into the inferior vena cava and allowed it to circulate for 120 min (Gurses et al., 2009), which is sufficient time to allow NaFlu to infiltrate the brain. After this, animals were deeply anesthetized again with pentobarbital before being sacrificed, and we collected blood for measuring S100B. Animals were perfused through the inferior vena cava with 150 ml 0.37% Na_2S solution and 150 ml 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 (Haug, 1967; van Vliet et al., 2011). The perfusion was done to wash the NaFlu out of the vessels so that the only NaFlu we measured was that in the brain parenchyma that had crossed the BBB. Animals were sacrificed by decapitation, and the brains were removed. Biofluorescence was quantified with IVIS Image Software Version 4.3.1. Brains were imaged using a standard set of IVIS Lumina II filters (Perkin Elmer) (excitation filter, 465 nm; emission filter, Cy5.5; exposure time, 5 s; bin, 2; f/stop, 2; field of view, 10 × 10 cm) (Burrell-Saward et al., 2015; Smith et al., 2012). Total Radiant Efficiency [Mp/sec]/[μW/cm^2] (TRE; fluorescence strength) was measured for each brain. To quantify autofluorescence, group 5 rats underwent the same protocol after ECS but without injection of NaFlu. In group 6, rats were treated with sham ECS followed by immediate injection of NaFlu (T = 0 min).

2.5. Fluorescence spectroscopy

After in vivo fluorescence imaging, brains were homogenized in 2.5 ml phosphate-buffered saline and mixed with a vortex for 2 min. Each sample was added to 2.5 ml distilled water, cooled for 30 min at 4 °C, and centrifuged at 14,000 × *g* for 10 min. The supernatant was collected and centrifuged at 14,000 × *g* for 10 min again. The concentration of NaFlu in the supernatant was measured at an excitation wavelength of 440 nm and emission wavelength of 525 nm using a fluorescence spectroscope (FlexStation II, Molecular Devices Inc.). NaFlu was expressed as ng/mg of brain tissue against a standard curve (Gurses et al., 2009).

2.6. A marker of BBB breakdown and astrocytes

Serum S100B is a standard marker of BBB breakdown (Kazmierski et al., 2012). Blood samples for measurement of S100B were collected in a vacuum blood collection tube (Venoject II Terumo Inc. Tokyo) from the inferior vena cava immediately after ECS (T = 0 min) and incubated for 1 h at room temperature. After that, serum samples were separated by centrifugation and stored at −80 °C. Serum S100B was measured with a Human S100B enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (BioVendor Inc. Cat. RD192090100R). Samples were diluted 4 × with dilution buffer, and 100 μl samples were pipetted into wells. Plates were incubated at room temperature for 120 min with shaking at 300 rpm on an orbital microplate shaker. The wells were then washed three times with wash solution, after which 100 μl biotin-labelled antibody solution was added to each well. Plates were then incubated at room temperature for 60 min with shaking at 300 rpm on an orbital microplate shaker, and then washed five times with wash solution. Streptavidin-horseradish peroxidase conjugate (100 μl) was then added to each well, and the plates were incubated at room temperature for 30 min with shaking at 300 rpm on an orbital microplate shaker. The wells were then washed five times with wash solution, and 100 μl substrate solution was added to each well. After incubation for 15 min at room temperature, colour development was terminated by adding 100 μl stop solution. The absorbance of each well was determined using a microplate reader set to 450 nm with the reference wavelength set to 630 nm (Spectra Max 340 PC384, Molecular Devices Inc.).