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Research Report

Pentylentetrazole-induced loss of blood–brain barrier integrity involves excess nitric oxide generation by neuronal nitric oxide synthase

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ABSTRACT

Dysfunction of the blood–brain barrier (BBB) is one of the major pathophysiological consequences of epilepsy. The increase in the permeability caused by BBB failure is thought to contribute to the development of epileptic outcomes. We developed a method by which the BBB permeability can be demonstrated by gadolinium-enhanced T_1 weighted imaging ($GdET_1WI$). The present study examined the changes in the BBB permeability in mice with generalized convulsive seizures (GCS) induced by acute pentylentetrazole (PTZ) injection. At 15 min after PTZ-induced GCS, the BBB temporarily leaks BBB-impermeable contrast agent into the parenchyma of the diencephalon, hippocampus and cerebral cortex in mice, and the loss of BBB integrity was gradually recovered by 24 h. The temporary BBB failure is a critical link to the glutamatergic activities that occur following the injection of PTZ. PTZ activates the glutamatergic pathway via the NMDA receptor, then nitric oxide (NO) is generated by NMDA receptor-coupled neuronal NO synthase (nNOS). To examine the influence of nNOS-derived NO induced by PTZ on the increases of the BBB permeability,

Abbreviations: AEDs, antiepileptic drugs; AMPA, DL-alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BBB, blood–brain barrier; CBZ, carbamazepine; CGP39551, (E)-(±)-2-amino-4-methyl-5-phospho no-3-pentenoic acid ethyl ester; DMSO, dimethyl sulfoxide; DETC, *N*,*N*-diethyldithiocarbamate Na; CBF, cerebral blood flow; CNS, central nervous system; CSM, cerebral smooth muscle; GABA, gamma-aminobutyric acid; Gd, gadolinium; Gd-HP-DO3A, gadolinium-1,4,7-tris (carbonylmethyl)-10-(2′-hydroxypropyl)-1,4,7,10-tetraazacyclo-dodecane; MK-801, (5S,10R)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]-cyclohepten-5,10-imine maleate; MRI, magnetic resonance imaging; GdET1WI, gadolinium-enhanced T1 weighted image; NBQX, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; nNOS, neuronal nitric oxide synthase; PTZ, pentylentetrazole; VPA, valproic acid; SI, signal intensities; TBARS, thiobarbituric acid-reactive substance

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GdET$_1$WI was performed using conventional nNOS gene-deficient mice with or without PTZ injection. The failure of the BBB induced by PTZ was completely protected by nNOS deficiency in the brain. These results suggest that nNOS-derived excess NO in the glutamatergic pathway plays a key role in the failure of the BBB during PTZ-induced GCS. The levels of NO synthetized by nNOS in the brain may represent an important target for the future development of drugs to protect the BBB.

1. Introduction

It has recently been suggested that blood–brain barrier (BBB) dysfunction is related to several central nervous system (CNS) diseases and injuries, including epilepsy, brain tumors, CNS infections, neurodegenerative disorders such as multiple sclerosis, Alzheimer's and Parkinson's diseases, and stroke (Zlokovic, 2008; Weiss, et al., 2009; Tomkins et al., 2011; Rosenberg, 2012; Marchi, et al., 2012). In epilepsy research, the clinical and experimental evidence supports the possibility that the BBB failure may trigger a chain of events leading to epilepsy, which may be causatively linked to seizures, although the occurrence of seizures does not always lead to a diagnosis of epilepsy. Neuropathological studies in the human epileptic brain have demonstrated structural evidence of an abnormal BBB, including increased micropinocytosis and fewer mitochondria in endothelial cells, a thickening of the basal membrane and the presence of abnormal tight junctions (van Vliet et al., 2007; Michalak, 2012). Although a link between BBB failure and seizures was shown by different experimental approaches (Friedman et al., 2009; Marchi et al., 2012), the mechanism underlying the BBB failure in seizures remains unknown.

A better understanding of the BBB failure may provide a variety of useful information, including the mechanisms responsible for the increase in the BBB permeability. The BBB comprises the microcirculation system of capillaries that are functionally coupled with parenchymal brain cells such as astrocytes and pericytes (Abbott et al., 2006; Neuwelt et al., 2011). Recently, the resistance levels of arterioles and capillaries were found to be regulated by the neuronal activities as a neurovascular unit (Hawkins and Davis, 2005). Although the neurovascular response to epileptic activity is largely unknown, these abnormal neurovascular responses were locally restricted to an area with BBB dysfunction (Stanimirovic and Friedman, 2012). This finding is interesting, because the anatomical overlaps alone suggest that pathological changes that disturb the neurovascular responses may also lead to dysfunction of the BBB and vice versa. Thus, it is suspected that the failure of the BBB may accompany abnormal neuronal activity.

Abnormal glutamatergic excitatory neurotransmission and GABAergic synaptic inhibition in the CNS can cause seizures and may be a major cause of epilepsy. BBB dysfunction has been suggested to play a role in the neuronal hyperexcitability underlying seizure precipitation and recurrence in symptomatic types of epilepsy (Friedman et al., 2009). In the present study, to mimic the generalized epileptic activity, pentylentetrazole (PTZ) was systemically injected into mice. Acute BBB dysfunction is known to cause brain edema and neuroinflammation by the extravasation of blood-borne cells and molecules into the brain parenchyma (Muldoon et al., 2013). This phenomenon, like brain inflammation, may promote the release of diffusible factors (e.g., nitric oxide (NO)) that have pathological effects on neuronal excitability. Small amounts of eNOS-synthesized NO in the cerebral smooth muscle (CSM) mediate the dilation or constriction of pial arteries and intraparenchymal arterioles depending upon the cerebral blood flow (CBF), and iNOS-derived excess NO generation by microglia and astrocytes under inflammatory conditions can mediate tissue injury, including BBB dysfunction (Wong et al., 2004). However, the relationship between the nNOS-derived excess NO generation and BBB dysfunction is currently unknown, although nNOS-derived NO generation has been demonstrated to be involved in the generalized convulsive seizures (GCS) induced by PTZ (Itoh et al., 2004; Itoh and Watanabe, 2009; Watanabe et al., in press). To evaluate the loss of BBB integrity *in vivo* we employed gadolinium (Gd)-enhanced magnetic resonance imaging (MRI) using T_1-weighted imaging (GdET$_1$WI) to non-invasively follow the signal changes induced by the extravasation of a particular BBB impermeable Gd contrast agent in a PTZ-induced GCS mouse model (Alvarez et al., 2010; Ichikawa and Itoh, 2011). In this study, we also examined whether the nNOS-derived excess NO in the glutamatergic pathway plays a role in the loss of BBB integrity during PTZ-induced GCS.

2. Results

2.1. *Acute PTZ injection induced the failure of the BBB*

We investigated the changes of the BBB permeability during the generalized seizures using acute PTZ-induced convulsive model mice (Löscher et al., 1991). The latency of GCS in the mice that were intravenously (i.v.) injected with PTZ was 4.75 ± 2.5 s at 40 mg/kg in comparison to 68.0 ± 9.1 s at 60 mg/kg (intraperitoneal (i.p.) injection). The duration of PTZ-induced GCS by i.v. and i.p. injection was 20.6 ± 1.13 and 28.5 ± 3.17 s, respectively. In this study, to perform GdET$_1$WI during PTZ-induced GCS, a mixture of Gd-HP-DO3A and saline or PTZ was intravenously injected as a bolus via a femoral vein. The i.v. injection with PTZ was invariably associated with a shorter latency and duration of GCS in comparison to the i.p. injection, although the GCS induced by intravenous PTZ at 40 mg/kg were equal to those induced by the i.p. injection of 60 mg/kg. The coronal GdET$_1$WI in control mice showed that