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Reduced expression of I_A channels is associated with post-ischemic seizures

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ABSTRACT

Purpose: Post-stroke seizures are considered as a major cause of epilepsy in adults. The pathophysiologic mechanisms resulting in post-stroke seizures are not fully understood. The present study attempted to reveal a new mechanism underlying neuronal hyperexcitability responsible to the seizure development after ischemic stroke.
Methods: Transient global ischemia was produced in adult Wistar rats using the 4-vessel occlusion (4-VO) method. The spontaneous behavioral seizures were defined by the Racine scale III–V. The neuronal death in the brain was determined by hematoxylin-eosin staining. The expression levels of A-type potassium channels were analyzed by immunohistochemical staining and western blotting.
Results: We found that the incidence of spontaneous behavioral seizures increased according to the severity of ischemia with 0% after 15-min ischemia and ~50% after 25-min ischemia. All behavioral seizures occurred with 48 h after ischemia. Morphological analysis indicated that brain damage was not correlated with behavioral seizures. Immunohistochemical staining showed that the expression levels of the A-type potassium channel subunit Kv4.2 was significantly reduced in ischemic brains with behavioral seizures, but not in ischemic brains without seizures. In addition, rats failing to develop spontaneous behavioral seizures within 2 days after ischemia were more sensitive to bicuculline-induced seizures at 2 months after ischemia than control rats. Meanwhile, Kv4.2 expression was decreased in brain at 2 months after ischemia.
Conclusion: Our results demonstrated the reduction of Kv4.2 expression might contribute to the development of post-ischemic seizures and long-term increased seizure susceptibility after ischemia. The mechanisms underlying post-stroke seizures and epilepsy is unknown so far. The down-regulation of I_A channels may explained the abnormal neuronal hyperexcitability responsible for the seizure development after ischemic stroke.

1. Introduction

Seizures are one of the most frequent complications in stroke patients and stroke is considered as a major cause of epilepsy in the adults (Hauser, 1992). The reported incidence of post-stroke seizures and epilepsy varies widely between studies due to many factors that include differences in study design, the patient population, diagnostic criteria, and the duration of follow-up. A large international prospective multicenter study reported that seizures occur in 8.9% of patients with stroke (10.6% with hemorrhagic and 8.6% with ischemic stroke) (Bladin et al., 2000). According to the temporal relation with the stroke onset, seizures are commonly classified as early- and late-onset, but there is no consistent definition how to separate early from late seizures. In most studies, early-onset is defined as the first seizure occurring within 2 weeks after stroke onset and late-onset is defined as the first seizure occurring more than 2 weeks after stroke (Bladin et al., 2000). Most early-onset seizures are likely to occur during the first 1–2 days after stroke onset. The risk of late-onset seizures and epilepsy is believed to increase when early-onset seizures occur (So et al.,

Abbreviations: 4-VO, 4-vessel occlusion; ACSF, artificial cerebrospinal fluid; BSA, bovine serum albumin; ECL, enhanced chemiluminescence; HRP, horseradish peroxidase; I_A, A-type K^+ currents; Kv, voltage-dependent K^+ currents; MCAO, middle cerebral artery occlusion; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; TBS, tris-buffered saline; TBST, TBS-0.1% Tween20.

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1996). It is widely accepted that post-stroke seizures are harmful and can exacerbate the injury processes, leading to higher morality and poor functional disability (Szaflarski et al., 2008). Therapeutic treatments for post-stroke seizures and epilepsy have been limited, because of the lack of a clear understanding of the pathophysiological mechanisms involved.

Although the relationship between a stroke and seizures has been described in both clinical studies and animal models (Kelly, 2002), the number of studies investigating the underlying pathophysiologic mechanisms leading to post-stoke seizures is sparse. This is partly due to the fact that animal models of post-stroke seizures is not well-established so far. The 4-vessel occlusion (4-VO) ischemia model described by Pulsinelli is the most used technique to produce transient global ischemia (Pulsinelli and Brierley, 1979). The percentage of animals developing behavioral seizures following 10, 20, or 30 min of four-vessel occlusion is 0, 8, and 40%, respectively (Pulsinelli et al., 1982). Unfortunately, ischemic animals with behavioral seizures are often died or excluded from experimental groups. Therefore, the pathologic changes in these post-ischemic animals with seizures have not been fully investigated.

Increase of neuronal excitability is a recognized cause of seizures generation. Spontaneous interictal epileptiform discharges in the hippocampus have been reported from ischemic rat brain (Epsztein et al., 2006). There are several mechanisms that may be associated with the neuronal hyperexcitability and the generation of epileptiform discharges in post-ischemic brain, one of which is the changes in intrinsic membrane properties (Congar et al., 2000). The transient A-type potassium currents (I_A currents) are critical to modulate of intrinsic membrane properties of neurons through the regulation of resting membrane potential, action potential (AP) half-width, frequency-dependent AP broadening and dendritic action potential propagation (Kim et al., 2005). In neurons, Kv4 subunits, together with, KChIPs and DPPX, give rise to the majority of somatodendritic I_A currents (An et al., 2000; Nadal et al., 2003; Sheng et al., 1992). In our previous studies, we found that the reduction of I_A currents and channels contributes to the seizure development after hyperglycemic ischemia (Lei et al., 2014). However, the involvement of I_A currents and channels in the post-ischemic seizures in rats with normal glucose levels has not been elucidated.

In the present study, we explored the association of I_A channels with the early-onset behavioral seizures and the long-term changes in seizure susceptibility after 4-VO ischemia. In addition to a description of the incidence and the characteristics of ischemia-induced behavioral seizures, we found that the down-regulation of I_A channels might be involved in the pathological process leading to post-ischemic seizures in both early and late stage.

2. Methods

2.1. The rat model of transient global ischemia

Transient global ischemia in rat was induced using the 4-VO occlusion as described in our previous study with modifications (Ruan et al., 2009). On the first day, adult male Wistar rats (150–200 g, Charles River) were anesthetized with 2% isoflurane via a nasal mask. Both vertebral arteries were electrocauterized. A length of silicone tubing (0.025″ I.D., 0.047″ O.D.) will be placed loosely around each common carotid artery and passed through two holes in a small teflon button before being tied in a loop. A suture line was tied at the end of the loop. The incision was closed with wound clips. The rats were then allowed to recover from anesthesia overnight. The next day, the carotid arteries were occlusion in the unanesthetized rats. The 2% lidocaine-HCl (Sparhawk Lab., Lenexa, KS, USA) was applied as a local anesthetic in the region of incision. The awake rats were hand-held in a simple restraint, the ventral neck suture removed. The silicone tubing was then threaded and drawn through a 2-cm plastic cylinder, compressing the artery against the Teflon button. During occlusion, the rectal temperature was maintained at 37 °C with a heating lamp via a feedback system, and the completeness of global ischemia was confirmed by testing the loss of righting and pupil reflexes. After the termination of 15, 20, 25 or 30-min occlusion, the silicone tubings, teflon buttons and sutures was removed and the incision was closed. The rats were then allowed to recover in a standard cage. Behavioral (i.e., convulsive) seizures include symptoms in a scale from III to V using the Racine scale (Racine, 1972). A class III seizure was characterized by forelimb clonus, an erect tail and lordotic posturing. A class IV was characterized by continued forelimb clonus and rearing on hindlimbs. Rats showing all of these behaviors in combination with a fall were defined as having a class V seizure. Experimental protocols were approved by the Institutional Animal Care and Use Committee of Indiana University School of Medicine in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Immunohistochemical staining

The rats were deeply anesthetized, perfused with PBS and fixed with 4% paraformaldehyde in PBS (Lei et al., 2012, 2010). After being postfixed overnight, six sets of coronal sections of the hippocampus were cut (40 μm) with a vibratome (Technical Products International) and collected in PBS. One set of sections will be stained with hematoxylin & eosin (H&E). Then, the sections from control and ischemia groups were stained together in each immunohistochemical session. The sections were incubated for 30-min in 0.3% H_2O_2 (Sigma) to quench the endogenous peroxidase activity. Subsequently, the sections were blocked and permeabilized in permeabilization solution (5% goat or horse serum, 0.1% Triton X-100 in PBS) for 1 h at room temperature. Thereafter, the sections were incubated with an antibody against Kv4.2, Kv4.3, or KChIP2 in permeabilization solution overnight at 4 °C. After being washed, the sections were incubated with biotinylated horse anti-mouse or goat anti-rabbit IgG (1:200; Vector) in blocking solution (5% horse or goat serum in PBS) for 1 h at room temperature. After three washes, the sections were processed with ABC and DAB reaction. All sections within the reaction were exposed to DAB for the exact same time. The sections were mounted onto slides, air dried, dehydrated in graded series of ethanol, infiltrated in xylene, and embedded in paraffin. The slides were then examined with a microscope (BX50; Olympus). Images were acquired with a digital camera coupled to control software (DP70-BSW; Olympus) at 4 and 20× magnification. The settings were kept constant throughout all experiments.

2.3. Western blotting

Brain slices were prepared using procedures similar to those previously described (Deng et al., 2009). Briefly, the animals were anesthetized with overdose of isoflurane and decapitated. The brains were quickly removed and immersed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 130 NaCl, 3 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 10 glucose (pH 7.4, 295–305 mOsm/L). Transverse brain slices containing the hippocampus (400 μm) were cut using a vibratome (VT 1000; Leica). Subsequently, the hippocampus was microdissected under a surgical microscope (Bausch & Lomb, Rochester, NY, USA) and frozen in liquid nitrogen. Tissues were lysed with ice-cold RIPA buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS; 5 mM EDTA, Boston BioProducts, Worcester, MA, USA) supplemented with a protease inhibitor cocktail