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Research report

Na^+, K^+-ATPase dysfunction causes cerebrovascular endothelial cell degeneration in rat prefrontal cortex slice cultures

Yuki Kurauchi [a], Akinori Hisatsune [b,c], Takahiro Seki [a], Hiroshi Katsuki [a,*]

[a] Department of Chemico-Pharmacological Sciences, Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto 862-0973, Japan
[b] Priority Organization for Innovation and Excellence, Kumamoto University, Kumamoto 860-8555, Japan
[c] Program for Leading Graduate Schools "HIGO (Health Life Science: Interdisciplinary and Glocal Oriented) Program", Kumamoto University, Kumamoto 862-0973, Japan

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ABSTRACT

Cerebrovascular endothelial cell dysfunction resulting in imbalance of cerebral blood flow contributes to the onset of psychiatric disorders such as depression, schizophrenia and bipolar disorder. Although decrease in Na^+, K^+-ATPase activity has been reported in the patients with schizophrenia and bipolar disorder, the contribution of Na^+, K^+-ATPase to endothelial cell dysfunction remains poorly understood. Here, by using rat neonatal prefrontal cortex slice cultures, we demonstrated that pharmacological inhibition of Na^+, K^+-ATPase by ouabain induced endothelial cell injury. Treatment with ouabain significantly decreased immunoreactive area of rat endothelial cell antigen-1 (RECA-1), a marker of endothelial cells, in a time-dependent manner. Ouabain also decreased Bcl-2/Bax ratio and phosphorylation level of glycogen synthase kinase 3β (GSK3β) (Ser9), which were prevented by lithium carbonate. On the other hand, ouabain-induced endothelial cell injury was exacerbated by concomitant treatment with LY294002, an inhibitor of phosphoinositide 3- (PI3-) kinase. We also found that xestospongin C, an inhibitor of inositol triphosphate (IP3) receptor, but not SEA0400, an inhibitor of Na^+, Ca^{2+} exchanger (NCX), protected endothelial cells from cytotoxicity of ouabain. These results suggest that cerebrovascular endothelial cell degeneration induced by Na^+, K^+-ATPase inhibition resulting in Ca^{2+} release from endoplasmic reticulum (ER) and activation of GSK3β signaling underlies pathogenesis of these psychiatric disorders.

1. Introduction

Vascular endothelial cell is one of the main components of cerebrovascular unit in the brain, which plays an important role in supplying nutrients to the brain as well as removing wastes from the brain. Several factors such as endothelins, prostaglandins (PGs) and nitric oxide (NO) released from vascular endothelial cells or surrounding pericytes regulate cerebral blood flow (CBF) or inflammation in the brain (Hall et al., 2014; Santhanam et al., 2010). Recently, imbalance of CBF has been observed in patients with several psychiatric disorders such as depression, schizophrenia, attention deficit hyperactivity disorder (ADHD) and bipolar disorder (Takizawa et al., 2014). In addition, disruption of cerebrovascular unit structure is one of the prominent findings of dementia or Alzheimer's disease (Kapasi and Schneider, in press; Nelson et al., in press; Zlokovic, 2011). Therefore, CBF change is considered to be the fundamentals of various neurological disorders.

Na^+, K^+-ATPase is a highly expressed membrane protein that hydrolyzes ATP to transducing Na^+ and K^+ (Aperia et al., 2016; Li and Langhans, 2015). Several lines of evidence show that dysfunction of Na^+, K^+-ATPase is involved in the pathogenesis of bipolar disorder, a mood disorder characterized by both manic and depressive symptoms (El-Mallakh and Wyatt, 1995; Goldstein et al., 2006; Mynett-Johnson et al., 1998; Rose et al., 1998). For example, pharmacological inhibition of Na^+, K^+-ATPase by intracerebroventricular injection of ouabain, a specific inhibitor of Na^+, K^+-ATPase, produced manic symptom of bipolar disorder in rats (El-Mallakh et al., 1995; Herman et al., 2007; El-Mallakh et al., 2003). Moreover, α3 subunit of Na^+, K^+-ATPase mutant mice showed several behavioral characteristics that resemble bipolar disorder (Kirshenbaum et al., 2014). However, detailed mechanisms that trigger in the onset of these symptoms are poorly

Abbreviations: CBF, cerebral blood flow; DMSO, dimethylsulfoxide; ER, endoplasmic reticulum; GSK3β, glycogen synthase kinase 3β; IP3 receptor, inositol triphosphate receptor; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; NCX, Na^+, Ca^{2+} exchanger; PI3-kinase, phosphoinositide 3-kinase; PDGFRβ, platelet-derived growth factor receptor β; PFC, prefrontal cortex; RECA-1, rat endothelial cell antigen-1; SDS, sodium dodecyl sulfate

* Corresponding author.
E-mail address: hkatsuki@gpo.kumamoto-u.ac.jp (H. Katsuki).

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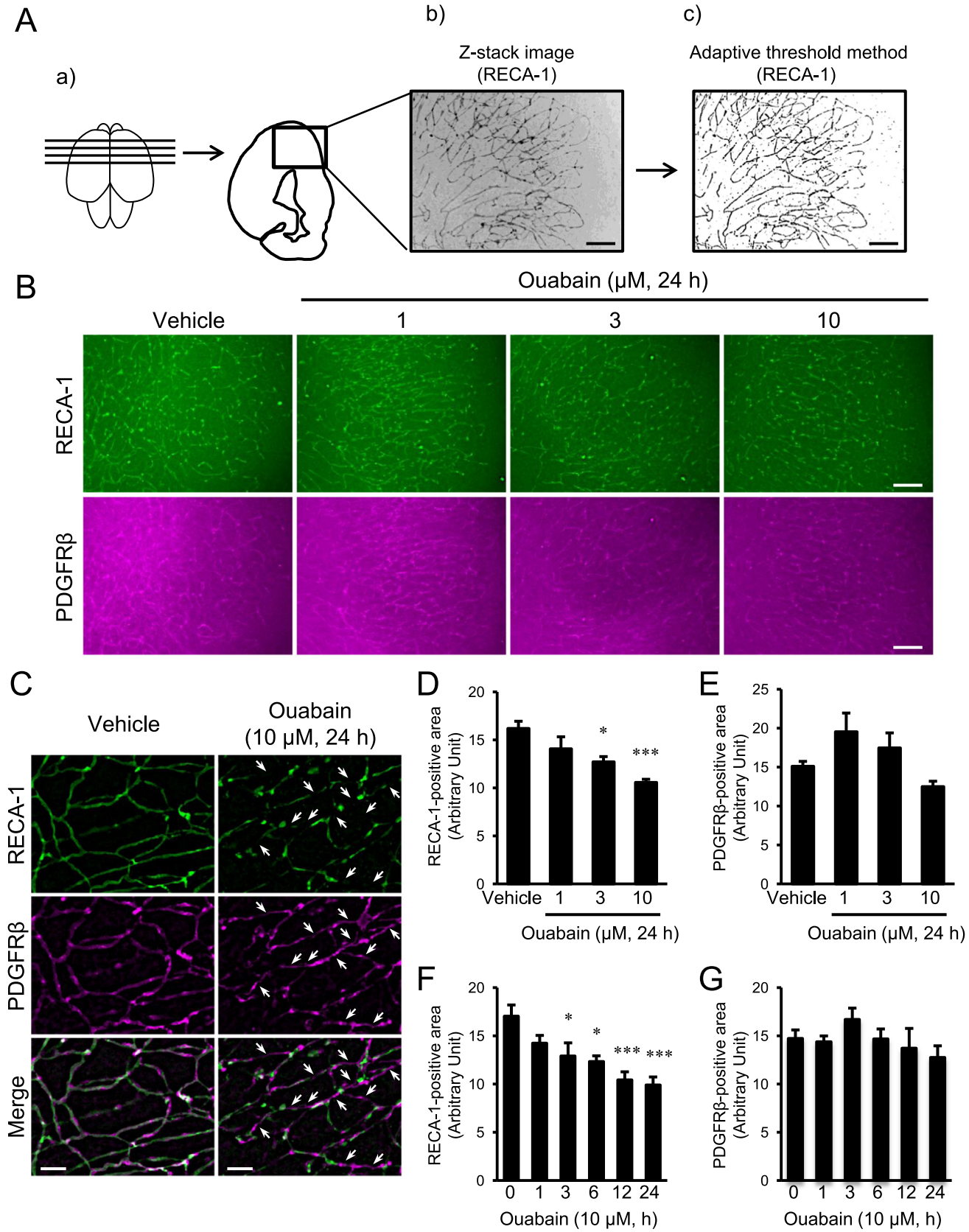

Fig. 1. Inhibition of Na^+, K^+-ATPase induces cerebrovascular endothelial degeneration. (A) Organotypic slice culture containing PFC region, and evaluation of RECA-1-positive area. Scale bars, 100 μm. (B) Representative photographs showing the results of immunofluorescence histochemistry on RECA-1 (upper panels) and PDGFRβ (bottom panels) after ouabain treatment. Slices were treated with ouabain (1–10 μM) for 24 h. Scale bars, 100 μm. (C) Representative photographs showing the results of immunofluorescence histochemistry on RECA-1 (upper panels), PDGFRβ (middle panels), and their merged image (bottom panels) after ouabain treatment. Slices were treated with ouabain (10 μM) for 24 h. Degenerating endothelial cells are indicated by arrows. Scale bars, 25 μm (D, E) Immunoreactive areas of RECA-1 (D) and PDGFRβ (E) were quantified. (F, G) Immunoreactive areas of RECA-1 (F) and PDGFRβ (G) were quantified. Slices were treated with ouabain (10 μM) for indicated periods. Results are expressed as means ± SEM. n = 6 slices for each treatment. Data sets were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. *, $^{***}p < 0.05$, 0.001 vs vehicle treatment.