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Research Report

Highly selective non-opioid kappa opioid receptor (KOR) agonist salvinorin A protects against forebrain ischemia-induced brain injury in rats

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ARTICLE INFO

Article history:
Accepted 14 February 2016
Available online 21 February 2016

Keywords:
Aquaporin-4 (AQP4)
Cerebral edema
Ischemia/reperfusion
Kappa opioid agonist
Salvinorin A
Motor function
Cognition

ABSTRACT

Objective: To investigate the effect of salvinorin A (SA) on brain injury and neurologic function post-brain ischemia/reperfusion (I/R) using a rat forebrain ischemia model and further explore the effect of kappa opioid receptor (KOR) inhibition by SA on aquaporin-4 (AQP4) expression in the hippocampus, cortex and striatum in the forebrain.

Methods: A forebrain ischemia model was established by colligating the bilateral common carotid arteries of SD rats for 10 min. The rats were randomized to receive dimethyl sulfoxide (DMSO), SA (1 μg/100 g body weight) or SA (onset of ischemia) plus SA antagonist nor-BIN (0.2 mg/100 g body weight. Rat brain water content was measured. Apoptotic neurons in the hippocampal CA1 region, cortex and striatum were enumerated. AQP4 in CA1, the cortex and the striatum were determined by immunoblotting assays and immunohistochemistry at 24 h post-ischemia. Neuromotor tests were performed on day 1, 2 and 5 post-ischemia. Water maze test was carried out on the 5th post-ischemia day.

Results: SA significantly attenuated I/R-induced increase in brain water content. Our immunoblotting assays and immunohistochemistry further revealed that SA effectively lessened I/R-induced upregulation of AQP4 expression in the hippocampus, cortex and striatum 24 h post-ischemia. SA also significantly reduced the percentage of dead and apoptotic neurons in these regions compared to DMSO. Moreover, SA partially reversed I/R-induced decline in rat motor function and cognition. The neuroprotective effects of SA were partially abolished by nor-BIN.

Conclusion: SA protects against I/R-induced brain injury by attenuating brain edema formation and inhibiting neuronal death and improves neurologic recovery of rats post-I/R.

1. Introduction

Stroke is the leading cause of adult disability and remains the third most common cause of death in industrialized nations and is also becoming an increasingly important disease in China (Gao et al., 2012). Malignant infarction, which is characterized by the formation of rapidly accumulating cerebral edema, occurs in 10–12% of stroke victims (Hacke et al., 1996). Brain edema following cerebral ischemia compromises

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http://dx.doi.org/10.1016/j.brainres.2016.02.024
0006-8993/

arterial inflow to surrounding tissues, leading to further ischemic damages and cerebral infarct enlargement, and is the major cause of stroke-related morbidity and mortality (McIntosh et al., 1996).

Despite the grave consequences of brain edema, preventive measures of cerebral edema are lacking, and therapeutic measures like mannitol and hypertonic saline and mechanical interventions such as decompressive craniectomy are largely reactive symptomatic interventions. The glial membrane water channel aquaporin-4 (AQP4) is largely expressed in astrocytic processes adjacent to cerebral capillaries and pial membranes lining the subarachnoid space and plays a critical role in maintaining cerebral water equilibrium (Hsu et al., 2011; Wen et al., 1999). Its importance in brain edema development following cerebral ischemia is being increasingly appreciated (Nito et al., 2012). AQP4 expression is associated with the inflammatory response (Tajuddin et al., 2013) and is upregulated following brain ischemia and is associated with aggravated brain injury and brain edema (Papadopoulos and Verkman, 2007). Inhibiting AQP4 expression has been shown to lessen brain edema following cerebral ischemia (Bhattacharya et al., 2013; Igarashi et al., 2011; Ribeiro Mde et al., 2006), suggesting that AQP4 may represent a novel preventative or therapeutic target in stroke management.

Kappa opioid receptor (KOR) plays an important role in the pathophysiological process of cerebral ischemia reperfusion injury (Chunhua et al., 2014). It has been shown to lessen post-cerebral ischemia injury and promote neurologic recovery (Birch et al., 1991; Chen et al., 2004; Mackay et al., 1993). Opioid KOR agonists could alleviate brain edema due to global and regional brain ischemia (Goyagi et al., 2003; Gueniau and Oberlander, 1997), but their clinical use is limited because of suppression of respiration and addiction. Salvinorin A (SA) is a highly selective non-opioid KOR agonist extracted from *Salvia divinorum* and, unlike other KOR agonists, has no respiration inhibitory effect and possesses low addictive potential (Vortherms and Roth, 2006). SA readily passes the blood brain barrier and also has a rapid onset of action. Our previous study showed that SA maintained brain autoregulation following brain ischemia in piglets (Su et al., 2012; Wang et al., 2012). We also found that SA maintained cerebral vasodilatation in both normal condition and during brain ischemia (Su et al., 2011). These findings suggest that SA can be a promising candidate for protecting and treating brain edema post-cerebral ischemia.

However, no study has been available on the relation between AQP4 and KOR inhibition by SA. We hypothesized that SA may lessen cerebral ischemia-induced brain injury by modulating AQP4 expression. In the current study, we investigated the effect of SA on brain edema and brain injury and neurocognitive function post-brain ischemia using a rat forebrain ischemia model and further explored the effect of KOR inhibition by SA on AQP4 expression in the hippocampus, cortex and striatum (Fig. 1).

2. Results

2.1. SA alleviates ischemia-induced brain edema in rats by downregulating AQP4 expression

As expected, forebrain ischemia resulted in a noticeable increase in the brain water content of rats in the I/R group compared to the sham operated rats ($P<0.01$) (Fig. 2A). This increase, however, was significantly attenuated by treatment with 1 μg/100 g body weight SA at 10 min after forebrain ischemia ($P<0.05$). The inhibitory effect of SA on brain edema was aborted by treatment with the SA antagonist nor-BIN ($P<0.05$). We then examined AQP4 expression in the hippocampus, the cortex and the striatum following forebrain ischemia. Our immunoblotting assays revealed that at 24 h after forebrain ischemia there was a significant increase in AQP4 expression in the hippocampus (1.97±0.16 vs. sham, 0.64±0.12; $P<0.01$), cortex (1.85±0.18 vs.

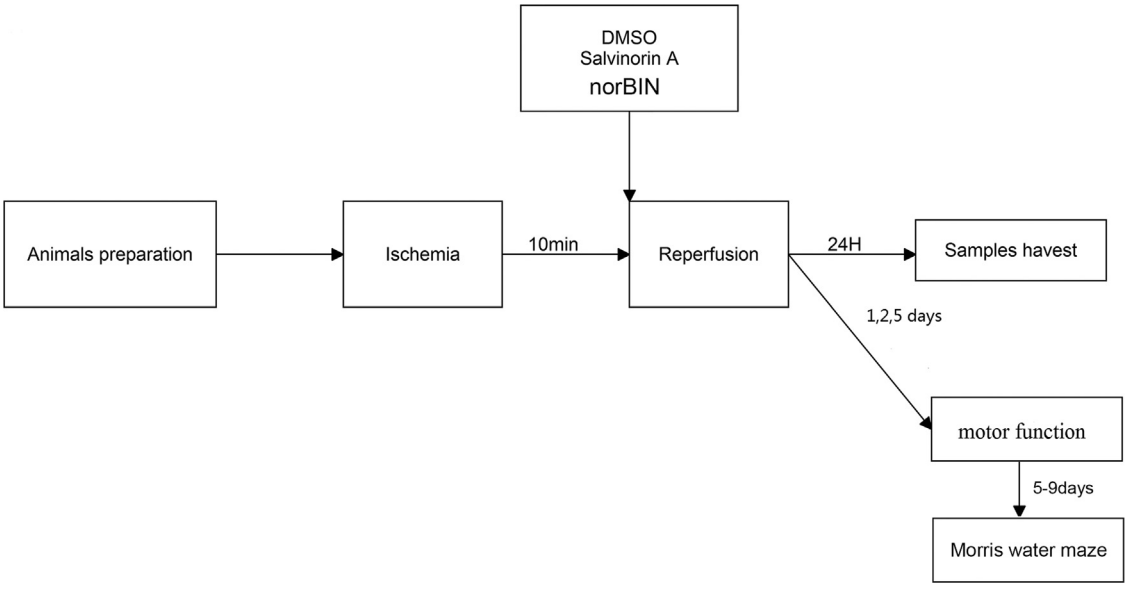

Fig. 1 – The study protocol flowchart. The bilateral common carotid arteries are colligated for 10 min to establish forebrain ischemia followed by reperfusion as detailed in Methods. The rats are given selective non-opioid Kappa opioid receptor (KOR) agonist Salvinorin A (SA) or SA plus KOR antagonist nor-BIN as described in Methods. DMSO: dimethyl sulfoxide.