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Original Article

# Effect of sevoflurane on the ATPase activity of hippocampal neurons in a rat model of cerebral ischemia-reperfusion injury *via* the cAMP-PKA signaling pathway

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**Abstract** We aim to investigate the effects of sevoflurane on the ATPase activity of the hippocampal neurons in rats with cerebral ischemia-reperfusion injury (IRI) *via* the cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) signaling pathway. Sixty rats were assigned into the normal, model and sevoflurane groups (n = 20, the latter two groups were established as focal cerebral IRI models). The ATPase activity was detected using an ultramicro Na (+)-K (+)-ATP enzyme kit. Immunohistochemical staining was used to detect the positive protein expression of cAMP and PKA. The hippocampal neurons were assigned to the normal, IRI, IRI + sevoflurane, IRI + forskolin, IRI + H89 and IRI + sevoflurane + H89 groups. qRT-PCR and Western blotting were performed for the expressions of cAMP, PKA, cAMP-responsive element-binding protein (CREB) and brain derived neurotrophic factor (BDNF). The normal and sevoflurane groups exhibited a greater positive protein expression of cAMP and PKA than the model group. Compared with the normal group, the expressions of cAMP, PKA, CREB and BDNF all reduced in the IRI, model and IRI + H89 groups. The sevoflurane group showed higher cAMP, PKA, CREB and BDNF expressions than the model group. Compared with

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the IRI group, ATPase activity and expressions of cAMP, PKA, CREB and BDNF all increased in the normal, IRI + sevoflurane and IRI + forskolin groups but decreased in the IRI + H89 group. It suggests that sevoflurane could enhance ATPase activity in hippocampal neurons of cerebral IRI rats through activating cAMP-PKA signaling pathway.


## Introduction

Ischemic stroke is regarded as one of the leading causes of mortality and disability in adults globally. In addition to its association with various serious physical deficits, including cognitive dysfunction, ischemic surgery can result in learning, and motor disabilities that may influence the patient's quality of life [1,2]. Ischemia-reperfusion injury (IRI) is a phenomenon that occurs in the event where the structure and function of ischemic organs and tissue cannot be recovered, following a period of inadequate blood supply followed by blood reperfusion and the larvaceous pathogenesis of neurological damage commonly referred to as stroke [3,4]. Reperfusion after cerebral ischemia can lead to additional brain damage, such as neuronal death, neurovascular injury, fatal cerebral edema and hemorrhagic transformation [5]. The mechanisms emphasized by which cell injury occurs in the event of cerebral IRI include inflammation, apoptosis, overproduction of reactive oxygen and nitrogen species (ROS/RNS), and excitotoxicity [6]. Although previous studies have shown that certain agents displayed promising neuro-protective roles, including Pim-1 kinase, cinnamophilin and epigallocatechin-3-gallate (EGCG), their protective roles have been identified to be limited and indirect [7,8] thus resulting in IRI remaining a leading medical problem requiring further study.

The sodium potassium adenosine triphosphatase ($Na^+$-$K^+$-ATPase) protein is an integral membrane protein located at the proximal tubules of the kidney acting to supply energy for the reabsorption and secretion of certain solutes [9]. Disturbances of $Na^+$-$K^+$-ATPase activity have been implicated in the pathophysiology of cerebral IRI [10]. IRI can lead to a functional reduction in the synthesis function of adenosine triphosphate (ATP) and further lead to a decline in ATPase activity [3]. Renal $Na^+$-$K^+$-ATPase has been shown to have a great effect on brain edema and neuronal damage in ischemic penumbra (IP) of cerebral IRI as well as a down-regulation in $Na^+$-$K^+$-ATPase activity in IP, representing a central pathogenesis of cerebral IRI [11]. Additionally the important role of $Na^+$-$K^+$-ATPase activity has been reported owing to its the protective effects of sevoflurane against IRI [2]. Sevoflurane is a volatile intravenous agent that is commonly used in the field of anesthesia [12]. It has been reported that sevoflurane preconditioning could induce endogenous neurogenesis against cerebral ischemic injury through promoting microglial activation [13]. Anesthesia by means of sevoflurane has been linked with postoperative cognitive dysfunction, however this can be relieved by inhibition of the NF-κB/P65 signaling pathway [14]. Previous reports have indicated that ATPase activity in cerebral IRI could be increased by sevoflurane [15]. As a regulator of the IRI cascade, cAMP-PKA activation has displayed promising signs in its ability to halt pathological cell sequestration, preventing destructive immune reactions, which ultimately promotes parenchymal cell survival [16]. Furthermore, the cAMP-PKA signaling pathway possesses the ability to enhance the ATPase activity *via* tyrosine phosphorylation [17—19].

Previous reports have provided evidence supporting the notion that MtCx43 could protect the neurovascular unit from the acute cerebral IRI by PKC, which is induced by the mito$K_{ATP}$ channel agonists [20]. A vast amount of research in rat models has been conducted in relation to the effects of propofol anesthesia on $Na^+$-$K^+$-ATPase activity during pneumoperitoneum [9]. Studies have also highlighted phosphodiesterases (PDEs) the enzymes that degrade cAMP, as playing a central role in regulating cAMP/PKA signaling cardiac myocytes [21]. However, little is known based on current research in regards to the influence of sevoflurane on the ATPase activity of hippocampal neuron in treating cerebral IRI. Furthermore, very little attention has been dedicated to understanding the interaction between sevoflurane and the cAMP-PKA signaling pathway in cerebral IRI. Therefore, during this study we established focal cerebral IRI rat models to determine the roles of by which sevoflurane affects the ATPase activity in the hippocampal neurons of rats with cerebral IRI *via* the cAMP-PKA signaling pathway.

## Materials and methods

### Ethical statement

The procedures and protocols of the study were in strict adherence with the principles and guidelines of the Declaration of Helsinki. Precautions were taken to minimize the pain and discomfort of all animals in the study.

### Establishment of focal cerebral IRI rat model

A total of sixty male specific pathogen-free (SPF) Sprague—Dawley (SD) rats weighing 250—300 g were selected for this study. All rats participating in the were provided by the Animal Experiment Center of General Hospital of Nanjing Military Area Command and housed in a SPF animal room under a 12 h light/dark cycle at 22 °C with a constant humidity of 50%—70%. For stability purposes the rats were conditioned by being adequately fed for 1 week and given free access to food and water. The middle cerebral artery