Involvement of the Paraventricular Nucleus in the Occurrence of Arrhythmias in Middle Cerebral Artery Occlusion Rats

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Background: Ischemic stroke complicating with arrhythmia is one of the main causes of sudden death. To investigate the association between ischemic stroke–induced arrhythmia and the activity of paraventricular nucleus (PVN), we used Fos protein as an objective indicator to illustrate the functional state of PVN neurons in middle cerebral artery occlusion (MCAO) rats, in single intracerebroventricular injection of L-glutamate rats and in application of MK-801 before L-glutamate injection and MCAO rats. *Methods:* The standard limb II electrocardiography was continuously recorded by a biological signal collecting and processing system. The experimental cerebral ischemic animal model was established by occluding the right middle cerebral artery. The Fos protein expression was detected by immunohistochemistry and Western blot. *Results:* The incidence of arrhythmia was significantly higher than that of controls (75.89% versus 0%), and Fos protein expression in the PVN also increased significantly in MCAO rats; both of them could be blocked by prior application of MK-801. Intracerebroventricular injection of L-glutamate induced changes in Fos protein expression and arrhythmia similar to that in the stroke, which could also be blocked by prior application of MK-801. *Conclusions:* It was concluded that activation of the PVN in MCAO rats is likely mediated by glutamate via activation of N-methyl-D-aspartic acid (NMDA) receptors, which causes arrhythmias. **Key Words:** Arrhythmia—glutamate—ischemic stroke—NMDA receptor—paraventricular nucleus.

Introduction

Ischemic stroke is a common life-threatening disease. Its high mortality is related to the lethal arrhythmia during the initial period of ischemic stroke.[1] Our previous studies proved that inward Na^+ current, transient outward K^+ current, and L-type calcium current channelopathy, and the Ca^{2+} overload in ventricular myocytes isolated from cerebral ischemic rats are likely the major ionic mechanisms underlying cerebrogenic cardiac arrhythmias.[2] However, what caused these changes in ventricular myocytes during ischemic stroke remains unknown. Arrhythmias are frequently seen in stroke patients, even in those without history or signs of primary heart diseases,[3] which suggest a central nervous system origin of these arrhythmias. Compelling evidence has revealed a close correlation between brain activation of unbalanced autonomic function and the onset of life-threatening cardiac arrhythmias, particularly sympathetic hyperactivity.[4] Recent studies further showed that the imbalance between sympathetic and parasympathetic activities does occur in those patients with cardiovascular disturbances in cerebrovascular diseases.[5] However, specific electrocardiographic (ECG) abnormality correlated with a localized intracranial pathologic change has not been established.

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Hypothalamic paraventricular nucleus (PVN) is a crucial region in brain control of autonomic functions and cardiovascular activity. In most instances, stimulation of the PVN could activate sympathetic output and induce arrhythmia.[6,7] In the heart, long-term activation of the sympathetic system can lead to uneven sympathetic innervations and the ensuing arrhythmia, even sudden cardiac death. The PVN receives glutamate innervations from forebrain and expresses large amount of N-methyl-D-aspartic acid (NMDA) receptors as well.[8] Microinjection of L-glutamate into the PVN could increase sympathetic nerve activity and cause positive cardiovascular response.[9] Earlier studies have also shown that ischemic stroke could increase levels of extracellular glutamate in the brain and sympathetic activity.[10,11] However, whether glutamatergic activation of the PVN is involved in the occurrence of arrhythmias after ischemic stroke and whether this activation is mediated by NMDA receptors remain to be determined.

For this purpose, we used Fos protein as an objective indicator to illustrate the functional state of PVN neurons in rats with middle cerebral artery occlusion (MCAO). We also compared the effects of single intracerebroventricular injection of L-glutamate on Fos expression and arrhythmias. Then we examined prior application of MK-801 on glutamate- and MCAO-evoked responses. The results indicated that activation of the PVN in MCAO rats is likely mediated by glutamate via activation of NMDA receptors, which causes arrhythmias.

Materials and Methods

Experimental Animals

Male Wistar rats (Experimental Animal Centre of Harbin Medical University, Harbin, China. Certificate No.09-2-1), 220-250 g, were used in this study. All rats were bred in an animal room with controlled temperature (23 ± 1°C), humidity (55 ± 5%), and food and water available ad libitum. All experimental protocols were preapproved by the Experimental Animal Ethic Committee of Harbin Medical University, China.

Experimental Groups

All rats were monitored the ECG for 20 minutes, and those with normal ECG were used in the following experiments. The standard limb II ECG was continuously recorded by a recorder (BL420; Chengdu TME Technology Co, Ltd, China). To observe the changes of the activity of PVN neurons after MCAO, 144 rats were randomly divided into the following 3 groups: (1) control (n = 16), (2) sham-operated (n = 16), and (3) right MCAO (n = 112). These rats in the third group were further randomly and equally divided into different time groups (n = 16 per group), 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, and 16 hours after MCAO. The rats in the control and sham-operated groups were sacrificed at 2 hours after anesthesia or operation, and the rats in MCAO groups were sacrificed at the corresponding time points after MCAO. The right (ischemic side) brain of each rat was used to detect Fos protein expression using immunohistochemistry and Western blot. To observe the effects of glutamate on arrhythmia induced by MCAO, 32 rats were randomly and equally divided into 4 groups (n = 8 per group): (1) saline (10 μL), (2) L-glutamate (.5 μmoles, 10 μL), (3) MK-801 (4 nmoles, 10 μL) before L-glutamate, and (4) MK-801 preceding MCAO. All rats were sacrificed at 30 minutes after single intracerebroventricular injection or surgery, and the right brain of each rat was used to detect Fos protein expression by Western blot. In determining the application amount of glutamate, we first calculated that in a total intracranial cerebrospinal fluid (CSF) of 200 μL in an adult rat,[12] injection of .5 μmoles of L-glutamate in 10 μL into the lateral ventricle would create a glutamate concentration of 2500 μmol/L after its even distribution in the CSF, which is much higher than 8.2 μmol/L, a positive predictive value for progressing stroke[13] and ensures sufficient glutamate diffused into the brain parenchyma from the CSF.

Preparation of Cerebral Ischemia Model by MCAO

Following an overnight fast, each rat was anesthetized with chloral hydrate (350 mg/kg, intraperitoneally). After exposure of the right common carotid artery, internal carotid artery, and external carotid artery surgically, a paraffin wax–coated fishing thread (diameter .28 mm) was aseptically introduced into the right common carotid artery, in an anterograde fashion toward the carotid bifurcation. It was then directed distally up the right internal carotid artery to a distance of 17.5 ± .5 mm from the carotid bifurcation to occlude the origin of the MCA. In sham-operated rats, the thread was immediately removed as soon as the origin of the MCA was reached.

Identification of Cerebral Infarction and Evaluation of Neurologic Deficit after MCAO

The neurologic deficits were evaluated by counting cumulative scales from scale 0-4, no visible neurologic deficits as the scale 0, forelimb flexion as the scale 1, contralateral forelimb grips weakly as the scale 2, if the animal was allowed to move around freely, it circled to the paretic side only when pulled by the tail as the scale 3, and spontaneous circling as the scale 4, respectively. For identification of the cerebral infarction, the brain was removed out and dissected into coronal sections of 2-mm thick after the animal was tested for neurologic deficits, then immersed into a saline solution containing 2% of 2, 3, 5-triphenyltetrazolium chloride at 37°C for 30 minutes. If the scale was over 2 and 2, 3, 5-triphenyltetrazolium chloride staining was significant, the model was successful (Fig 1).