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Short communication

Prevention of brain infarction by postischemic administration of histidine in rats

Naoto Adachi*,[1], Keyue Liu, Tatsuru Arai

Department of Anesthesiology and Resuscitology, Ehime University School of Medicine, Shitsukawa, Touon-shi, Ehime 791-0295, Japan

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Abstract

Focal cerebral ischemia for 2 h by occlusion of the right middle cerebral artery provoked severe brain infarction in the rat brain after 24 h. Intraperitoneal administration of histidine, a precursor of histamine, immediately and 6 h after reperfusion, alleviated brain infarction. The infarct size in the histidine (200 mg/kg, 500 mg/kg, and 1000 mg/kg, each time) groups was 71%, 39%, and 7% of that in the control group, respectively. Although intracerebroventricular administration of mepyramine (3 nmol), an H_1 antagonist, did not affect the morphologic outcome in histidine-treated rats, ranitidine (30 nmol), an H_2 antagonist, completely abolished the alleviation caused by histidine. These findings indicate that postischemic administration of histidine prevents development of brain infarction by stimulating central histamine H_2 receptors.

Theme: Disorders of the nervous system
Topic: Ischemia

Keywords: Cerebral ischemia; Histamine; Histidine; H_2 receptors; Middle cerebral artery; Rats

The pathogenesis of brain infarction is a macroscopic tissue necrosis caused by prolonged cerebral ischemia. Despite successful reperfusion of cerebral blood flow, there are several factors that harm neurons during reperfusion phase. Since brain infarction is usually treated after the onset of ischemia, prevention of reperfusion injury may be a principal therapy for brain infarction. In our previous study, we found that postischemic administration of histidine, a precursor of brain histamine, prevented delayed neuronal death in striatal neurons caused by transient occlusion of the middle cerebral artery for 15 min [2]. The improvement of histologic outcome may be caused by facilitation of central histaminergic activity, because blockade of central histamine H_2 receptors completely abolished the beneficial effect by histidine. On the other hand, histamine H_2 action suppresses inflammation by suppressing neutrophil infiltration and cytokines, whereas histamine H_1 action augments allergic inflammation [8]. Since inflammation during reperfusion phase is involved in the development of reperfusion injury, it is likely that postischemic administration of histidine alleviates the outcome caused by prolonged ischemia as well as delayed neuronal death. In the present study, therefore, the effect of postischemic administration of histidine on acute brain infarction was evaluated using an animal model of focal ischemia for 2 h. To examine the relationship with brain histamine, the effects of intracerebroventricular administration of histamine antagonists were assessed in histidine-treated rats.

This study was approved by the Committee on Animal Experimentation at Ehime University School of Medicine, Ehime, Japan. In experiment 1, the effects of postischemic administration of histidine on the infarct size were evaluated. In experiment 2, the effects of intracerebroven-

* Corresponding author. Fax: +81 89 960 5386.
E-mail address: nadachi@m.ehime-u.ac.jp (N. Adachi).
[1] Present address: Medical Division, Mabuchi Clinic, Kyoto 600-8357, Japan.

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tricular administration of histamine antagonists were examined in histidine-treated rats.

In experiment 1, forty-three male Wistar rats (300 g) were allocated to one of four groups; saline-injected control (n = 12), histidine (200 mg/kg) (n = 8), histidine (500 mg/kg) (n = 10), and histidine (1000 mg/kg) (n = 13) groups. Animals were anesthetized with 2% halothane in a gas mixture with 50% nitrous oxide in oxygen, and they breathed spontaneously. With the rats in supine position, the skin was incised along the median line of the neck, and the right common carotid artery was exposed. The thermocouple needle probe was inserted into the rectum to maintain rectal temperature. After an intraperitoneal injection of heparin (100 units), the root of the right middle cerebral artery was occluded by insertion of a silicone-coated 4–0 nylon thread from the bifurcation of the internal and external carotid arteries [12]. The tip of the thread was placed 18 mm distal from the bifurcation. After the surgical incision was sutured, animals were allowed to recover from anesthesia. During surgery, the rectal temperature was maintained at 37.0 ± 0.1 °C with a heating lamp. All rats showed paralysis of the contralateral limbs after recovery from anesthesia.

Animals were anesthetized again 5 min before reperfusion of the blood flow. After the skin was reopened, cerebral blood flow was resumed 2 h after middle cerebral artery occlusion by pulling the thread by 5 mm. Then, the surgical incision was sutured again, and the animals were intraperitoneally injected with saline or histidine (200, 500, or 1000 mg/kg). After recovery from anesthesia, the animals were allowed access to food and water ad libitum. The intraperitoneal injection was repeated 6 h after reperfusion.

The animals that underwent 24 h of reperfusion were anesthetized with an intraperitoneal injection of sodium pentobarbital. Then, the brains were perfused with heparin-prepared saline, and the animals were decapitated. The brains were rapidly removed and rinsed in saline. Brain slices, 2-mm thick, between the coronal planes at the optic chiasma and caudal edge of the mammillary body were incubated for 30 min with 2% triphenyltetrazolium chloride in 0.1 mol/L phosphate buffer (pH 7.4) at 37 °C. Triphenyltetrazolium chloride is reduced by dehydrogenase enzymes, which exist in viable cells and result in a formazan precipitate, thereby turning the tissue a deep red color. In contrast, the nonviable cells in the infarcted area show a pale gray color with this procedure. The tissue was stored overnight in 10% formalin. The infarct size was then determined using computer-aided planimetry by an investigator who was unaware of the particular treatment group.

In experiment 2, twenty-six rats (300 g) were allocated to one of three groups; saline (n = 7), mepyramine (n = 8), and ranitidine (n = 11) groups. Animals were anesthetized and subjected to occlusion of the middle cerebral artery for 2 h by an identical procedure to the one described above. Then, the animals were placed in a stereotaxic apparatus in the prone position under halothane anesthesia. The skull was exposed and a small burr hole for drug administration was drilled in the left hemisphere contralateral to the ischemic side (0.8 mm posterior and 1.5 mm lateral to the bregma) to avoid the effect of a wave of spreading depression [11]. A 27-gauge needle was inserted through the burr hole at a depth of 5.0 mm below the skull surface. Animals were intracerebroventricularly injected with saline (20 μL), mepyramine (3 nmol), or ranitidine (30 nmol) in a duration of 1 min. In this experiment, all animals were intraperitoneally injected with histidine (1000 mg/kg) after intracerebroventricular administration. Then, the animals were allowed to recover from anesthesia. Histidine (1000 mg/kg) was administered again 6 h after reperfusion. The brains were observed after a triphenyltetrazolium stain 24 h after reperfusion.

The data were analyzed by Scheffé's test to detect differences among groups.

In experiment 1, focal cerebral ischemia for 2 h provoked brain infarction in the striatum and surrounding cerebral cortex in saline-injected control animals (Fig. 1). Postischemic loading with histidine dose-dependently suppressed ischemic brain damage in both the striatum and cerebral cortex. The total volume of brain infarction in each histidine group was 71%, 39%, and 7% of that in the control group, respectively. Brain infarction was not observed on the nonischemic side.

In experiment 2, intracerebroventricular administration of mepyramine (3 nmol) did not affect the morphologic outcome in histidine-treated rats (Fig. 2). However, blockade of histamine H_2 receptors with ranitidine (30 nmol) completely abolished the improvement by histidine. Brain infarction was not found on the nonischemic side in the three groups.

Brain histamine is not transported from plasma, but is formed in the brain from L-histidine by a specific enzyme, L-histidine decarboxylase. Since L-histidine does not saturate L-histidine decarboxylase under normal physiologic conditions, histidine loading increases synthesis of brain histamine [14,15]. Considering that acute administration of histidine after ischemia improved the outcome in the present study, facilitation of central histaminergic activity may contribute to the prevention of reperfusion injury, and the beneficial effect of histidine may be associated with stimulation of histamine H_2 receptors in the brain.

Restoration of blood flow after prolonged ischemia initially results in hyperperfusion followed by sustained and progressive hypoperfusion [6,18]. The delayed hypoperfusion is a crucial factor in the poor outcome in postischemic recovery, and the magnitude of postischemic hypoperfusion depends on the severity and duration of proceeding ischemia [9]. In a rat model of transient occlusion of the middle cerebral artery, postischemic hypoperfusion has been shown to be prominent after 2 h of ischemia [18]. In the present experimental procedure, therefore, postischemic hypoperfusion may have contributed to the aggravation of brain infarction. Although the