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Basic Investigation

Effect of Batroxobin on Expression of Neural Cell Adhesion Molecule in Temporal Infarction Rats and Spatial Learning and Memory Disorder

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The effect of Batroxobin expression of neural cell adhesion molecule (NCAM) in left temporal ischemic rats with spatial memory disorder was investigated by means of Morri's water maze and immunohistochemical methods. The results showed that the mean reaction time and distance of temporal ischemic rats for searching a goal were significantly longer than those of sham-operated rats and at the same time NCAM expression of left temporal ischemic region was significantly increased. However, the mean reaction time and distance of Batroxobin-treated rats were shorter and they used normal strategies more often and earlier than those of ischemic rats. The number of NCAM immune reactive cells of Batroxobin-treated rats was more than that of ischemic group. In conclusion, Batroxobin can improve spatial memory disorder of temporal ischemic rats and the regulation of the expression of NCAM is probably related to the neuroprotective mechanism.

Snake venom is a well-known traditional Chinese medicine used to treat some diseases including ischemic cerebrovascular diseases. Batroxobin is a thrombin-like enzyme isolated from snake venom which enables to remove specifically fibrinogen. Our previous studies found that Batroxobin was an excellent drug for treatment of acute ischemic cerebrovascular diseases and can rapidly stops transient ischemic attacks [1,2]. Batroxobin could attenuate cerebral edema in ischemic gerbils and influence c-fos, arginine vasopression (AV), ET1 and NOS expression as well as effect heat shock protein 70 (HSP70), basic fibroblast growth factor BFGF) expression of rat brains during ischemia and reperfusion[3~9]. Batroxobin could influence monoamines and their metabolism levels in extracellular fluid of rat brains[10], apoptosis[11] and decrease NO toxicity[12]. We also found that Batroxobin could improve learning and memory disorder and effect c-Jun expression of in

rats with left temporal ischemia[13-14]. The protective effect of Botroxobin was proved by pathological evidences observed with light microscope, electromicroscope and immunocytochemical studies[15-16].

Neural cell adhesion molecule (NCAM) belongs to the cell adhesion molecule of the immunoglobulin superfamily characterized by the Ig domain. The Ig domain contains approximately 100 amino acids forming two β-sheets. In brain, NCAM is found in three major forms that are either transmembrane (NCAM-180, NCAM-140) or attached to the membrane via a glycosylphosphatidyl inositol anchor (NCAM-120). NCAM is important not only during brain development but also in synaptic plasticity in the adult brain associated with regeneration and learning, but the mechanisms are poorly understood [17]. On the other hand, the association of memory disorder induced by temporal ischemia and NCAM expression as well as the effect of Batroxobin are unclear.

A recently developed spatial memory task is Morri's water maze (MWM)[7] which has been proven very useful for characterizing the neurochemical basis of spatial learning and memory in rodents[8]. In this study we used Morri's water maze to investigate the effect of Batroxobin on the expression of NCAM in temporal ischemic rats with spatial learning and memory disorders.

Materials and Methods

Animal and grouping Thirty-six male Wistar rats weighing 180~220g were randomly divided into three groups, ischemia group (n=12), Batroxobin-treated group (n=12) and sham-operated group (n=12). The rats were in experimental environment for two days for habituation and then a five-days behavioral training was carried out during 10:00 am ~ 5:00 pm. The rats were free for food and water during the whole time of experiment. After the behavioral experiment had finished, the rats were killed and NCAM expression of brain tissue was studied by immunohistochemical method.

Animal model: Selective left temporal ischemia of rat model was induced by the photochemical method[5]. Each rat was anaesthetized with 10% chloral hydrate (0.3g/kg, i.p.). The rats were placed in a stereotaxic frame and rectal temperature was kept at 36.5 ℃ ± 0.7 ℃ with a heating lamp. The left temporal scalp incision was performed to expose the skull surface. For skull illumination, standard equipment was used. A light guide fiber cool light was positioned on the left temporal skull surface. Rose bengal dye (Sigma Chemical Ltd; 20mg/ml in sterile 0.9% NaCl) was injected slowly via a lateral tail vein (20mg/kg), and the left temporal skull surface was illuminated for 20minutes.The light was then interrupted, the scalp wound closed with sutures, and the animal allowed to recover. Batroxobin-treated rats received an injection of Batroxobin (8BU/kg, i.p.) 30min before operation and two days later. Sham-operated control animals were prepared with the same procedures except illumination.

Morri's water maze behavioral experiment[7]: The behavioral experiment equipment consists of Morri's water maze and Morri's Maze Experimental Assistant System (MMEAS). Morri's water maze is a large circular pool (d=94mm, h=55cm) filled with water rendered opaque with milk powder. Submerged just below the water surface somewhere within the pool is a platform (s=20cm^2) onto which the rat can climb to emerge from the cool water and escape from the necessity of swimming. MMEAS consists of image collect card, video camera and automated computer tracking system. The experimental data were collected and analyzed automatically[19].

The behavioral experiment was started on the fourth day after operation and continued for five days including 1 day of pretraining trial and 4-day training trial. Each rat accepted 6 trials per day with a 15 min interval for each and the total number of training trial is 24. The experiment date included response time (s),

distance (cm) and strategies. The mean value of three trials for response time and distance was considered as one unit respectively. F and X^2 test were analyzed with SPSS software package.

Immunohistochemistry: Six rats from each group were anaesthetized with 10% chloral hydrate (0.3g/kg, ip), brains were fixed by transcardiac perfusion of 4% paraformaldehyde 40 ml, then the brains were quickly removed and kept in the fixation of 4% paraformaldehyde at 4 ℃. They were subsequently cryostat sectioned (slice thickness,30 μm) and stained for NCAM by using monoclone antibody (Santa Cruz Ltd) and SP kit[9]. The slices of ischemic brain tissue were observed under microscope.

Results

Morri's water maze behavioral experiment: In brief, the mean reaction time and distance of temporal ischemic rats for searching a goal were significantly longer than those of sham-operated rats. However, the mean reaction time and distance of Batroxobin-treated rats were shorter and they used normal strategies more often and earlier than those of ischemic rats (details see elsewhere [19]).

Expression of NCAM: The expression of NCAM of left temporal ischemic rats was significantly increased as compared with that of sham-operated group. However, expression of NCAM in Batroxobin-treated rats was more than that of ischemic group (Fig.).

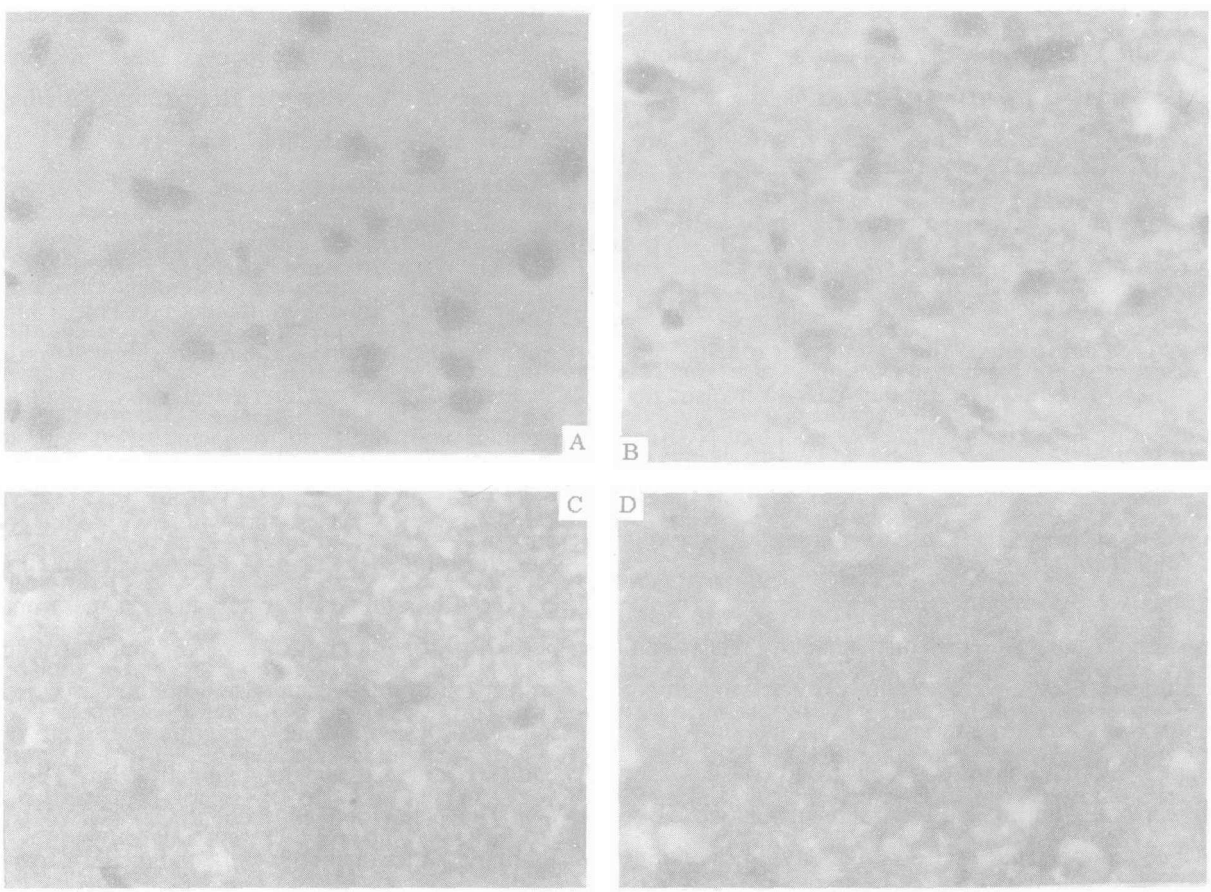

Fig. The NCAM expression of rat temporal cortex in various groups.
A. negative control (without NCAM antibody) B. Sham-operation group
C. ischemic group D. Batroxobin-treated group

Discussion

In the present Morri's water maze behavioral experiment and immunohistochemical study, we found that the spatial learning and memory ability was disordered in left temporal ischemic rats and the NCAM expression of ischemic temporal cortex was increased. This result was in accordance with our previous study that c-fos and c-Jun gene expression was increased during cerebral ischemic-reperfusion[5-14]and the present result further suggested that there may be a relation among left temporal ischemia, spatial memory disorders and NCAM expression.

The classical role of NCAM is to mediate cell adhesion through a homophilic action, NCAM on one cell binding to NCAM on another. It is believed that homophilic NCAM binding activates signal transduction. *In vitro*, NCAM or NCAM antibodies have been shown to result in increased intracellular calcium in certain neuronal cells. It has been proposed that NCAM binds to the fibroblast growth factor receptor (FGF-R), thereby activating intracellular signaling. It is believed that in a learning process neuronal connection are subject to structural changes to establish long-term memory. Some studies indicated a role for NCAM in learning and establishment of long-term memory. First, intracranial injection of NCAM antibodies in rats and chicks has been showed to inhibit consolidation of a passive avoidance task, when administered in a discrete time window after the learning session. Injection of NCAM antibodies in the six to eight hours posttraining period impaired establishment of long-term memory for a passive avoidance response, when tested at the 48-h recall time. In addition, it has been shown that NCAM is synthesized with other glycoproteins, in a glycoprotein-dependent phase of establishment of long-term memory occurring six to eight hours after the learning session. Second, NCAM knock-out mice have shown deficiency in spatial learning when tested in Morris water maze. Third, changes in the posttranslational modification of NCAM have been observed following learning [20,21].

Our MWM study has found that the mean reaction time and distance of Batroxobin-treated rats are shorter and they use normal strategies more often and earlier than those of ischemic rats[18]. The result is in accordance with our previous study that Batroxobin may ameliorate the dysfunction of learning and memory in temporal ischemic rats measured by step-down methods[12]. The MWM result further indicates that the Batroxobin may also ameliorate the dysfunction of spatial learning and memory in temporal ischemic rats measured by Morri's water maze. It has been demonstrated that Batroxobin can reduce the morbidity and mortality of ischemic stroke in gerbils and rats, ameliorate cerebral edema, and attenuate the dysfunctions of arginine vasopression, excitatory amino acids and nitric oxide, and down-regulation of endothelin-1 gene expression. Based on these mechanisms, it is reasonably proposed that Batroxobin may be of regulation of NACM expression in ischemic brain tissue and ameliorate ischemic brain injury, and may therefore improve the spatial learning and memory disorder of left temporal ischemic rats.

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