Effect of Interleukin-1β on the Variation of Adenylyl Cyclase Expression in Rats with Seizures Induced by L-Glutamate*

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Summary: To explore the mechanism of interleukin-1beta (IL-1β) in the onset of seizure and the effect of IL-1β on the expression of adenylyl cyclase (AC) in rats with seizure induced by L-glutamate. Experimental rats were first injected with IL-1β and then L-glutamate (a dose under the threshold) was injected into the right lateral ventricle. The rats were sacrificed 4 h after the onset of epileptic activity and examined for changes in behavior, immunohistochemistry and compared with those with seizure induced by L-glutamate alone. It was found that the expression of AC in hippocampal and neocortex of rats with seizure induced by IL-1β and L-glutamate were stronger than that of control group (P<0.05), without significant difference found between the L-glutamate group and IL-1β plus L-glutamate group in the expression of AC, the latent period and the severity of seizure. When IL-1ra were given (i. c. v.) first, there was no epileptic activity and the expression of AC did not increase. There were no differences in the expression of AC of rats with IL-1ra and that of control rats. But when 2-methyl-2-(carbocyclopropyl)glycine (MCCG) was given (i. c. v.) first, the strongest expression of AC, the shortest latent period and the most serious seizure activities were observed. The results indicated that IL-1β could facilitate the onset of epilepsy induced by L-glutamate through IL-1R, metabotropic glutamate receptors might work with IL-1R and the increased expression of AC might be involved in the process.

Key words: seizure; interleukin-1beta; adenylyl cyclase; rat

Epilepsy is a kind of severe neural system disease. In the past, researches had already demonstrated that the onset of epilepsy was related to the imbalance of immune-neuro-endocrine network. IL-1, as an important cytokine, exists extensively in the brain and is involved in the regulation of the network by receptor IL-1R. The IL-1 family currently comprises IL-1α, IL-1β and the IL-1 receptor antagonist, IL-1ra. IL-1α and IL-1β could all act on the brain, but the action of IL-1β is predominant. Some investigators found that the amount of IL-1 in the cerebrospinal fluid increased in adult rats when seizure was induced and in epileptic experiment animal model. IL-1R and GluR worked together to adjust the cellular function via interaction1−4. There was, however, no report on the interaction between IL-1R and mGluRs and their relation with adenyl cyclase in the experimental animals with epilepsy. In present study IL-1β, IL-1α and MCCG were injected into the lateral ventricle of rats and the changes in the expression of AC in the hippocampus and cerebral cortex, were examined to explore the function of IL-1β in epileptogenesis.

1 MATERIALS AND METHODS

1.1 Animal and Grouping

Forty normal male Sprague Dawley rats, weighing 170−240 g were used. All animals were provided by the Animal Center of Tongji Medical College of Huazhong University of Science and Technology, Wuhan, China. They were divided into 5 groups at random with eight rats in each group: Rats were anaesthetized by sodium pentobarbital (40 mg/kg, ip), and were injected normal saline 6 μl in group 1 as control group 1, 6 μl 50 mg/ml L-Glutamate in group 2, 500 U/2.5 μl IL-1β (Beijing Baosai Company, China), and 3 μl 50 mg/ml L-Glutamate (group 3). IL-1ra (Beijing Baosai Company, China) 2.5 μg/2.5 μl, IL-1β 500 U/2.5 μl and 50 mg/ml L-Glutamate 3 μl (group 4), and MCCG (Sigma Company, USA) 40 nmol/2.5 μl, IL-1β 500 U/2.5 μl and 3 μl 50 mg/ml L-Glutamate (group 5) into the lateral ventricle. The stereotaxic coordinates were 0.8 mm posterior to bregma, 1.2 mm later to the midline and 3.7 mm below the dura, the biauricular line was set 2.4 mm below the incicor bar.

1.2 Behavior Observation

Behavior of seizure was classified using criteria previously described by Schultz-Krohn. The behavioral performance of each group was observed and the latent period and lasting time were recorded.

1.3 Immunocytochemistry

Four h after treatment, rats were anaesthetized with sodium pentobarbital (40 mg/kg, ip) and perfused through ascending aorta with 100 ml 0.9 % normal saline, followed by 400 ml 4 % paraformaldehyde in phosphate buffer (PB, 0.1 mol/L, pH=7.4). The brains were removed and post-fixed in foregoing paraformaldehyde solution for 3 h, then immersed in 20 % sucrose solution overnight at 4 °C. Sections of 20 μm were cut coronally in a cryostat. The sections contained dorsal hippocampus were collected in phosphate buffer...
buffer for immunocytochemical staining by the method of SABC. The primary antibody was rabbit anti-AC (1:100, Santa Cruz, USA) and stained in solution containing 0.5 mg/ml dianinobenzidine, 0.05 mol/l Tris-HCl (pH = 7.6), 0.03 % H2O2 at room temperature for 15 min. The sections were thoroughly washed several times with PBS after each step. Subsequently the sections were mounted, dehydrated, cleared, cover-slipped and observed under light microscopy.

1.4 Control Test

In replacing test, sections were incubated with normal rabbit serum instead of primary antibody. In empty test, sections were incubated without primary antibody.

1.5 Western Blotting

Four h after treatment, rats were sacrificed by decapitation and the hippocampus and cerebral cortex were removed. The brain tissues were minced and homogenized with ice-cold buffer by using glass homogenizers for 10 min. The homogenates were centrifuged with 12000 g for 10 min at 4°C. The supernatants were solubilized (1:1, v/v) in loading buffer and then were boiled for 10 min. After cooling, the samples were loaded (15 µg protein per lane) on standard SDS-PAGE gels, proteins were then transferred to polyvinylidene difluoride membranes. Blocking of non-specific binding sites was performed by incubating the membrane for 1 h at 37°C in 5% non-fat dry milk dissolved in PBS. The incubation with the adenylyl cyclase antibody (1:200, Santa Cruz, USA) was carried out for 2 h at 37°C. Biotinylated goat anti-rabbit IgG in PBS (1:200, Beijing Zhongshan Company, China) was utilized as the secondary antibody at 37°C for 1 h, and then the membranes were incubated with horseradish peroxidase streptavidin complex (1:200, Beijing Zhongshan Company, China) at 37°C for 1 h and staining solution containing 0.6 mg/ml dianinobenzidine, 0.01 mol/l Tris-HCl (pH = 7.6), 0.03 % H2O2 at room temperature for 1–2 min. The membranes were thoroughly washed several times with PBS after each step. The resultant products appeared as brown belt in membranes.

1.6 Image Analyses

Fifty fields to be evaluated were chosen from immunocytochemical stained slices of each group at random. Identically-sized images of these fields were captured on a microscope fitted with a CCD and connected to a computer and the belt of Western blotting membranes were scanned. Images were analyzed by using an HMIA-2000 analyzer, USA. The mean values of optic density of the cells and area integrated optical density of the belt of the Western blotting of the hippocampus and cerebral cortex were calculated. Values were compared by t-test.

2 RESULTS

2.1 Behavior Observation

The group 1 showed no epileptic seizure, the group 2 had rhythmical spasm of muscles of face, forelimb and trunk as well as piloerection, corresponding stage 3–4, with the latent period being about 2 min and the lasting time 20 min. Rats of the group 3 showed no clinical manifestation after infection of II-1β, but showed facial, trunk and limb tonus after the administration of L-glutamate.

There was no statistical difference between the group 2 and the group 3 (P>0.05). The seizure did not occur in the group 4 either. The group 5 showed convulsion (stage 4–5) after injection of MCCG, II-1β and L-glutamate in turn. Compared with the group 2, its latent period was shorter and lasting time became longer (P<0.05, table 1).

2.2 Immunocytochemistry

The positive immunoreactivity (IR) products of adenylyl cyclase showed brown color, fine paritcles located mainly in the cytoplasm and nuclei (fig. 1). The expression of AC of the group 2 and the group 3 was higher than that of the control group in the hippocampus area CA3 (P<0.05). The AC content of the group 4 in the cortex and hippocampus was almost as same as the control group (P>0.05). But the group 5 showed the highest levels among the 5 groups. There were significant differences as compared with the control group (P<0.05, table 1).

Table 1 The latent period and lasting time (min) of seizure and the average A of the immunoreaction of AC in the cortex and hippocampus of the 5 groups (x±s)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Latent period</th>
<th>Lasting time</th>
<th>Cerebral Cortex</th>
<th>Hippocampal area CA3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.0719±0.0019</td>
<td>0.0985±0.0065</td>
</tr>
<tr>
<td>2</td>
<td>2.0±0.3</td>
<td>21.0±1.3</td>
<td>0.1228±0.0119</td>
<td>0.1305±0.0045</td>
</tr>
<tr>
<td>3</td>
<td>1.8±0.2</td>
<td>22.5±1.5</td>
<td>0.1163±0.0119</td>
<td>0.1298±0.0083</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0.0629±0.0042</td>
<td>0.0912±0.0172</td>
</tr>
<tr>
<td>5</td>
<td>1.1±0.2</td>
<td>16.1±1.6</td>
<td>0.1610±0.0112</td>
<td>0.1761±0.0269</td>
</tr>
</tbody>
</table>

P<0.05 as compared with the group 1

2.3 Western Blotting

The expression of AC of the group 2 and the group 3 was higher than that of the control group in the cortex and hippocampus (P<0.05), and the AC content of the group 4 in the cortex and hippocampus was almost as same as the control group.