BIOCHEMICAL ASPECTS OF PENTYLENE-TETRAZOLE INDUCED SEIZURE

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Abstract.....At the onset of pentylentetrazole induced convulsions, the adenylate cyclase activity and phosphodiesterase activity were increased. The former was markedly stimulated in the brain stem of rats. In the cerebral cortex and brain stem, the glucose level was significantly decreased, and the concentration of glucose-6-phosphate was increased. However, the definite changes in energy reserve system of the brain could not be observed at the onset of pentylentetrazole induced seizures. The present study revealed some correlation between pentylentetrazole convulsions and the adenylate cyclase activity and glycometabolism.

Key words: pentylentetrazole seizure, adenylate cyclase, glycometabolism, energy metabolism

INTRODUCTION

The occurrence mechanisms of epilepsy and/or convulsions were not clearly understood. To clarify the relationship between onset of convulsion and biochemical changes prior to convulsion, many experimental models of epilepsy were generally used. However, the biochemical or neurochemical characterization of these experimental epilepsy was not yet available. Withdrawal seizure of barbital was one of the experimental epilepsy, and pentylentetrazol seizure was also used for the experimental convulsion. These models for epilepsy have been examined in our laboratory.

On epileptic seizure, rate of glycolysis and hexokinase activity is depressed (Jinnai et al.; 1960). Hypoglycemia is able to induce the convulsion (Tower; 1960). Carbohydrate metabolism in nervous system was sensitively influenced by various neural activity
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(Lowry et al.; 1964, Collins et al.; 1970, Ferrandell and McDougal; 1971), neurotropic drug dosing (Strang and Bachelard; 1973, Bachelard and Lindsay; 1966), etc. The metabolism in nervous system is one of the important markers of neuronal activity.

In this report, the effects of pentylenetetrazole on the glycometabolizing system and on the adenylate cyclase activity were examined.

MATERIALS AND METHODS

Male Sprague-Dawley rats (5.5 week age) were used for experiments. Pentylenetetrazole (80 mg/kg) was injected intraperitoneally. On clonic-tonic convulsion, the rat was sacrificed by decapitation and the brain was immediately removed. The brain was dissected into 3 portions; the cerebral cortex, brain stem, and cerebellum.

For measuring the metabolites of glycometabolism and energy metabolism, the tissue was homogenized by 4 volume of 0.4N perchoric acid using Polytroin (Kinematica Co., Switzerland). For assay of enzyme activities, the tissue was homogenized by 4 volume of 0.32M sucrose solution (pH 7.4; containing 2mM Tris, 40μM EGTA) also by using Polytroin. In all process before homogenization, tissue was kept in the condition cooled by dry-ice and liquid nitrogen respectively for assay of enzyme activity and metabolites. The protein content in sample was measured by the method of Lowry (1951). The method for measuring enzyme activity and each component was previously used. (Iwata et al.; 1978).

Assay of adenylate cyclase activity: The standard assay system (final volume 0.5ml) for measurement of adenylate cyclase activity contained (in mmole/liter): Tris-maleate (pH 7.4), 80; ATP, 0.3; MgSO₄, 1.2; isobutylmethylxanthine, 1; EGTA, 0.6; plus 50 μl of homogenate. The reaction was carried out for 2.5 min at 30°C and stopped by placing the tube in boiling-water bath for 3 min. The amount of cyclic AMP formed in each tube was measured on duplicate 50 μl aliquots by "Amersham cyclic AMP assay kit". Interference of test substance in the binding protein assay was negligible. Under the experimental conditions used, enzyme activity was proportional to the time of incubation and enzyme concentration.

Assay of phosphodiesterase activity: In order to measure the activity of c-AMP phosphodiesterase, 10 μl of homogenate was incubated in the following medium (Baba et al.; 1978), final volume 0.1 ml containing (in mmole/liter) adenosine-3',5'-monophosphate, 1; Tris-HCl buffer (pH 8.0), 40; MgCl₂, 5; CaCl₂, 0.5; plus 10 μl homogenate. After incubation for 10 min at 30°C, the tube was placed in boiling water for 2 min to stop the reaction. The volume of aliquot was adjusted to 0.5 μl and adenosine-5'-monophosphate content of this sample was analyzed (Jaworek 1974). Phosphodiesterase activity was represented as amount of produced adenosine-5'-monophosphate (nmoles/mg protein/10min). These biochemical measurements were performed under duplicated condition. Student's t-test was used for statistics analysis.

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RESULTS

Table 1 showed the effect of pentylenetetrazole induced convulsions on cyclic-AMP system. Both in the cerebral cortex and brain stem, pentylenetetrazole had a tendency of increasing the adenylate cyclase activity (197 percent of control, 225 percent, respectively). Phosphodiesterase activity was also stimulated by pentylenetetrazole seizure (140 percent of control).

Table 1. Effect of pentylenetetrazole on cyclic AMP system of rat brain (cerebral cortex, brain stem).

<table>
<thead>
<tr>
<th></th>
<th>AC</th>
<th>PDE</th>
</tr>
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<tbody>
<tr>
<td>cerebral corex</td>
<td>16.455±5.499</td>
<td>651.86±163.658</td>
</tr>
<tr>
<td></td>
<td>(197.51%)</td>
<td>(140.77%)</td>
</tr>
<tr>
<td>brain stem</td>
<td>16.839±4.491*</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>(225.39%)</td>
<td></td>
</tr>
</tbody>
</table>

The activity was shown as the mean±S. E. M. of duplicated 6 observations. The unit of activities are pmole c-AMP/mg protein for adenylate cyclase activity (AC) and produced AMP nmole/mg protein/10 min for phosphodiesterase activity (PDE). Numbers in parenthesis are percent change of control.

*p<0.10, compared to control.

Effects of pentylenetetrazole on central glycometabolism of rats were shown in table 2. The seizure decreased the glucose level in the cerebral cortex (20 percent of control, p<0.01) and the brain stem (53 percent of control, p<0.01). In the cerebral cortex and brain stem, the concentration of glucose-6-phosphate was increased. In the former, 135 percent of control was observed, while in the latter 134 percent of control was observed. The glycogen concentration was not changed in three portions. Little changes were observed in the cerebellum. The lactate level was not significantly changed in the cerebral cortex, brain stem, and cerebellum.

Table 2. Effect of pentylenetetrazole on glycometabolism.

<table>
<thead>
<tr>
<th></th>
<th>Glycogen</th>
<th>Glucose</th>
<th>G-6-p</th>
<th>Lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td>1.6167±0.1421</td>
<td>0.8150±0.1619**</td>
<td>0.1400±0.0145*</td>
<td>1.6167±0.3130</td>
</tr>
<tr>
<td></td>
<td>(102.21%)</td>
<td>(20.07%)</td>
<td>(135.00%)</td>
<td>(93.90%)</td>
</tr>
<tr>
<td>Brain stem</td>
<td>1.6750±0.4659</td>
<td>2.2383±0.2011**</td>
<td>0.1728±0.0180*</td>
<td>1.3317±0.1780</td>
</tr>
<tr>
<td></td>
<td>(87.16%)</td>
<td>(53.04%)</td>
<td>(134.26%)</td>
<td>(72.31%)</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>1.3917±0.0704</td>
<td>2.2117±0.3202</td>
<td>0.1342±0.0199</td>
<td>1.7817±0.4603</td>
</tr>
<tr>
<td></td>
<td>(79.30%)</td>
<td>(76.05%)</td>
<td>(91.11%)</td>
<td>(143.30%)</td>
</tr>
</tbody>
</table>

The unit of component is μmole/g wet weight. Each data is the mean±S. E. M. of duplicated 6 observations. The numbers in parenthesis are the percent change of control. G-6-p: glucose-6-Phosphate.

*p<0.10, **p<0.01, compared to control.
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In table 3, the effects of pentylentetrazole on central energy reserve system were shown. In three portions, creatine phosphate (CP), adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) were examined. On convulsions, the ATP level the in brain stem was decreased (62 percent of control, p<0.02). The AMP level in the cerebellum was increased (292 percent of control, p<0.10). However, the definite tendency could not generally be observed.

Table 3. Effect of pentylentetrazole on energy metabolism. The unit of component is nmoles/mg wet weight of tissue.

<table>
<thead>
<tr>
<th></th>
<th>CP</th>
<th>ATP</th>
<th>ADP</th>
<th>AMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td>3.3067±0.1140 (96.78%)</td>
<td>2.1650±0.2300 (105.61%)</td>
<td>0.4350±0.0457 (105.53%)</td>
<td>0.2235±0.1152 (49.30%)</td>
</tr>
<tr>
<td>Brain stem</td>
<td>2.5717±0.5135 (90.50%)</td>
<td>1.5917±0.2594** (62.87%)</td>
<td>0.7183±0.1724 (177.36%)</td>
<td>0.2767±0.1492 (367.92%)</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>2.7833±0.3745 (131.20%)</td>
<td>1.9600±0.3477 (92.60%)</td>
<td>0.4717±0.0995 (86.82%)</td>
<td>0.1540±0.0532* (292.22%)</td>
</tr>
</tbody>
</table>

* p<0.10, ** p<0.02, compared to control.

**DISCUSSION**

Pentylentetrazole is a central nervous system stimulant whose use in experimental models of epilepsy has been investigated. Previously reported about the biochemical aspects about seizure induced by barbital withdrawal (Yanaura et al.; 1982). In those reports, glycometabolism, energy reserve system, and cyclic-AMP system were sensitively influenced. The assay of these biochemical systems was the objective and quantitative index of barbital dosing and withdrawal. In the present study, the effects of pentylentetrazole on the glycometabolizing system and on the adenylate cyclase activity were examined. At the onset of convulsions, the adenylate cyclase activity increased, while the glucose level decreased. However, energy reserve system was not markedly involved in pentylentetrazole induced seizures. In this respect, pentylene -tetrazole seizures were different from barbital withdrawal seizures.

The mechanism of pentylentetrazole convulsant action in still undefined. However, Gross et al (1980) demonstrated that both reserpine and aminophylline inhibit seizure-induced cyclic-AMP elevations and concomitantly hasten the appearance of tonic seizures after pentylentetrazole injection. Cyclic-AMP in brain may thus have an antiepileptic effect and perhaps have some role in mechanisms leading to seizure attenuation or termination. Large increase in central nervous system levels of cyclic AMP was also associated with electrically induced seizures (Sattin; 1971, Lust et al.; 1972, 1976).
On the other, Jinnai et al (1960) reported that glycolysis and hexokinase activity were depressed by epileptic seizure. Whatever the mechanism it seems quite evident that the present results revealed some correlation between pentylentetrazole induced seizure and the increase of adenylate cyclase activity and glycometabolism.

REFERENCES


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