LACK OF ALTERATION IN REGIONAL BRAIN ADENOSINE 3',5'-CYCLIC MONERPETIC
Previously reported studies have suggested that acute and chronic treatment with ethanol induces alterations in adenosine 3', 5'-cyclic monophosphate (c-AMP) levels in the brain. Because the methods used to minimize postmortem accumulation of c-AMP are now considered to be inadequate, the effects of ethanol were reinvestigated using focused microwave fixation to prevent postmortem c-AMP accumulation. These studies were extended to include measurements in seven
20. ABSTRACT (continued)

areas of the rat brain and in animals rendered ethanol-dependent. Three treat-
ment groups were examined: acutely treated (6 g/kg, p.o.), ethanol-dependent
while still intoxicated, and ethanol-dependent while undergoing a withdrawal
syndrome. No changes in Ė-AMP levels were observed in any of the brain areas
studied after any of the ethanol treatments. The data suggest that changes in
Ė-AMP levels in the brain do not play any role in the acute and chronic effects
of ethanol. The results of this study provide further information on the develop-
ment and exploration of models for chronic insults to the brain, such as long-
term exposure to toxic chemicals and ionizing and nonionizing radiation.
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## TABLE

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INTRODUCTION

Over the past several years a number of studies have appeared which attempted to implicate adenosine 3', 5'-cyclic monophosphate (c-AMP) in the actions of ethanol. A single dose of ethanol has been reported to selectively deplete c-AMP in the cerebellum but not in the cerebrum, pons, or medulla oblongata. Chronic feeding of ethanol, on the other hand, was shown to elevate c-AMP levels.

The proper measurement of c-AMP in the brain has been the subject of a great deal of controversy. It has been demonstrated that brain c-AMP levels increase rapidly after death and that the rapid fixation of brain tissue is important in obtaining c-AMP values which approach those present in the living animal. Previous experiments cited above which studied the effects of ethanol on c-AMP levels employed either conventional microwave fixation or immersion of the whole animal into liquid nitrogen, procedures that obtain less than optimal results. Microwave ovens can be modified to focus the beam over a small area and focused microwave fixation now appears to provide c-AMP values which approximate most closely the values to be encountered in vivo.

In the study to be presented here we have reinvestigated the effects of ethanol on brain c-AMP after acute and chronic administration using focused microwave fixation as a means of maintaining c-AMP levels. Also, the experiments have been extended to include measurements in seven brain areas and in ethanol-dependent animals.

METHODS

Male Sprague-Dawley rats were treated either acutely or chronically with 20 percent (w/v) ethanol solutions by means of intragastric intubation. For acute experiments a single dose of ethanol (6 g/kg) was administered 2 hours before the end of the experiment. In the chronic studies ethanol dependence was induced by the method of Majchrowicz, which involves giving 9-15 g/kg of ethanol per day in three to five fractions for 4 days.

For c-AMP determinations the rats were euthanatized by focused microwave fixation (Litton Menumaster 70/50, modified by Medical Engineering Consultants,
Lexington, Massachusetts; 1.3 kW; 3.5-sec exposure time). The excised brains were dissected into the following parts: cerebellum, brain stem, hypothalamus, thalamus, hippocampus, caudate nucleus and cerebral cortex. c-AMP was purified by the method of Mao and Guidotti. The tissue was homogenized in 1 ml of 0.4 N perchloric acid and centrifuged at 30,000 x g for 20 minutes. The supernatant was neutralized with 0.15 ml of 3 M Tris base and poured onto an alumina column (0.4 x 4.3 cm) equilibrated with 0.06 M Tris-HCl buffer (pH 7.5). c-AMP was eluted with 0.6 M Tris HCl buffer (pH 7.5) onto a Dowex 1 x 2 column (0.4 x 8.0 cm, 200-400 mesh) equilibrated with water. The resin was washed with 4 ml of water and the c-AMP eluted with 3 ml of 0.05 HCl. This fraction was neutralized with 0.2 ml of 0.6 M Tris base and placed on a Dowex 50W-X8 column (0.4 x 3.0 cm, 200-400 mesh H+ form) equilibrated with water. After washing the resin with 2 ml of water, the c-AMP was eluted with another 2.5 ml of water. c-AMP was then measured by the competitive protein binding assay of Gilman.

Ethanol was measured enzymatically in the blood of rats at the time of death using the Calbiochem Ethanol Stat-Pack. Statistical comparisons between means were made using Student's "t" test.

RESULTS AND DISCUSSION

C-AMP levels in seven areas of the rat brain were determined after acute and chronic treatment with ethanol. In acute studies a single dose of ethanol (6 g/kg) was administered and the animals euthanatized 2 hours later. In the chronic experiments rats were rendered ethanol-dependent and euthanatized either while still intoxicated or during the withdrawal syndrome. The withdrawal syndrome exhibited the signs and symptoms as outlined previously. Under the various conditions of ethanol treatment studied here, c-AMP levels were unaltered in any of the seven areas of the brain (Table 1). These results are not in agreement with previous reports which claimed reduced cerebellar c-AMP after a single dose of ethanol and elevated cerebral c-AMP after chronic feeding.
Table 1. Brain c-AMP Levels in Discrete Areas After Various Conditions of Ethanol Treatment

<table>
<thead>
<tr>
<th>Brain Area</th>
<th>Controls (pmoles/mg protein ± S.E.)</th>
<th>Acute (pmoles/mg protein ± S.E.)</th>
<th>Chronic Intoxicated (pmoles/mg protein ± S.E.)</th>
<th>Withdrawal Syndrome (pmoles/mg protein ± S.E.)</th>
</tr>
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<tr>
<td>Cerebellum</td>
<td>3.4 ± 0.16</td>
<td>3.2 ± 0.08</td>
<td>3.5 ± 0.34</td>
<td>3.7 ± 0.19</td>
</tr>
<tr>
<td>Brain Stem</td>
<td>3.1 ± 0.17</td>
<td>3.0 ± 0.08</td>
<td>3.2 ± 0.11</td>
<td>3.0 ± 0.24</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>3.2 ± 0.32</td>
<td>3.2 ± 0.19</td>
<td>3.3 ± 0.10</td>
<td>3.4 ± 0.26</td>
</tr>
<tr>
<td>Thalamus</td>
<td>4.5 ± 0.51</td>
<td>4.5 ± 0.36</td>
<td>4.3 ± 0.30</td>
<td>4.3 ± 0.34</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>3.7 ± 0.09</td>
<td>3.7 ± 0.39</td>
<td>3.8 ± 0.14</td>
<td>3.8 ± 0.24</td>
</tr>
<tr>
<td>Caudate Nucleus</td>
<td>3.8 ± 0.27</td>
<td>3.5 ± 0.18</td>
<td>3.7 ± 0.15</td>
<td>3.3 ± 0.24</td>
</tr>
<tr>
<td>Cerebral Cortex</td>
<td>7.2 ± 0.36</td>
<td>7.2 ± 0.28</td>
<td>7.1 ± 0.43</td>
<td>6.9 ± 0.46</td>
</tr>
</tbody>
</table>

Each group consisted of 5-10 animals. Blood ethanol levels in acutely treated animals were 373 ± 8.9 mg/dl, while in chronic intoxicated animals they were 334 ± 22.9 mg/dl.

The discrepancies among the reported studies are not clear. However, the main difference in experimental design involves the manner in which the animals were killed. Postmortem elevation of c-AMP in the brain can be quite dramatic especially in the cerebellum. Cerebellar c-AMP levels have been reported to rise tenfold 2 minutes after decapitation. Volicer and Gold have claimed that single doses of ethanol induce selectively a reduction in cerebellar c-AMP levels in a dose-dependent manner when using conventional microwave fixation. They also showed that ethanol depresses the rate of postmortem c-AMP accumulation. If, with the use of conventional microwave fixation, there is a postmortem accumulation of c-AMP which seems likely, the inhibition by ethanol of this accumulation would make it appear as if a reduction of c-AMP levels had taken place. Also acute ethanol treatment has been reported not to influence the activity of adenylate cyclase or phosphodiesterase. Therefore, observed reductions in c-AMP levels following ethanol treatment may be artifactual.

Although we found no alteration in c-AMP levels in ethanol-dependent rats, a role of this cyclic nucleotide in the chronic effects of ethanol cannot be excluded.
French and his co-workers have noted a number of interesting findings relative to the sensitivity of the c-AMP generating system to putative neurotransmitters in cortical brain slices. They found that chronic administration of ethanol for 9-14 weeks results in a noradrenergic subsensitivity when measured in animals still intoxicated. However, 3 days after withdrawal the reverse occurs, i.e., noradrenergic supersensitivity. Furthermore, an increased sensitivity was also observed with histamine and serotonin. Since ethanol dependence can be induced in only a few days, it is not clear whether this is sufficient time for the reported alterations in the c-AMP generating system to develop. These studies should be repeated using the newer methods for inducing ethanol dependence in order to relate these findings more precisely to the dependence process.
REFERENCES


12. Mao, C. C. and Guidotti, A. Simultaneous isolation of adenosine 3',5'-cyclic monophosphate (cAMP) and guanosine 3',5'-cyclic monophosphate (cGMP) in small tissue samples. Anal. Biochem. 59:63-68, 1974.
