EXPERIMENTAL ALCOHOLISM IN RATS: RNA CONTENT AND COMPOSITION IN ISOLATED CEREBELLAR PURKINJE CELLS AFTER LONG-TERM TREATMENT*

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INTRODUCTION

As long ago as the beginning of this century cortical cerebellar degeneration could be correlated with a clinically well known cerebellar syndrome characterized by instability when standing and walking, and incoordination of the lower extremities (1). Morphological studies showed the degeneration to be topographically restricted to the anterior vermis and neighbouring hemispheres and to affect Purkinje cells and granular cells especially. These findings were confirmed in later studies and have been reviewed by Allop and Turner (2). In 1930 and thereafter several authors were able to correlate this cerebellar degeneration with chronic alcoholism (2, 3, 4). This type of degeneration has been regarded as specific and different from late cerebellar atrophy, since it occurs at a younger age than the more unspecified late atrophy, and because the clinical condition improves if the patient becomes abstinent. In addition, the symptoms progress with continued abuse.

In clinical material it is always difficult to decide whether the ethanol itself or in combination with malnutrition causes the observed changes. Studies by Lhermitte et al. (5) indicated that degenerative changes occurred in rabbit Purkinje cells after ethanol treatment, and that these changes were less pronounced if the diet was supplemented with thiamine. Dreyfus (6) investigated the distribution of thiamine in the nervous system of the rat and found the concentration to be highest in the cerebellar vermis. He also noted that this storage was seriously depleted if the diet was poor in thiamine. After intraperitoneal injection of ethanol in rats Kiessling (7) showed that acetaldelyde, the primary metabolite of ethanol, was concentrated in the cerebellum. It was also found that acetaldelyde inhibits pyruvate oxidation, and that cerebellar mitochondria were more susceptible to this inhibition than mitochondria from cerebrum, liver, kidney and muscle.

A number of studies (8, 9) have shown that the RNA content and composition in cerebellar Purkinje cells can be used as a parameter of cell function. In view of the accumulated data, summarized above, on disturbed cerebellar function as a result of ethanol intoxication, it was considered relevant to study the RNA content and composition in rat cerebellar Purkinje cells by means of a method which allows analyses to be performed at the single cell level. In the

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present study rats were given ethanol as the only source of liquid for 2 and 8 months, respectively, and the effect on Purkinje cell RNA was then determined according to Edström (10).

MATERIAL AND METHODS

Albino rats of the Sprague-Dawley strain were used. The two groups of experimental animals were given 15% ethanol ad libitum as the only source of liquid during 2 and 8 months, respectively, and received an otherwise adequate diet. The rats were litters from parents which had also been subjected to an alcohol diet. The eight month old ethanol-treated rats were divided into two groups, one having free access to alcohol during the entire period, while the other was given water instead of alcohol during the last 21 hours before sacrifice. The controls were divided in three groups. One group was 2 months old, the second group was 8 months old, and the rats in the third group had the same weight as the eight month old ethanol-treated rats but were somewhat younger due to the reduced weight of rats undergoing long-term alcohol treatment. Samples for blood ethanol concentration tests were taken by cardiac puncture in the anesthetized animal, and determinations were carried out through the courtesy of the State Laboratory of Legal Medicine. The rats were sacrificed by decapitation under ether anesthesia and the anterior and posterior cerebellar vermis and pieces from hemispheres near the anterior lobe were rapidly dissected out and fixed in Carnoy's solution (absolute ethanol, chloroform, glacial acetic acid, 6/3/1 by volume). After washing in absolute ethanol and clearing in benzene, the tissue pieces were embedded in paraffin and cut into 50 µ slices. Purkinje cells were isolated by micromanipulation, and the RNA was subsequently extracted by three ribonuclease digestions from two-cell samples in an oil chamber. The collected extracts were evaporated on a quartz glass and then redissolved in saturated ammonium acetate to form lens-shaped drops. The drops were photographed in ultraviolet light at a wavelength of 257 ma, together with a reference system. The amounts of RNA in the ultra-violet-absorbing spots, each spot representing two cells, were determined by a photometric system (10). Other groups of Purkinje cells were extracted in the same way as previously described. These evaporated extracts were hydrolyzed in 4-N hydrochloric acid for subsequent determination of base composition (10).

RESULTS

Animals. The consumption of liquid and pelleted food was always lower in rats receiving alcohol as the only source of fluid compared to controls. After weaning the daily intake of 15% ethanol was around 50% of that of water intake, and the consumption increased to 50–70% of that of water. The ethanol-treated rats ate about 30% less solid food compared to their controls. The low food intake resulted in a markedly reduced weight increase, and at the time of sacrifice the ethanol-treated rats had a body weight which was 20–30% lower than that of the controls. In spite of the high blood alcohol concentration (0.20–0.25%) the rats did not show any signs of impaired normal motor or vestibular function.

RNA analyses. The cytochemical results are presented in Tables I–III. The regional localization of the areas from which cells were dissected, are referred to according to the nomenclature proposed by Larsell (11). As seen from table I, two month old ethanol-treated rats have decreased amounts of total RNA in Purkinje cells from the areas investigated compared to controls. The differences are most pronounced in the most anterior part of the anterior vermis and
TABLE I

RNA Content in Purkinje Cells from 2 Month Old Ethanol-treated Rats and Controls
Determinations carried out on samples of two cells.

\[ \text{Mean values in pg ± S.E.M.} \]

<table>
<thead>
<tr>
<th>Area</th>
<th>Alcohol rats</th>
<th>N.</th>
<th>Controls</th>
<th>N.</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lobulus I-II</td>
<td>152 ± 7.3</td>
<td>4</td>
<td>176 ± 5.4</td>
<td>4</td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td>Lobulus IX-X</td>
<td>143 ± 6.5</td>
<td>4</td>
<td>157 ± 14.4</td>
<td>4</td>
<td>P &lt; 0.025</td>
</tr>
<tr>
<td>Hemisphere</td>
<td>130 ± 7.9</td>
<td>4</td>
<td>101 ± 8.3</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

N. represents the number of animals. 6-9 samples were determined in each animal.

TABLE II

RNA Content in Purkinje Cells from 8 Month Old Ethanol-treated Rats, Abstinent Rats, Age Control Rats and Weight Control Rats
Determinations carried out on samples of two cells.

\[ \text{Mean values in pg ± S.E.M.} \]

<table>
<thead>
<tr>
<th>Area</th>
<th>Alcohol</th>
<th>N.</th>
<th>Abstinent</th>
<th>N.</th>
<th>Age contr.</th>
<th>N.</th>
<th>Weight contr.</th>
<th>N.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-II</td>
<td>146 ± 11.0</td>
<td>5</td>
<td>151 ± 3.7</td>
<td>6</td>
<td>151 ± 3.3</td>
<td>8</td>
<td>171 ± 8.5</td>
<td>6</td>
</tr>
<tr>
<td>IX-X</td>
<td>134 ± 6.2</td>
<td>5</td>
<td>135 ± 5.3</td>
<td>6</td>
<td>137 ± 5.2</td>
<td>8</td>
<td>146 ± 9.3</td>
<td>6</td>
</tr>
</tbody>
</table>

N. represents the number of animals; 6-9 samples were determined in each animal.

TABLE III

Base Composition of Purkinje Cell RNA from Ethanol-treated Rats and Weight Controls, Expressed as Molar Proportions in Per Cent of the Sum

<table>
<thead>
<tr>
<th>Area</th>
<th>A</th>
<th>G</th>
<th>C</th>
<th>U</th>
<th>( \frac{A + G}{C + U} )</th>
<th>N.</th>
<th>N.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alc. I-II</td>
<td>20.4</td>
<td>30.1</td>
<td>29.5</td>
<td>20.0</td>
<td>1.02</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Alc. IX-X</td>
<td>21.3</td>
<td>29.2</td>
<td>29.0</td>
<td>20.5</td>
<td>1.02</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Contr. I-II</td>
<td>22.7</td>
<td>28.9</td>
<td>29.1</td>
<td>19.3</td>
<td>1.07</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Contr. IX-X</td>
<td>20.9</td>
<td>29.9</td>
<td>28.0</td>
<td>21.1</td>
<td>1.03</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

N. 1 represents the number of animals.
N. 2 represents the number of hydrolysates. Each hydrolysis value is the mean of 2-5 microelectrophoretic separations.

adjacent cerebellar hemisphere, lobuli I-III. The decrease observed in the uvula and nodulus (Larsell's lobules IX-X) is only minor and insignificant.

Table II shows the results from the eight month old rats. No significant changes in the Purkinje cell RNA content were found between the ethanol-treated and control groups. Some determinations of the amount of RNA in cells from the hemisphere were made in this case but no differences were observed between any of the groups.

Determination of the base composition of Purkinje cell RNA from lobulus I-II and lobulus IX-X in ethanol-treated rats and weight controls showed almost identical values for the different groups (Table III).
DISCUSSION

The aim of the present study was to determine whether a clinical syndrome characterized by instability when standing and walking and cerebellar degeneration restricted to the anterior vermis and neighbouring hemispheres (1, 2), could be induced experimentally. As reviewed by Victor et al. (3, 4) this syndrome has been attributed to chronic alcoholism and therefore rats ingesting alcohol chronically were used in the present study.

The results show that after two months of drinking ethanol, the Purkinje cell RNA content decreased in the anterior part of the cerebellar vermis and adjacent areas in the hemisphere while no significant differences in the posterior vermis were observed between ethanol-treated rats and controls. After eight months the differences between ethanol-treated rats and controls no longer existed. The Purkinje cell RNA base composition was also investigated, and the values for experimental and control rats were almost identical. In one experimental group the rats were given water instead of alcohol during the final 24 hours preceding sacrifice (abstinent rats). This was done because it has been shown that protein synthesis in cerebral and cerebellar cortex increases above the normal value if an ethanol-treated rat is allowed free access to water instead of alcohol prior to sacrifice (12). The abstinent rats did not differ from either ethanol-treated rats or controls in the present study.

There may be several explanations for the finding that alcohol intoxication lowered the Purkinje cell RNA content in the “sensitive” cerebellar areas after two months of alcohol intake but not after eight months. The shorter period involves the time when the central nervous system matures, and in the rat especially the cerebellum matures at a rather late period. It has been shown by Ringborg (13), Haltia (14) and Jarlstedt (unpublished) that the neuronal RNA content increases during the first weeks of life, and it may be argued that the neurons are more vulnerable during this maturation period. Thus a depressing drug such as ethanol may retard the increase in neuronal RNA and result in the observed lower RNA content in ethanol-treated rats. However, this does not explain why some areas are more affected than others. Another reason for the differences between two and eight month ethanol-treated rats may be that ethanol actually depresses the cerebellar Purkinje cell RNA content but that this effect is counteracted by increased peripheral proprio- and exteroceptive impulses, due to efforts to maintain balance during alcohol-intoxication, which in turn increase Purkinje cell RNA (8). The view that cerebellar depression occurs in ethanol-treated rats is supported by Gordon (16), who found a 60% reduction in the transmitter γ-aminobutyric acid, which is believed to be synthetized by the Purkinje cells, after in vivo administration of ethanol in rats.

Histological examination of sections from the cerebellar areas investigated did not reveal any signs of degeneration in spite of the large proportion of their life-span during which the rats were heavily intoxicated with ethanol. The possibility exists that this observation is due to species differences, but another explanation can be offered. Chronic alcoholics very often suffer from malnutri-
tion and a vitamin deficiency state, which in themselves may be harmful to the Purkinje cells. It was recently shown (15) that folie acid deficiency reduces the accumulation of RNA in cerebellar Purkinje cells in growing chicks. Thus, it seems quite possible that vitamin deficiency aggravates the effect of alcohol on the cerebellum, and that this may be the reason for the correlation between cerebellar degeneration and chronic alcoholism. We hope to be able to elucidate this mechanism by further studies.

SUMMARY

1. Experimental alcoholism was produced in rats by supplying them with 15% ethanol as the only source of liquid for 2 and 8 months, respectively. The diet was otherwise adequate.

2. The RNA content in isolated Purkinje cells from the anterior vermis, anterior portions of the cerebellar hemispheres and the posterior vermis was determined using Edström's microtechniques.

3. After two months the ethanol-treated rats had a lower Purkinje cell RNA content in cells from the anterior vermis and the anterior portions of the cerebellar hemispheres as compared to controls. After 8 months the amount of cellular RNA and its base composition were similar in ethanol-treated rats and controls.

4. The ethanol-treated rats consumed about 30% less food and liquid than control rats. The body weight after eight months was 20–30% lower in ethanol-treated rats as compared to controls.

REFERENCES


