Neurotransmitter interactions in the striatum and hypothalamus of mice after single and repeated ethanol treatment

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Abstract

In single treatment study, ethanol was administered intraperitoneally to ICR mice (about 34 g) in the amounts of 1.0, 2.0, 3.0 or 4.0 g/kg body weight. The 3,4-dihydroxyphenylacetic acid (DOPAC) + homovanillic acid (HVA) concentration in the striatum was elevated with 3.0 and 4.0 g/kg of ethanol. In the hypothalamus, the DOPAC, HVA and 5-hydroxyindoleacetic acid concentrations were increased after injection of 3.0 and 4.0 g/kg of ethanol. Furthermore, the acetylcholine (ACh) and gamma-aminobutyric acid (GABA) concentrations were also increased following the injection of 1.0, 2.0, 3.0 and 4.0 g/kg. To study the effects of repeated administration, mice were injected intraperitoneally with 1.0 or 2.0 g/kg of ethanol once daily for 7 days. The DOPAC + HVA level in the striatum was elevated after injection of 1.0 and 2.0 g/kg of ethanol. The GABA and ACh concentrations in the hypothalamus were decreased after repeated injections of ethanol. These results suggest that ethanol significantly alters the utilization of dopamine, ACh and GABA in the hypothalamus. This may partially explain why ethanol has such profound effects on emotional behavior and mood.

KEYWORDS: ethanol, dopamine, serotonin, ?-aminobutyric acid, acetylcholine, striatum, hypothalamus

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Neurotransmitter Interactions in the Striatum and Hypothalamus of Mice after Single and Repeated Ethanol Treatment

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In single treatment study, ethanol was administered intraperitoneally to ICR mice (about 34 g) in the amounts of 1.0, 2.0, 3.0 or 4.0 g/kg body weight. The 3,4-dihydroxyphenylacetic acid (DOPAC) + homovanillic acid (HVA) concentration in the striatum was elevated with 3.0 and 4.0 g/kg of ethanol. In the hypothalamus, the DOPAC, HVA and 5-hydroxyindoleacetic acid concentrations were increased after injection of 3.0 and 4.0 g/kg of ethanol. Furthermore, the acetylcholine (ACh) and γ-aminobutyric acid (GABA) concentrations were also increased following the injection of 1.0, 2.0, 3.0 and 4.0 g/kg. To study the effects of repeated administration, mice were injected intraperitoneally with 1.0 or 2.0 g/kg of ethanol once daily for 7 days. The DOPAC + HVA level in the striatum was elevated after injection of 1.0 and 2.0 g/kg of ethanol. The GABA and ACh concentrations in the hypothalamus were decreased after repeated injections of ethanol. These results suggest that ethanol significantly alters the utilization of dopamine, ACh and GABA in the hypothalamus. This may partially explain why ethanol has such profound effects on emotional behavior and mood.

Key words: ethanol, dopamine, serotonin, γ-aminobutyric acid, acetylcholine, striatum, hypothalamus

The acute or chronic administration of ethanol has been shown to alter several neurotransmitter systems in the brains of mice and rats (1-12). These neurotransmitter changes may contribute directly to ethanol's profound behavioral effects. The nigrostriatal system is a loop in the basal ganglia involving a sequence of dopaminergic, cholinergic and GABAergic neurons (9). However, a clear and undisputed role has yet to emerge for monoamines or any other neurotransmitter in the mediation of the effects of ethanol. Thus, when studying the role of ethanol on neurotransmitter function it seems reasonable to study the effect on more than one area of the brain.

The purpose of the present study was to assess the interactions of ethanol exposure to brain monoamine neurotransmitter systems or any other neurotransmitter systems in the brain regions. Since many of the studies involving ethanol and neurotransmitters have been concerned with only one dose of ethanol or one neurotransmitter, we studied dopamine (DA), serotonin (5-HT) and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA), γ-aminobutyric acid (GABA), acetylcholine (ACh) and choline (Ch) concentrations in the striatum and hypothalamus of mice following single and repeated exposure to ethanol.

Materials and Methods

Male ICR mice (about 34 g; Charles River Japan, Inc. Japan) were used. They were housed 6 per cage and kept at a room temperature of 24°C on a 12-h light-dark cycle, and were allowed free access to food (MF: Oriental yeast Co., Ltd., Tokyo, Japan) and water. In the single treatment experiment, ethanol was administered intraperitoneally to mice in the amount of 1.0, 2.0, 3.0 and 4.0 g/kg body weight as a diluted solution in saline, while only saline was given to control animals in an equal volume. The mice were killed 2h after dosing with ethanol or saline. In the repeated treatment experiment,
mice were injected intraperitoneally with 1.0 or 2.0 g/kg of ethanol once daily for 7 days, while control animals were treated with an equal volume of saline according to the same schedule. The mice were killed 2 h after the last injection of ethanol or saline. At the completion of each experiment, the head of mice were irradiated with a focused microwave apparatus for 1.6 sec (3.8 kw microwave, 2450 MHz; Toshiba, Tokyo, Japan). The whole brains were rapidly removed and frozen on dry ice, and stored at −70°C until dissection into discrete brain nuclei. Trunk blood was collected from each animal immediately after decapitation into a test tube for ethanol assay.

The stored whole brains were sliced into coronal sections of 1 mm thick on a cryostat kept at −20°C. Sliced tissues were dissected at −20°C into striatum (dorsal-lateral region) and hypothalamus (anterior hypothalamic region). All procedures were carried out with brain tissue in a frozen state. Brain samples were again stored −70°C until assayed for the content of monoamines and their acid metabolites, ACh, Ch and GABA.

Tissue was homogenized with 0.05 N HCl and the homogenate was centrifuged at 20,000 × g for 20 min. An aliquot of supernatant was used for assay of neurotransmitters. DA, 5-HT, 5-HIAA, DOPAC and HVA were determined by high-performance liquid chromatography (HPLC) with electrochemical detection (13). The ACh and Ch levels were estimated by HPLC coupled with a styrene polymer chromatographic column, immobilized-enzyme column (AC-Enzyme pak; EICOM, Kyoto, Japan) and electrochemical detector (14). The GABA level was estimated using the method of Okada et al. (15). Ethanol concentration in serum was determined by using a commercial kit (Boehringer-Mannheim GmbH, Mannheim, Germany). The protein content of the homogenate was determined by the procedure of Lowry et al. (16).

Data are expressed as the mean ± S.D. The results were statistically analyzed using Student’s t-test and the Aspin-Welch test by the Muscot series program (Y.D.K. Co., Ltd., Tokyo, Japan) on a computer.

Results

In the single-dose experiment, the effects of single treatment with four different doses (1.0, 2.0, 3.0 and 4.0 g/kg body weight) on serum ethanol level are shown in Table 1. The changes in the serum ethanol level increased in a dose-dependent manner. In the striatum, the DOPAC + HVA concentration was markedly elevated after injection of 3.0 and 4.0 g/kg of ethanol, as compared to control animals (Fig. 1). The 5-HIAA concentration was elevated after injection of 3.0 g/kg of ethanol (Fig. 1). The concentrations of ACh and GABA in the striatum were unchanged 2 h after the injection of four different doses of ethanol (data not shown). In the hypothalamus, the DOPAC, HVA and 5-HIAA concentrations were increased after injection of 3.0 and 4.0 g/kg, and the ACh concentration was increased following the injection of 1.0, 3.0 and 4.0 g/kg. The GABA concentration was also increased following the injection of 1.0, 2.0 and 3.0 g/kg (Table 2).

In the repeated treatment experiment, ethanol was not detected in the serum after repeated injection of 1.0 g/kg of ethanol. However, the average serum ethanol level was 0.70 ± 0.08 g/l after repeated injection of 2.0 g/kg of ethanol. The DOPAC + HVA level in the striatum were elevated after repeated treatment with 1.0, 2.0 g/kg of ethanol (Fig. 2). The concentrations of ACh and GABA in the striatum were unchanged after injection of 1.0 and 2.0 g/kg of ethanol (data not shown). In the hypothalamus, the GABA concentration was decreased after repeated injection of 2.0 g/kg of ethanol, and the ACh and Ch concentrations in the hypothalamus were decreased after repeated injection of 1.0 g/kg of ethanol (Table 3).

Discussion

In single treatment with ethanol, the results showed that the higher doses (above 3.0 g/kg) of ethanol increased the DOPAC and HVA concentrations in the striatum and
Fig. 1  Effects of single ethanol administration on the DA, DOPAC + HVA, 5-HT and 5-HIAA concentrations in the striatum of mice. Ethanol was administered intraperitoneally to ICR mice in the amount of 1.0, 2.0, 3.0 or 4.0 g/kg body weight. Mice were killed 2 h after each different dose of ethanol or saline. Results are expressed as percentages of mean levels in the control group. Each value is the mean of 5 mice. Significantly different from the control group (Student’s t-test or Aspin-Welch test: * * P < 0.01). Abbreviation: DA, dopamine; DOPAC, 3, 4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid.

Ethanol (g/kg): 0 ( ); 1 ( ); 2 ( ); 3 ( ); 4 ( )

<table>
<thead>
<tr>
<th>Neurotransmitter Concentrations</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>35.5 ± 0.8</td>
<td>45.7 ± 7.3*</td>
<td>40.8 ± 13.4</td>
</tr>
<tr>
<td>DA</td>
<td>18.2 ± 6.4</td>
<td>26.2 ± 8.2</td>
<td>21.5 ± 5.8</td>
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<tr>
<td>DOPAC</td>
<td>7.5 ± 1.1</td>
<td>8.4 ± 1.5</td>
<td>8.2 ± 1.7</td>
</tr>
<tr>
<td>HVA</td>
<td>9.0 ± 2.4</td>
<td>13.6 ± 3.4</td>
<td>13.0 ± 4.3</td>
</tr>
<tr>
<td>5-HT</td>
<td>46.6 ± 14.4</td>
<td>62.5 ± 21.4</td>
<td>54.3 ± 9.4</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>23.5 ± 3.1</td>
<td>35.9 ± 12.3</td>
<td>26.4 ± 5.4</td>
</tr>
<tr>
<td>ACh</td>
<td>228.4 ± 42.2</td>
<td>347.0 ± 106.1*</td>
<td>292.5 ± 75.2</td>
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<tr>
<td>Ch</td>
<td>312.1 ± 86.9</td>
<td>488.6 ± 238.6</td>
<td>387.7 ± 101.9</td>
</tr>
<tr>
<td>GABA</td>
<td>23.6 ± 7.0</td>
<td>45.3 ± 11.7*</td>
<td>37.0 ± 6.2*</td>
</tr>
</tbody>
</table>

Ethanol was administered intraperitoneally to mice in the amount of 1.0, 2.0, 3.0 or 4.0 g/kg body weight as a diluted solution in saline, while only saline was given to control animals an equal volume. Mice were killed 2 h after dosing of ethanol or saline. Results are expressed as the mean ± S.D. of 5 mice in units of pmol/mg protein or nmol/mg protein (GABA). Significantly different from the control group (Student’s t-test of Aspin-Welch test: * P < 0.05). Abbreviation: NA, noradrenaline; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; ACh, acetylcholine; Ch, choline; GABA, γ-aminobutyric acid.
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Fig. 2 Changes in DA, DOPAC + HVA, 5-HT and 5-HIAA concentrations of the striatum of mice with 1.0 or 2.0 g/kg body weight of ethanol daily for 7 days. ICR mice were injected intraperitoneally with 1.0 or 2.0 g/kg of ethanol once daily for 7 days. Mice were killed at 2h after the final injection of ethanol. Results are expressed as percentages of the mean level in the control group. Each value is the mean of 6-8 mice. Significantly different from the control group (Student's t-test or Aspin-Welch test: *P < 0.05). Abbreviations are the same as those in Fig. 1. Ethanol (g/kg): 0 ( ); 1 ( ); 2 ( ).

Table 3 Changes in neurotransmitter concentrations in the hypothalamus with 1 or 2 g/kg body weight of ethanol daily for 7 days

<table>
<thead>
<tr>
<th>Neurotransmitter</th>
<th>Neurotransmitter concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (Control)</td>
</tr>
<tr>
<td>NA</td>
<td>35.6 ± 13.8</td>
</tr>
<tr>
<td>DA</td>
<td>29.3 ± 24.0</td>
</tr>
<tr>
<td>DOPAC</td>
<td>7.0 ± 3.3</td>
</tr>
<tr>
<td>HVA</td>
<td>13.9 ± 6.8</td>
</tr>
<tr>
<td>5-HT</td>
<td>51.7 ± 6.4</td>
</tr>
<tr>
<td>ACh</td>
<td>376.6 ± 69.9</td>
</tr>
<tr>
<td>Ch</td>
<td>466.4 ± 93.3</td>
</tr>
<tr>
<td>GABA</td>
<td>44.1 ± 8.1</td>
</tr>
</tbody>
</table>

Mice were injected intraperitoneally with 1 or 2 g/kg of ethanol once daily for 7 days. Mice were killed at 2h after the final injection of ethanol. Results are expressed as the mean ± S.D. of 6-8 mice in units of pmol/mg protein or nmol/mg protein (GABA). Significantly different from the control group (Student's t-test or Aspin Welch test: *P < 0.05). Abbreviations are the same as those in Table 2.

hypothalamus, whereas the lower doses had no effect. These findings of elevated levels of DA metabolites after high-dose ethanol are generally in agreement with the report by Dar and Woolles (11). Furthermore, Barbaccia et al. (10) reported that acute ethanol treatment increased striatal DA turnover, expressed as the DOPAC concentration. Our data also showed that the repeated treatment with ethanol had a similar effect on the concentrations of
DA and its metabolites in the striatum. Neurotransmitter alteration in the striatum may contribute to the changed locomotor activity which are often associated with ethanol administration. In addition, the incertohypothalamic dopaminergic system also appears to be activated by high doses of ethanol, as indicated by the higher levels of DOPAC and/or HVA in the hypothalamus of following single treatment with ethanol.

The present data also show that ethanol affects the serotonergic system in the striatum and some other brain regions. An increased concentration of 5-HIAA without any change in the 5-HT concentration by single treatment with ethanol (3.0 and 4.0 g/kg) was shown in both the striatum and the hypothalamus. Similar findings of the increased concentration of 5-HIAA following acute ethanol treatment was reported by Pohorecky et al. (5). Khatib et al. (17) have suggested that the results of study for the serotonin (5-HT) acidic metabolite (5-HIAA) are not likely due exclusively to inhibition of efflux of 5-HIAA from the brain. Furthermore, there is a report (8) of an increase in 5-HT turnover in the striatum following ethanol treatment. Therefore, one possible interpretation of the present findings is that single ethanol may increase the activity of specific 5-HT pathways.

The present data showed that single administration of ethanol increased the concentrations of ACH and GABA in the hypothalamus. These results suggest that, after single ethanol treatment, both GABAergic and cholinergic systems in the hypothalamus may be activated concomitantly. It is also of interest that repeated ethanol administration significantly altered the utilization of both ACH and GABA in the hypothalamus. This may partially explain why ethanol has such profound effects on emotional behavior and mood. However, it remains to be seen why the GABA concentration in the hypothalamus was altered differently following the single or repeated ethanol administration.

In conclusion, the present study suggests that the action of single and repeated ethanol treatment on the central nervous system may result from the interaction among neurotransmitter systems. It seems that the major transmitter involved in these reactions is dopamine in the striatum. However, in the hypothalamus, the direct or indirect role of dopamine remains to be established for the action on both GABA and ACH neurons.

References


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