EFFECTS OF BRAIN BIOGENIC AMINES ON ETHANOL WITHDRAWAL REACTIONS AND THE DEVELOPMENT OF ETHANOL DEPENDENCE IN MICE

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Abstract—Mice were made physically dependent on ethanol by a four-day period of ethanol inhalation. A single injection of L-dopa tended to enhance ethanol withdrawal reactions and 5HTP tended to suppress it. Daily treatment with PCPA and L-dopa or pretreatment with 60HDA tended to modify the severity of withdrawal reactions. PCPA slightly decreased the severity, 60HDA tended to aggravate the severity, and L-dopa tended to decrease the severity. The simultaneous estimation of brain biogenic amine levels suggests that 5HT and DA may be involved in the development of physical dependence on ethanol. Treatment with neuropharmacological drugs during ethanol withdrawal modified the severity of ethanol withdrawal reactions. Pentobarbital and diazepam completely suppressed ethanol withdrawal reactions. GHBA and reserpine suppressed the severity and haloperidol, imipramine, and methylphenidate aggravated it. The simultaneous estimation of brain catecholamine levels suggests that suppression of ethanol withdrawal reactions consistently results from the decreased activities of either noradrenergic or dopaminergic neurons. However, the effects of biogenic amines on ethanol withdrawal reactions may be different from those on the development of physical dependence on ethanol.

There has been considerable interest in the possible involvement of central biogenic amines in the development of ethanol dependence. However, there is no general agreement on the effects of chronic ethanol exposure on the brain biogenic amine system. Brain norepinephrine levels have been reported to be unchanged (1, 2) or increased (3, 4) after chronic exposure to ethanol. Similarly, dopamine levels were found to be increased (4, 5) or unchanged (1). Serotonin levels have been also reported to be unchanged (6–8) or elevated (2, 3). Catecholamine turnover appears to be increased after chronic ethanol treatment (2, 9, 10), and serotonin turnover has been reported to be decreased (11).

Since Goldstein reported the effects of drugs which modify neurotransmission on ethanol withdrawal reactions (12), several papers on the effects of biogenic amines on the reactions have appeared (13–17). The results, however, are inconsistent. The purpose of this paper is to elucidate the possible role of biogenic amines on ethanol withdrawal reactions and physical dependence on ethanol.

MATERIALS AND METHODS

Ethanol administration: Male ICR strain mice with an initial body weight of approx. 35 g were used. Mice were made physically...
dependent on ethanol by the method of Goldstein (18). Mice were exposed to ethanol vapor for 4 days in a plastic chamber. Pellet food and drinking water were given ad lib. A microtube pump delivered absolute ethanol at 3 ml/hr onto a filter paper wick in a flask. Air was delivered through the flask and into the chamber at 3 l/min by monitoring with a gas flow meter. Pyrazole injections, 50 mg/kg i.p., were daily at 10.00 a.m. At the start of each experiment, a priming dose of ethanol at 2 g/kg was given i.p. together with the first injection of pyrazole.

Effects of single injection of the drugs which modify brain biogenic amine contents on withdrawal reactions in mice: Ethanol-dependent mice were intraperitoneally injected with p-chlorophenylalanine (PCPA) (300 mg/kg), 5-hydroxytryptophan (5HTP) (75 mg/kg), α-methyl-p-tyrosine (AMPT) (200 mg/kg), or L-dihydroxyphenylalanine (L-dopa) (50 mg/kg) 4 hr after ethanol withdrawal. These four drugs were chosen to increase or decrease endogenous serotonin or catecholamines. PCPA was suspended in normal saline containing one drop of Tween 20. AMPT was made up according to Spector et al. (19). The other two drugs were dissolved in normal saline. The injection volume was 20 ml/kg. The severity of withdrawal reactions was estimated by the scoring system according to Goldstein (18), and it was observed every hour until 10 hr and 24 hr after ethanol withdrawal. The weight loss was also observed 24 hr after ethanol withdrawal.

Effects of drugs which modify biogenic amine contents on the development of physical dependence on ethanol in mice: Mice were intraperitoneally injected PCPA (300 mg/kg/day), 5HTP (75 mg/kg/day), and L-dopa (50 mg/kg/day) for four days during ethanol inhalation. 6-Hydroxydopamine hydrobromide (6OHDA) (50 μg/mouse) was intracerebrally injected four days prior to ethanol vapor exposure according to Haley et al. (20). 6OHDA was chosen instead of AMPT since the renal toxicity of AMPT has been described (21). 6OHDA was made up to a concentration of 2 mg base/ml in normal saline containing 0.1% ascorbic acid. The injection volume was 25 μl. One hr after the last injection, the mice which were not withdrawn from ethanol vapor exposure were killed by decapitation, and brain serotonin (5HT), 5-hydroxyindoleacetic acid (5HIAA), nor-epinephrine (NE), and dopamine (DA) were assayed in order to investigate possible interactions of these drugs and ethanol. Twenty four hr after the last injection, the mice of the other group were withdrawn from ethanol vapor exposure 5 hr prior to sacrifice. After the severity of withdrawal reactions was estimated, the mice were quickly killed by decapitation, and brain 5HT, 5HIAA, NE, and DA were determined. 5HT and 5HIAA were assayed by the method previously described (11), and NE and DA were assayed by the method of Chang (22).

Effects of neuropharmacological drugs on withdrawal reactions and catecholamine levels: Each drug was administered at the divided doses to mice in the intervals as described in the results to ensure the continuous maximum effect. All drugs except methylphenidate which was applied orally were injected i.p. in a volume of 20 ml/kg. The drugs used were pentobarbital sodium (Abbott Lab.), diazepam (Takeda), gamma-hydroxybutyric acid sodium salt (GHBA) (Wako), haloperidol (Dainippon), reserpine (Takeda), lithium chloride (Wako), imipramine (Fujisawa), and methylphenidate (Takeda). After four-day ethanol vapor inhalation, mice were removed from the ethanol vapor chamber and immediately administered the drugs as described in the results. The severity of their withdrawal reactions was estimated 5 hr after ethanol
withdrawal, and then brain NE and DA levels were immediately determined.

RESULTS

Effects of single injection of drugs which modify brain biogenic amine contents on the severity of withdrawal reactions in mice: Figure 1 shows time courses of the severity of withdrawal reactions and weight losses after 24 hr during withdrawal reactions. Control groups were mice that had been exposed to four-day ethanol inhalation and received no drugs after withdrawal. They developed a typical pattern of seizure scores, and their peak height of the severity of withdrawal reactions were attained 6 hr after ethanol withdrawal. Their mean score was a 2.8±0.20 point. The mean scores then decreased to 2.4±0.24 and 2.2±0.20 at 8 and 10 hr after ethanol withdrawal, respectively. Five mice were used in each group. When the drugs were administered, the peak height was not different from the control. However, the mean scores at 8 and 10 hr after ethanol withdrawal tended to be decreased by 5HTP (2.4±0.24 and 2.2±0.20, respectively). When AMPT and L-dopa were administered, the peak height of the score was prolonged until 10 hr after ethanol withdrawal. Weight losses during withdrawal reactions did not seem to correlate with the severity of withdrawal reactions.

Effects of drugs which modify biogenic amine contents on the development of physical dependence on ethanol in mice: Daily pyrazole injections did not seem to influence the biogenic amine contents. As shown in Table 1, the brain 5HT level was increased after ethanol withdrawal (0.467 ±0.009 μg/g versus 0.617±0.027 μg/g). In PCPA- or 5HTP-treated mice, however, brain 5HT levels were decreased after ethanol withdrawal. Even during ethanol inhalation, treatment with PCPA caused continuous decreases in brain 5HT and 5HIAA, and treatment with 5HTP caused transient increases in brain 5HT and 5HIAA followed by a decrease in brain 5HT. The severity of withdrawal reactions tended to be diminished by treatment with PCPA (1.9±0.2 points versus 1.6±0.1 points). 5HTP, however, did not influence the severity. As shown

Fig. 1. Effects of single injections of the drugs which modify brain biogenic amine levels on ethanol withdrawal reactions in mice. Mice undergoing ethanol withdrawal reactions were treated at 4 hr after withdrawal with the drugs. Ordinate, mean scores of five animals based on the rating system according to Goldstein. Abscissa, hours after ethanol withdrawal. Ethanol administration and the doses of the drugs are shown in the “Methods” section.
Table 1. Effects of PCPA and 5HTP on brain 5HT and 5HIAA contents and the ethanol-induced convulsions in mice after ethanol inhalation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5HT</th>
<th>5HIAA</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (8)</td>
<td>0.485±0.023</td>
<td>0.239±0.007</td>
<td></td>
</tr>
<tr>
<td>Pyrazole (8)</td>
<td>0.467±0.009</td>
<td>0.241±0.007</td>
<td></td>
</tr>
<tr>
<td>Ethanol (before withdrawal) (8)</td>
<td>0.548±0.027</td>
<td>0.414±0.031</td>
<td></td>
</tr>
<tr>
<td>+PCPA¹ (7)</td>
<td>0.305±0.025*</td>
<td>0.142±0.022*</td>
<td></td>
</tr>
<tr>
<td>+5HTP¹ (7)</td>
<td>0.823±0.061*</td>
<td>2.433±0.188*</td>
<td></td>
</tr>
<tr>
<td>Ethanol (5 hr after withdrawal) (7)</td>
<td>0.617±0.027**</td>
<td>0.368±0.032</td>
<td>1.9±0.2</td>
</tr>
<tr>
<td>+PCPA² (8)</td>
<td>0.283±0.013*</td>
<td>0.125±0.013*</td>
<td>1.6±0.1</td>
</tr>
<tr>
<td>+5HTP² (8)</td>
<td>0.499±0.012*</td>
<td>0.347±0.017</td>
<td>1.8±0.3</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E.
Concentrations are expressed as µg/g.
The number of animals is shown in parentheses.
¹ 1 hr after last injection.
² 24 hr after last injection.
*significantly different from the ethanol control, P<0.05.
**significantly different from the pyrazole control, P<0.05.
Methods of administration of ethanol and the drugs are shown in the text.

Table 2. Effects of 60HDA and L-dopa on brain NE and DA contents and the ethanol-induced convulsions in mice after ethanol inhalation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NE</th>
<th>DA</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (8)</td>
<td>0.254±0.017</td>
<td>1.054±0.045</td>
<td></td>
</tr>
<tr>
<td>Pyrazole (8)</td>
<td>0.257±0.018</td>
<td>1.105±0.044</td>
<td></td>
</tr>
<tr>
<td>Ethanol (before withdrawal) (8)</td>
<td>0.152±0.011**</td>
<td>1.042±0.055</td>
<td></td>
</tr>
<tr>
<td>+60HDA (7)</td>
<td>0.079±0.012*</td>
<td>0.688±0.054*</td>
<td></td>
</tr>
<tr>
<td>+L-dopa¹ (7)</td>
<td>0.116±0.016</td>
<td>1.122±0.067</td>
<td></td>
</tr>
<tr>
<td>Ethanol (5 hr after withdrawal) (7)</td>
<td>0.145±0.011**</td>
<td>0.955±0.054</td>
<td>2.2±0.2</td>
</tr>
<tr>
<td>+60HDA (12)</td>
<td>0.085±0.009*</td>
<td>0.799±0.057</td>
<td>2.4±0.1</td>
</tr>
<tr>
<td>+L-dopa² (7)</td>
<td>0.179±0.020</td>
<td>0.947±0.065</td>
<td>1.9±0.3</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E.
Concentrations are expressed as µg/g.
¹ 1 hr after last injection.
² 24 hr after last injection.
*significantly different from the ethanol control, P<0.05.
**significantly different from the pyrazole control, P<0.05.
Methods of administration of ethanol and the drugs are shown in the text.

In Table 2, the brain NE level was markedly decreased during ethanol inhalation, and this tendency was more significant after ethanol withdrawal (0.257±0.018 µg/g versus 0.145±0.011 µg/g). Pretreatment with 60HDA caused a significant continuous decrease in brain NE and a moderate decrease in brain DA, even during ethanol inhalation. Treatment with L-dopa caused a transient increase in brain DA, but did not alter the brain NE level. The severity of withdrawal reactions tended to be augmented by treatment with 60HDA (2.2±0.2 points versus 2.4±0.1 points) and diminished with L-dopa (2.2±0.2 points versus 1.9±0.3 points).

Relationships between biogenic amine
contents and the severity of withdrawal reactions: Figure 2 shows correlations between brain biogenic amine contents and the severity of withdrawal reactions in the control group and groups with drug administration. A negative correlation in the case of 5HT (r\(=-0.9055\), \(P<0.01\)) and a positive one in the case of DA (r\(=0.8765\), \(P<0.01\)) were demonstrated in the control group. These correlations were not observed in the groups with drug administration.

Effects of neuropharmacological drugs on withdrawal reactions and catecholamine contents: The mean score and brain NE and DA levels were determined 5 hr after ethanol withdrawal. Control groups were mice that had been exposed to four-day ethanol inhalation and received no drugs after withdrawal. The mean score was a 2.39±0.07 points. Brain NE and DA levels at that time were 0.146±0.003 \(\mu\)g/g and 1.008±0.018 \(\mu\)g/g, respectively. The values were lower than those in alcoholic mice which were not withdrawn from ethanol vapor (i.e., 0.165±0.005 \(\mu\)g/g and 1.085±0.022 \(\mu\)g/g for NE and DA, respectively). Results are shown in Table 3 and summarized in Fig. 3.

Pentobarbital, 30 mg/kg i.p. at 0, 1.5, 3, and 4.5 hr after ethanol withdrawal, completely suppressed withdrawal reactions but did not alter the levels of brain NE and DA. Diazepam, 1 mg/kg i.p. at 0, 2.25, and 4.5 hr after ethanol withdrawal, also completely suppressed withdrawal reactions. The brain NE level was not altered, but the brain DA level was slightly increased, and this difference was statistically significant. GHBA, 250 mg/kg i.p. at 0, 2.25, and 4.5 hr after ethanol withdrawal, moderately suppressed withdrawal reactions. This acid moderately decreased the brain NE level and significantly increased the brain DA level under these conditions. Haloperidol, 2.5 mg/kg i.p. at 0, 2.25 and 4.5 hr after ethanol withdrawal, aggravated withdrawal reactions. Under these conditions, brain NE and DA levels were significantly decreased, while it might not alter those of normal mice, at least with the dose employed. Reserpine, 1 mg/kg i.p. at 0 and 4 hr after ethanol withdrawal, tended to suppress withdrawal reactions. This drug effectively lowered brain NE and DA levels. LiCl was injected i.p. twice daily for 7 days in a dose of 2 mEq/kg. After the

![Fig. 2](image-url)  
**Fig. 2.** Relationships between brain biogenic amine levels and the severity of ethanol withdrawal reactions in mice. Mice undergoing ethanol withdrawal reactions were treated for 4 days with the drugs. Ordinate, the score at 5 hr after ethanol withdrawal based on the rating system according to Goldstein. Abscissa, each brain biogenic amine level which is expressed as \(\mu\)g/g. Each point shows the score and the amine level for each animal. Values represent correlation coefficients.
Table 3. Effects of neuropharmacological drugs on the severity of ethanol withdrawal reactions and brain catecholamine levels in mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>N</th>
<th>Score mean±S.E.</th>
<th>Norepinephrine mean±S.E.</th>
<th>Dopamine mean±S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>65</td>
<td>2.39±0.07</td>
<td>0.146±0.003</td>
<td>1.008±0.013</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td>9</td>
<td>0</td>
<td>0.146±0.010</td>
<td>1.062±0.040</td>
</tr>
<tr>
<td>Diazepam</td>
<td>9</td>
<td>0</td>
<td>0.148±0.008</td>
<td>1.107±0.044*</td>
</tr>
<tr>
<td>GHBA</td>
<td>11</td>
<td>2.03±0.13*</td>
<td>0.123±0.009*</td>
<td>1.320±0.043*</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>16</td>
<td>2.77±0.13*</td>
<td>0.073±0.004*</td>
<td>0.677±0.029*</td>
</tr>
<tr>
<td>Reserpine</td>
<td>7</td>
<td>2.08±0.09</td>
<td>0.085±0.004*</td>
<td>0.814±0.035*</td>
</tr>
<tr>
<td>LiCl</td>
<td>8</td>
<td>2.28±0.10</td>
<td>0.148±0.007</td>
<td>1.048±0.066</td>
</tr>
<tr>
<td>Imipramine</td>
<td>10</td>
<td>2.76±0.13*</td>
<td>0.154±0.005</td>
<td>0.996±0.038</td>
</tr>
<tr>
<td>Methylphenidate</td>
<td>12</td>
<td>2.78±0.14*</td>
<td>0.151±0.003</td>
<td>1.054±0.046</td>
</tr>
</tbody>
</table>

*significantly different from the controls, P<0.05.

N: number of animals.

The simultaneous estimation of the severity of ethanol withdrawal reactions and brain catecholamine levels was performed 5 hr after ethanol withdrawal. The severity was estimated as mean scores based on the rating system according to Goldstein (18). Administration of ethanol and the drugs is shown in the text.

Fig. 3. Effects of neuropharmacological drugs on the severity of ethanol withdrawal reactions and brain catecholamine levels in mice. Ordinate, percent of control obtained from the values in Table 3. Each bar represents the mean±S.E. *Significant difference from the control, P<0.05.

DISCUSSION

Single injection of the 5HT synthesis inhibitor, PCPA; the 5HT precursor, 5HTP; first 3-day treatment, mice were exposed to ethanol vapor for 4 days. LiCl had no effect on withdrawal reactions and the levels of brain NE and DA. Imipramine, 25 mg/kg p.o., and methylphenidate, 50 mg/kg p.o. at 0 and 4 hr after ethanol withdrawal, aggravated withdrawal reactions, but did not alter the levels of brain NE and DA.
the catecholamine synthesis inhibitor, AMPT; and its precursor, L-dopa had no consistent significant effect on ethanol induced withdrawal convulsions as Goldstein has reported (12). However, slightly prolonged effects by AMPT and L-dopa and a tendency for the decreased effect by 5HTP were observed. The effects of L-dopa and 5HTP are likely since a negative correlation for 5HT and a positive one for DA were demonstrated between brain amine contents and the severity of withdrawal reactions. Wood et al., however, emphasized the depletion of NE to aggravate withdrawal reactions (17). Indeed, a marked depletion of brain NE was observed after ethanol withdrawal. Pohorecky et al. also concluded that the withdrawal symptoms and the activation of noradrenergic neurons during ethanol withdrawal were caused by the sudden lack of ethanol in the system (23).

After ethanol withdrawal, a slight elevation of the brain 5HT level, a marked depletion of brain NE, and a slight decrease in brain DA were demonstrated. These data are inconsistent with most of the results found by others that were described in the introduction. A main reason of this discrepancy may be the difference of the methods used for ethanol administration. Daily treatment with PCPA and L-dopa or pretreatment with 60HDA tended to modify the severity of withdrawal reactions, which may be a main manifestation of physical dependence. PCPA slightly decreased the severity, suggesting that 5HT may be involved in the development of physical dependence since a continuous decrease in brain 5HT by PCPA was observed even during ethanol inhalation. Inconsistent results, however, have been reported (12, 15, 24), although the author has demonstrated the decrease in 5HT turnover during chronic ingestion of ethanol (11). 60HDA tended to aggravate withdrawal reactions, and L-dopa tended to decrease the severity. Similar results have been obtained using AMPT (13) and DA (14). These results suggest that dopamine may be partially involved in the development of physical dependence since L-dopa did not alter the brain NE level. Recently, it has been reported that chronic ingestion of ethanol by mice resulted in subsensitive dopamine receptors (25-27). If this effect is responsible for the development of physical dependence, a decrease in DA may accelerate it, and vice versa. The present data were consistent with this suggestion.

Treatment with some neuropharmacological drugs modified the severity of withdrawal reactions. Pentobarbital and diazepam suppressed ethanol withdrawal reactions under the present experimental conditions. Similar results have been reported by Goldstein (28). Actions of pentobarbital and diazepam were indicated to be mediated by the central GABA-ergic system (29-32). It has been suggested that the GABA pathway may suppress ethanol withdrawal reactions. However, the results of the possible role for GABA in ethanol withdrawal symptoms still remain equivocal (33-35). Diazepam has been indicated to slow down the turnover rate of catecholamines (36). The present data that diazepam slightly elevated the brain DA level may be explained by this action.

GHBA suppressed ethanol withdrawal reactions. A slight decrease in brain NE and a moderate increase in brain DA were observed at that time. It has been reported that GHBA causes a marked and selective increase in brain DA and no significant increase in brain NE (37). It has been also suggested that the central nervous system depressant properties of GHBA may be related to the blockade in the release of brain DA (37, 38). Therefore, the present data suggest that ethanol withdrawal reactions may be suppressed by decreased activities
of dopaminergic neurons. This is likely since L-dopa tended to aggravate ethanol withdrawal reactions.

Haloperidol which has been demonstrated to block DA receptors in brains (39) aggravated ethanol withdrawal reactions. Similar results have been reported using chlorpromazine or haloperidol (14, 28). A recent review, however, summarizes that neuroleptics, in general, are ineffective or have minor effects on electroshock and pentetrazol convulsions (40). Haloperidol has usually been considered to exert little or no effect on brain levels of catecholamines (41). In the present experiment, however, marked decreases in brain NE and DA were observed after haloperidol injections during ethanol withdrawal, indicating that the increased release of brain NE and DA might occur. Therefore, this possible release of brain NE and DA may aggravate ethanol withdrawal reactions.

Reserpine which is known as a depletor of catecholamines and serotonin suppressed ethanol withdrawal reactions. Goldstein has reported that reserpine greatly aggravated and prolonged withdrawal reactions (28). Many other investigators have reported that reserpine induced a facilitation of experimental seizures (40). However, Koslow and Roth have found that large doses of reserpine decreased the duration of the tonic extensor seizure in electroshock (42). In the present study, reserpine exerted the depletion of brain NE and DA, indicating that decreased activities of noradrenergic or dopaminergic neurons may produce the suppression of ethanol withdrawal reactions.

Imipramine and methylphenidate aggravated ethanol withdrawal reactions, but they did not alter the levels of brain NE and DA. Imipramine was demonstrated to exert the antidepressant effect by adrenergic potentiation via a blockade of NE uptake (43). The central stimulant action of methylphenidate has been considered to result from its ability to preferentially release dopamine from stored pools (44).

Therefore, suppression of ethanol withdrawal reactions consistently results from the decreased activities of either the noradrenergic or the dopaminergic neurons. The present data suggest that the effects of biogenic amines on ethanol withdrawal reactions may be different from those on the development of physical dependence on ethanol since single injection of L-dopa, e.g., tended to aggravate ethanol withdrawal reactions, but daily treatment with L-dopa tended to suppress them. Moreover, suppression of ethanol withdrawal reactions was demonstrated to result from the decreased activities of dopaminergic neurons by treatment with the neuropharmacological drugs.

Although these effects were not sufficient to definitively support a direct role of central catecholamines and serotonin in the development of physical dependence on ethanol, these amines may have some influence on the severity of ethanol withdrawal reactions or the development of physical dependence on ethanol.

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