EFFECTS OF ETHANOL ON CATECHOLAMINE LEVELS AND RELATED ENZYME ACTIVITIES IN DIFFERENT BRAIN REGIONS OF RATS

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Abstract—The effects of ethanol on the contents of norepinephrine (NE) and dopamine (DA) and the activities of related enzymes in the various regions of rat brains with different doses and mode of administration of ethanol were investigated. In acute ethanol intoxication, steady-state levels of NE were not altered. Continuous ethanol intoxication, however, significantly reduced NE contents and tended to decrease the activity of dopamine-β-hydroxylase in the hippocampus. The decrease in NE contents became more significant during ethanol withdrawal, especially in the medulla oblongata and the striatum. DA contents were increased in the brain-stem region in all ethanol-treated rats. The increase in DA contents correlated with the increase in tyrosine hydroxylase activity. The present data suggest that the dopaminergic system may contribute to the development of physical dependence on ethanol.

There is no general agreement on the effects of ethanol on catecholaminergic systems in the central nervous system. Main reasons for this may be differences in the doses or methods used to administer ethanol. Moreover, norepinephrine (NE) and dopamine (DA) are found to be unequally distributed in various regions. It was also revealed that these amines are specifically localized in the complex systems of neurons in the brain, i.e., noradrenergic neurons have terminals concentrated in the hypothalamus, whereas dopaminergic neurons have terminals concentrated in the striatum. Therefore, the present experiments were conducted to study the effects of ethanol on regional distributions of NE and DA in rat brains with different doses or modes of administration of ethanol. Activities of related enzymes, tyrosine hydroxylase (TH), dopamine-β-hydroxylase (DBH) and monoamine oxidase (MAO), were also assayed in various regions of rat brains.

MATERIALS AND METHODS

Ethanol treatment: Male Wistar strain rats with an initial body weight approx. 250 g were used. In the acute study, rats were injected i.p. with 1.5 and 4.0 g/kg of ethanol 30 min prior to sacrifice for moderate and severe intoxication, respectively. Animals for continuous intoxication were given ethanol vapor according to the method of Goldstein (1). Rats 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 a respirator. Pyrazole,
an inhibitor of ethanol metabolism, was injected daily at 10:00 a.m. at 50 mg/kg i.p. to stabilize blood ethanol levels. At the start of each experiment, a priming dose of ethanol at 1.5 g/kg was given i.p., together with the first injection of pyrazole. The ethanol-withdrawn rats were removed from the ethanol vapor chamber 5 hr prior to sacrifice.

Dissection of brain regions: After the rats were killed by decapitation, the brains were carefully removed, blotted, and chilled. Dissections were performed on an ice-cooled glass plate according to the method of Glowinski and Iversen (2). Five regions were separated: medulla oblongata, hypothalamus, midbrain, striatum, and hippocampus.

Determination of blood ethanol concentration: Blood ethanol concentrations were determined by an enzymic method (3). A tenth ml of blood was deproteinized by adding 5 ml of 0.45% ZnSO₄ and 1 ml of 0.1 N NaOH. The incubation mixture for ethanol determination consisted of 1 ml of the deproteinized sample, 0.1 M sodium pyrophosphate buffer containing semicarbazide, pH 8.7, 200 µl of 10 mg/ml NAD, and 100 µl of 5 mg/ml of lyophilized alcohol dehydrogenase from yeast. The optical density at 340 nm was measured after overnight incubation at room temperature. Standard solutions of ethanol were run at the same time.

Determination of catecholamines: Tissue pieces were weighed and homogenized in 4 ml of chilled acidified n-butanol. NE and DA were assayed by the method of Chang (4).

Determination of tyrosine hydroxylase activity: TH activity was determined by measuring ¹⁴C-dopa formed from ¹⁴C-tyrosine principally according to the method of Nagatsu et al. (5). Tissue pieces were homogenized in 3 ml of chilled 0.05 M tris-HCl buffer, pH 6.0, containing 0.2% Triton X-100. The homogenates were centrifuged at 10,000×g for 10 min, and the supernatant was used as an enzyme preparation. The incubation mixture consisted of 200 µl of the enzyme preparation, 0.1 M β-mercaptoethanol, 0.2 M Na-acetate buffer, pH 6.0, 2 mM dimethyltetrahydropterine, 1 mM FeSO₄, and 25 µM tyrosine (+0.1 µCi ¹⁴C-tyrosine). The total volume was 0.5 ml. Incubations were carried out at 37°C for 30 min in a shaking water bath and terminated by adding 0.5 ml of 0.8 N perchloric acid containing 0.5 mg/ml of EDTA Na₂. The supernatant was adjusted to pH 5 with 6 N K₂CO₃ and centrifuged. The supernatant was adjusted to pH 8.4 and placed on a column of 0.2 g of alumina. The effluent was decanted. The column was washed with 5 ml of water, and dopa was eluted from the column with 2 ml of 0.2 N acetic acid into a counting vial. Radioactivity was counted by a liquid scintillation counter.

Determination of dopamine-β-hydroxylase activity: Tissue pieces were homogenized in 3 ml of chilled 0.1 M phosphate buffer pH 7.4, containing 0.1% Triton X-100 and centrifuged at 23,000×g for 30 min. The supernatant was used as an enzyme preparation. DBH activity was measured by the method of Kato et al. (6) using a dual wavelength spectrophotometer.

Determination of monoamine oxidase activity: The homogenate of a tissue piece in 10 vol. of chilled 1/15 N phosphate buffer, pH 7.4, was used as an enzyme preparation. MAO activity was measured by the method of Mayerson et al. (7) using an ammonia electrode. One mM tyramine was used as a substrate. The incubations were carried out at 37°C for 30 min. The electrode potential was measured 2 min after immersing the electrode.

Determination of protein contents: Protein contents were measured by the method of Lowry et al. (8).
RESULTS

Blood levels: The blood samples of 4 rats in each group were taken via the jugular vein and blood ethanol levels were measured. Ethanol was not detected in the saline or pyrazole controls. The blood ethanol levels of the ethanol-treated rats are shown in Table 1. During exposure to ethanol vapor, the rats were visibly intoxicated and ataxic.

Their blood ethanol levels were similar to those obtained at 30 min after i.p. injection of 1.5 g/kg of ethanol in rats (namely, the moderately intoxicated group). Severely intoxicated rats were comatose. Ethanol-withdrawn rats seemed somewhat excited, but withdrawal symptoms were not observed 5 hr after the removal from ethanol vapor.

Effects of pyrazole: Since pyrazole was administered to the rat during ethanol vapor exposure, it was determined whether pyrazole could modify the effects of ethanol. Pyrazole was injected daily at a dose of 50 mg/kg i.p. for continuous intoxication.

Table 1. Blood levels of ethanol in ethanol-treated rats

|                  | Moderate intoxication | Severe intoxication | Continuous intoxication |
|------------------|-----------------------|---------------------|------------------------|
|                  | Ethanol was given      |                     |                        |
| moderate         | 1.5 g/kg i.p. of      |                     | 1.38±0.25              |
|                  | ethanol 30 min prior  |                     | 0.68±0.31 mg/ml        |
|                  | to sacrifice          |                     |                        |
| severe           | 4.0 g/kg i.p. of      |                     |                        |
|                  | ethanol 30 min prior  |                     |                        |
|                  | to sacrifice          |                     |                        |
| intoxicated      | 4 day inhalation of    |                     |                        |
|                  | ethanol vapor (ethanol,|                     |                        |
|                  | 3 ml/hr and air, 3 l/min) |                 |                        |
| withdrawn        | withdrawn from ethanol |                     |                        |
|                  | vapor 5 hr prior to    |                     |                        |
|                  | sacrifice after 4 day  |                     |                        |
|                  | inhalation of ethanol  |                     |                        |
|                  | vapor                  |                     |                        |

Fig. 1. Effects of ethanol on norepinephrine contents in five brain regions of the rat. Each bar represents the mean±S.E. of six determinations. Ethanol was given as described in the caption to Table 1. Control for acute intoxication: saline instead of ethanol. Control for continuous intoxication: daily pyrazole injections and no ethanol inhalation. *significant difference from the control (P<0.05)
exposure, effects of pyrazole on NE and DA contents and related enzyme activities were investigated. Although the NE contents in each region except the medulla oblongata tended to decrease by 10 to 30% of the saline controls, these differences were not statistically significant. DA contents tended to increase in the medulla oblongata, but these were not significantly different from the saline controls. Activities of TH, DBH, and MAO also tended to increase in some regions, but these increases were not statistically significant.

Effects of ethanol on NE contents: Effects of ethanol on NE contents in five brain regions of the rat are shown in Fig. 1. In moderate or severe acute intoxication, NE contents were not different from saline controls in any region. However, by 4 days of continuous intoxication, 20 to 50% decreases in NE contents were observed in the hypothalamus, midbrain, and hippocampus, compared with those of the pyrazole controls. These differences were statistically significant in the hippocampus. When ethanol was withdrawn, more marked decreases in NE contents were seen and were statistically significant in the medulla oblongata and the striatum, even compared with those of the continuously intoxicated rats. Figure 1 also shows the different regional distributions of NE between groups of the intoxicated and withdrawn rats. NE levels in the continuously intoxicated rats were significantly lower in the hippocampus. On the other hand, those in the ethanol-withdrawn rats were significantly lower in brain-stem areas.

Effects of ethanol on DA contents: Effects of ethanol on DA contents in five brain regions of the rat are shown in Fig. 2. In

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![Fig. 2. Effects of ethanol on dopamine contents in five brain regions of the rat. Each bar represents the mean±S.E. of six determinations. Ethanol administration and controls are shown in Table 1 and Fig. 1, respectively. *significant difference from the control (P<0.05).](image)
moderate acute intoxication, DA contents in the medulla oblongata and the hypothalamus increased. In severe acute intoxication, significant increases of DA contents were observed in the medulla oblongata, hypothalamus, midbrain, and striatum. However, those in the hippocampus were not altered. Figure 2 also shows the similar regional distributions of the DA in the moderately and severely intoxicated rats. In continuous intoxication, DA contents in the medulla oblongata, hypothalamus and midbrain were significantly increased, but those in other regions were not altered. There was no difference in DA contents between the continuously intoxicated and withdrawn rats.

Effects of ethanol on activities of TH, DBH, and MAO: Effects of ethanol on TH activity are shown in Fig. 3. Significant increases were seen in the medulla oblongata of all ethanol-treated rats and in the midbrain of moderately intoxicated rats. Tendencies to increase were also observed in the hypothalamus and the midbrain of acutely intoxicated groups. No change, however, was seen in the striatum and the hippocampus of all ethanol-treated groups. These changes of TH activity by ethanol administration were similar to those of DA contents.

As shown in Fig. 4, DBH activity was not altered in acutely intoxicated groups. However, DBH activity in the hippocampus of continuously intoxicated rats tended to decrease.

MAO activity in the medulla oblongata of moderately intoxicated rats was decreased, but there was no change in other regions (Fig. 5).

DISCUSSION

Pyrazole did not alter the steady-state levels of catecholamines at the dose used in the present experiment, although Brown et
al. have reported an increase in NE (9). This discrepancy may be due to the different methodology. Activities of TH, DBH, and MAO were not affected by pyrazole administration. Determinations of blood ethanol levels indicated that continuously intoxicated rats were moderately intoxicated.

In either of rats with moderate or severe acute ethanol intoxication, steady-state levels of NE in each group were not altered. Continuous ethanol intoxication, however, significantly reduced NE contents in the hip-
pocampus. This effect may be due to ethanol per se and not due to acetaldehyde (10) since a combined dose of pyrazole inhibits ethanol oxidation to acetaldehyde. The decreases in NE contents may be interpreted as manifestations of an acceleration of NE turnover (11, 12) or an increase in a release of NE (13). Simultaneous assay of DBH activity, however, revealed that its activity tended to decrease in this region. The decreases in NE contents became more significant during ethanol withdrawal, especially in the medulla oblongata and the striatum. NE turnover has been shown to increase during ethanol withdrawal (14). Therefore, these effects may indicate the activation of noradrenergic neurons. The present data, however, suggest that the effects of ethanol withdrawal may be dissimilar to those of ethanol per se since different regional distributions of NE were observed under these two conditions. Pohorecky et al. also concluded that the withdrawal symptoms and the activation of noradrenergic neurons during the withdrawal of ethanol were caused by the sudden lack of ethanol in the system (15).

DA contents were increased in the brain-stem region in all ethanol-treated rats. Those in the hippocampus, however, were not altered. The regional distributions of DA were not similar to those of NE. These increases seemed to be dose-related, and distributions were similar between the moderately and severely intoxicated rats. Simultaneous assay of TH activity in each region revealed that the increase in DA contents correlated with the increase in TH activity. These data suggest that DA synthesis may be accelerated in the brain-stem region by ethanol administration. However, the results of ethanol effects on DA synthesis or TH activity in the striatum were inconsistent (16–18). In the present studies, TH activity in the striatum was not altered by ethanol administration, whereas that in the medulla oblongata of all ethanol-treated rats and the midbrain of moderately intoxicated rats was increased.

MAO activity in the medulla oblongata of moderately intoxicated rats was slightly decreased, whereas those in other regions were not altered. Wiberg et al. have also reported that chronic ethanol treatment in rats did not alter brain MAO activity (19).

Recently, chronic ingestion of ethanol by mice has been reported to result in subsensitive dopamine receptors (20–22). Moreover, the previous study suggested that the dopaminergic system may partially contribute to the development of physical dependence on ethanol (23). The present results may be considered additional evidence for this suggestion.

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