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Monoamine and Metabolite Levels in CNS Regions of the P Line of Alcohol-Preferring Rats After Acute and Chronic Ethanol Treatment[1]

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MURPHY, J. M., W. J. McBRIDE, L. LUMENG AND T.-K. LI. *Monoamine and metabolite levels in CNS regions of the P line of alcohol-preferring rats after acute and chronic ethanol treatment.* PHARMACOL BIOCHEM BEHAV **19**(5) 849–856, 1983.—Levels of norepinephrine (NE), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were determined in 8 brain regions of the P line of alcohol-preferring rats following: (a) an IP injection of 2.5 g ethanol/kg body wt; (b) 8 and 15 weeks of chronic free-choice drinking of 10% ethanol; (c) 15 weeks of chronic free-choice drinking of 10% ethanol and 24 hours of withdrawal; and (d) 7 weeks of forced administration of 5% ethanol in liquid diet. One hour after IP injection of 2.5 g ethanol/kg body wt, the levels of DOPAC and HVA increased 20–45% in the cerebral cortex (CTX) and striatum (STR). A 20% lower content of NE in the CTX of the ethanol group was the only other statistically significant difference observed. Chronic free-choice drinking of 10% ethanol for 8 weeks (6.5±0.4 g ethanol/kg/day) or 15 weeks (7.8±0.2 g ethanol/kg/day) and the chronic forced administration of ethanol in liquid diets (up to 13.2±0.2 g ethanol/kg/day) did not produce any consistent pattern of alterations in the levels of the monoamines or their metabolites in the 8 CNS regions. After 15 weeks of chronic free-choice drinking of 10% ethanol, withdrawal from alcohol also did not produce alterations in the content of the monoamines or their metabolites. These data indicate that acute administration of hypnotic doses of ethanol increases the metabolism of specific dopaminergic neurons in the CNS of the P rat, but monoamine levels and metabolism are not altered after chronic (7–15 weeks) alcohol consumption.

Alcohol-preferring rats Acute ethanol treatment Chronic ethanol treatment CNS monoamines Norepinephrine Dopamine Serotonin 3,4-Dihydroxyphenylacetic acid Homovanillic acid 5-Hydroxyindoleacetic acid

NUMEROUS studies have suggested the involvement of CNS norepinephrine (NE), dopamine (DA) and serotonin (5-HT) transmitter systems in alcohol sensitivity, tolerance and dependence [14, 19, 25, 30]. However, a clear and undisputed role has yet to emerge for these monoamines or any other neurotransmitter in the mediation of these effects of ethanol. Most of the studies on the neurochemical consequences of ethanol have utilized animals that normally would not consume significant amounts of alcohol by free-choice drinking. Consequently, alcohol has had to be given by forced administration. Only a few studies have examined the neurochemical effects of ethanol in animals that show an innate preference for alcohol [1, 2, 23].

There is now compelling evidence for a genetic predisposition in the etiology of alcoholism and in alcohol drinking behavior [6, 9, 27, 28]. Moreover, some findings have suggested that alcoholics or individuals with a genetic predisposition to alcoholism may respond differently to alcohol than do nonalcoholics [8,27]. In this context, the study of selectively bred animals that have an innate preference for alcohol appears to be particularly relevant as a means to discern possible neurochemical correlates of alcohol drinking behavior and the effects of alcohol. In our laboratory, two rat lines have been selectively bred for their alcohol-preferring and -nonpreferring drinking behavior [18, 20, 21]. In free choice between 10% (V/V) ethanol and water, with food available ad lib, the alcohol-preferring P line of rats voluntarily consumes amounts of alcohol (>5 g/kg body weight/day) that approach their metabolic capacity [17,18].

The purpose of the present study was to assess the effects

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of ethanol exposure on brain monoamine neurotransmitter systems in the P line of rats. The content of NE, DA, 5-HT and the metabolites, 5-hydroxyindoleacetic acid (5-HIAA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were determined in eight discrete CNS regions of alcohol-naive P rats and of P rats following acute and chronic exposure to ethanol.

METHOD

The selectively bred P line originated from a randomly bred Wistar colony at the Walter Reed Army Institute of Research [20]. Rats used in the present study were of the S20 and 21 generations. All rats were housed individually; a normal 12 hour light-dark cycle was maintained beginning at 0800 hours. At the completion of each experiment, the rats were killed by the near-freezing method [31]. The animals were handled daily for at least seven days prior to killing and were adapted to the killing apparatus to minimize stress. The brains were rapidly removed and dissected at −20°C or lower into eight brain regions: cerebral cortex, striatum, hypothalamus, thalamus, hippocampus, midbrain, pons-medulla, and cerebellum. The brain parts were stored at −70°C until assayed for the content of DA, NE, 5-HT, DOPAC, HVA and 5-HIAA by HPLC with electrochemical detection. To facilitate chromatographic separation of the metabolites, the assay procedure described previously [22] was modified by the use of a mobile phase (pH 3.5) containing 17.5% (V/V) methanol, 29.6 mM citric acid, 14.3 mM disodium phosphate, 0.275 mM octyl sodium sulfate and 0.1 mM EDTA.

Statistical differences were determined with the standard, independent *t*-test for two groups and with a one-way ANOVA and Newman-Keuls post-hoc tests when three or more groups were used. All data are presented as means±SEM.

Acute Ethanol Administration

Six male, alcohol-naive P rats (300–325 g) received a single IP injection of 2.5 g ethanol/kg body weight as a 12 g% solution in saline. Littermates matched for body weight served as saline-injected controls. All animals were killed one hour after injection and trunk blood was collected for the determination of blood alcohol concentrations (BACs).

Chronic Ethanol Exposure

Free choice for 8 weeks. Adult, male rats of the P line (300–325 g) were randomly assigned to two groups of ten. The control group received H_2O as its sole source of fluid for 8 weeks, while the experimental group had free access to H_2O and a 10% (V/V) ethanol solution. Food (Wayne Lab Blox) was available ad lib to both groups. Fluid intake and body weights were monitored throughout the experiment. After eight weeks, all rats were killed between 1300 and 1500 hours.

Free choice for 15 weeks. Adult, male rats of the P line (300–325 g) were allowed 15 weeks of free access to H_2O and a 10% ethanol solution containing 0.125 g% sodium saccharin which was added to enhance ethanol consumption [16]. Paired littermate controls were matched for body weight and were given H_2O and an isocaloric sucrose solution (14.2 g%) containing sodium saccharin (0.125 g%). The sucrose solution was given in a volume equal to that of the alcohol solution consumed daily by the experimental animal. Food (Wayne Lab Blox) was available to both groups ad lib. At the end of the 15 weeks, all animals were killed between 0800 and 0900 hours at the end of the dark cycle. Half of the experimental group (N=7) was killed while still on free-choice drinking (the ethanol-maintained group) and half was killed after the ethanol had been removed for 24 hours (the withdrawn group). Rats in the withdrawn group were matched for ethanol intake and body weights to the rats remaining on free choice. The withdrawn rats were observed for signs of physical dependence [13] at two hour intervals between 2–10 hours after ethanol was removed. Trunk blood was collected for the determination of BACs only from the group remaining on free choice at the time of killing.

Comparison of Chronic Free-Choice Ethanol Drinking with Forced Administration of Ethanol in Liquid Diets for 7 Weeks

Adult, female rats of the P line (200–225 g) were divided into six groups each containing 6 animals. The first group (control) was given H_2O as its sole source of fluid and had food (Wayne Lab Blox) available ad lib. The second group (FC) had free-choice access to both H_2O and 10% ethanol, and food (Wayne Lab Blox) was also available ad lib. A third group (LD-E) received a liquid diet which contained ethanol (Bio-Mix No. 711, Bio-Serv Inc., Frenchtown, NJ). The diet consisted of 36% of the total calories as ethanol, 18% as protein, 35% as fat, and 11% as carbohydrates. The fourth group (LD) was the pair-fed control of the third group and it was given the same liquid diet except that ethanol was isocalorically replaced by carbohydrates. The caloric density of the control and alcohol-containing liquid diet was 1 kcal/ml. Two additional groups were given the commercial liquid diet supplemented with zinc and protein. It was prepared by adding 20 mg of zinc and 50 g of casein per liter of diet. The fifth group (LDS-E) received the supplemented liquid diet containing ethanol. Its caloric density was 1.21 kcal/ml, with 29% as ethanol, 32% as protein, 29% as fats, and 10% as carbohydrates. The sixth group (LDS) served as the pair-fed control of the fifth group and received the same supplemented diet except that ethanol was isocalorically replaced by carbohydrates. All 36 rats were given their respective feeding regimens for seven weeks. Body weights, caloric intake and ethanol consumption were recorded throughout the experiment. BACs were measured at 0600, 1400 and 2200 hours on three consecutive days in the second and the sixth week. Animals were killed between 1300 and 1500 hours after seven weeks.

RESULTS

Effects of Acute Ethanol Administration

One hour after the injection of 2.5 g of ethanol/kg body weight, the levels of the DA metabolites, DOPAC and HVA, were significantly (20–45%) elevated in the cerebral cortex and striatum as compared with the saline-injected controls (Table 1). A 20% decrease in the content of NE in the cerebral cortex of the ethanol group was the only other significant effect observed. Trunk blood samples collected from the ethanol-injected rats at the time of killing showed an average BAC of 277±14 mg%.

Effects of Chronic Free-Choice Ethanol Consumption

The overall average amount of ethanol consumed (g/kg body weight/day) by the male rats on free choice was

TABLE 1

LEVELS OF NE, DA, DOPAC, HVA, 5-HT AND 5-HIAA IN EIGHT CNS REGIONS OF THE P RATS 1 HR AFTER AN INJECTION OF SALINE OR 2.5 g ETHANOL/kg

	nmole/g tissue (mean ± S.E.M.; N=6)					
	NE	DA	DOPAC	HVA	5-HT	5-HIAA
Cerebral Cortex						
Saline	1.97 ± 0.14	4.61 ± 0.32	0.57 ± 0.02	0.29 ± 0.01	3.41 ± 0.10	1.98 ± 0.14
Ethanol	1.54 ± 0.08*	4.40 ± 0.13	0.70 ± 0.02†	0.43 ± 0.04†	3.48 ± 0.06	2.10 ± 0.22
Striatum						
Saline	N.D.	48.94 ± 1.27	4.35 ± 0.13	2.89 ± 0.14	3.81 ± 0.10	3.06 ± 0.11
Ethanol	—	47.97 ± 1.64	5.51 ± 0.27†	3.83 ± 0.34*	4.15 ± 0.19	3.55 ± 0.28
Hypothalamus						
Saline	9.50 ± 0.51	3.42 ± 0.06	0.58 ± 0.03	N.D.	6.69 ± 0.16	3.43 ± 0.12
Ethanol	8.62 ± 0.36	3.40 ± 0.24	0.68 ± 0.07	—	6.65 ± 0.16	3.64 ± 0.28
Thalamus						
Saline	1.96 ± 0.11	1.30 ± 0.24	0.31 ± 0.03	0.11 ± 0.01	4.83 ± 0.22	3.78 ± 0.24
Ethanol	1.91 ± 0.07	1.47 ± 0.38	0.38 ± 0.05	0.15 ± 0.04	5.02 ± 0.27	4.30 ± 0.45
Hippocampus						
Saline	0.97 ± 0.14	0.14 ± 0.01	N.D.	N.D.	2.54 ± 0.09	1.82 ± 0.14
Ethanol	1.30 ± 0.20	0.16 ± 0.02	—	—	2.72 ± 0.08	2.06 ± 0.13
Midbrain						
Saline	3.44 ± 0.10	1.30 ± 0.14	0.29 ± 0.02	0.15 ± 0.01	6.05 ± 0.11	4.73 ± 0.32
Ethanol	3.62 ± 0.12	1.22 ± 0.09	0.34 ± 0.02	0.17 ± 0.02	6.22 ± 0.16	4.97 ± 0.26
Pons-Medulla						
Saline	2.94 ± 0.13	0.22 ± 0.02	0.12 ± 0.01	0.26 ± 0.01	4.00 ± 0.09	2.92 ± 0.19
Ethanol	2.75 ± 0.08	0.25 ± 0.03	0.16 ± 0.02	0.30 ± 0.02	4.18 ± 0.13	3.12 ± 0.25
Cerebellum						
Saline	0.96 ± 0.07	0.05 ± 0.01	N.D.	0.11 ± 0.02	0.52 ± 0.02	0.38 ± 0.04
Ethanol	1.29 ± 0.14	0.04 ± 0.01	—	0.12 ± 0.01	0.56 ± 0.04	0.45 ± 0.08

*$p<0.05$ and †$p<0.01$ by t-test for ethanol compared to saline group.
N.D. indicates that the compound was not detected with the instrument sensitivity and HPLC conditions used.

6.5±0.4 for the unflavored ethanol over eight weeks, and 7.8±0.2 for the rats allowed to drink the saccharin-flavored 10% ethanol solution over 15 weeks. Throughout both of these free-choice experiments, body weights increased consistently and did not differ between the ethanol and control groups. Body weights at the end of eight weeks of free choice were 434±15 g for the control group and 424±11 g for the experimental group. In the 15 week experiment, the body weights of the pair-fed sucrose control group, the ethanol-maintained group and the ethanol-withdrawn group were 465±6, 456±8 and 455±13 g, respectively. Animals in the ethanol-maintained group at the time of killing exhibited an average BAC of 36±15 mg%. The ethanol withdrawn group showed signs of withdrawal, Stage II–III in severity, 2 to 10 hours following ethanol removal.

The levels of NE, DA, DOPAC, HVA, 5-HT and 5-HIAA determined in the eight brain regions of the male P rats generally were unaltered by 8 and 15 weeks of free-choice ethanol consumption (Tables 2 and 3). Only a few statistically significant differences were found. The single significant effect in the eight week free-choice experiment was a 10% decrease in the level of 5-HIAA in the striatum of the ethanol group (Table 2). In the 15 week free-choice experiment (Table 3), three statistically significant differences were observed. HVA levels of the midbrain were higher in the ethanol-maintained group than in both the H_2O and the withdrawn groups, and 5-HIAA content of the pons-medulla was 10% lower in the withdrawn group than in the ethanol-maintained group.

Comparison of the Effects of Chronic Free-Choice Versus Forced Ethanol Consumption

The body weights of the control, LD and LDS groups and the body weights and ethanol consumption of the FC, LD-E and LDS-E groups of female P rats are shown in Table 4. The control and LDS groups exhibited an average increase of 55 and 53 g in body weight over the 7-week period, whereas the LD group showed no change in body weight. The FC and LDS-E groups exhibited about the same weight gain as their respective controls, whereas the LD-E group had only a small (15 g) increase in body weight.

The LD-E and LDS-E groups consumed larger amounts of ethanol than the FC group after one and 7 weeks (Table 4). The average ethanol intake over the 7-week period did not change for the FC group, increased 22% for the LD-E animals, and decreased 19% for the LDS-E group. Approximately 70% of the differential change in ethanol intake over the 7 weeks can be accounted for by the differences in the weight gain of the LD-E and LDS-E groups.

TABLE 2

LEVELS OF NE, DA, DOPAC, 5-HT AND 5-HIAA IN EIGHT BRAIN AREAS OF P LINE OF RATS MAINTAINED FOR EIGHT WEEKS ON WATER OR FREE CHOICE BETWEEN WATER AND 10 PERCENT ETHANOL

	nmol/g wet wt. (Mean ± SEM; N=10)				
	NE	DA	DOPAC	5-HT	5-HIAA
Cerebral Cortex					
H_2O	1.70 ± 0.08	5.51 ± 0.34	0.28 ± 0.03	3.40 ± 0.05	1.29 ± 0.03
Ethanol	1.71 ± 0.13	5.93 ± 0.26	0.31 ± 0.02	3.34 ± 0.07	1.31 ± 0.03
Striatum					
H_2O	N.D.†	60.78 ± 2.48	4.12 ± 0.23	3.45 ± 0.12	2.26 ± 0.06
Ethanol	N.D.	56.70 ± 2.33	3.74 ± 0.22	3.17 ± 0.12	2.05 ± 0.07*
Hypothalamus					
H_2O	10.32 ± 0.50	1.75 ± 0.11	0.22 ± 0.12	5.81 ± 0.13	2.01 ± 0.07
Ethanol	9.72 ± 0.57	1.72 ± 0.10	0.14 ± 0.05	5.58 ± 0.15	1.88 ± 0.07
Thalamus					
H_2O	2.61 ± 0.28	1.69 ± 0.28	0.10 ± 0.04	4.68 ± 0.14	2.61 ± 0.06
Ethanol	2.16 ± 0.25	1.79 ± 0.27	0.14 ± 0.05	4.62 ± 0.25	2.52 ± 0.11
Hippocampus					
H_2O	1.31 ± 0.09	N.D.	N.D.	2.78 ± 0.08	1.51 ± 0.06
Ethanol	1.20 ± 0.09	N.D.	N.D.	2.57 ± 0.08	1.37 ± 0.04
Midbrain					
H_2O	2.35 ± 0.15	0.72 ± 0.10	N.D.	6.08 ± 0.16	3.05 ± 0.14
Ethanol	2.36 ± 0.17	0.69 ± 0.08	N.D.	5.82 ± 0.19	3.16 ± 0.11
Pons-Medulla					
H_2O	2.51 ± 0.10	0.24 ± 0.01	N.D.	3.92 ± 0.10	2.23 ± 0.06
Ethanol	2.47 ± 0.13	0.23 ± 0.01	N.D.	3.92 ± 0.11	2.26 ± 0.07
Cerebellum					
H_2O	0.60 ± 0.06	N.D.	N.D.	0.63 ± 0.05	0.42 ± 0.04
Ethanol	0.58 ± 0.07	N.D.	N.D.	0.59 ± 0.04	0.33 ± 0.02

*$p<0.05$ by t-test for ethanol group compared to H_2O control group.

†N.D. indicates that the compound was not detected with the instrument sensitivity and HPLC conditions used.

BACs were determined at 0600, 1400 and 2200 hours in the second and the sixth week. Rats in the FC and LDS-E groups had mean BACs that ranged from 37–61 mg% at 0600, 17–28 mg% at 2200 hours and to 0–15 mg% at 1400 hours. Animals in the LD-E group had mean BACs of 119–165 mg% and did not show diurnal variation.

In general agreement with the findings for the male P-rats on free choice (Tables 2 and 3), no significant differences were found in any brain region of the female P-rats given the solid food and H_2O only (control) and the group given solid food and free choice between H_2O and 10% ethanol (Table 5; data for the free-choice group are not shown). No significant differences were observed among the solid food control group and the LD and LDS groups. In addition, no differences were observed between the LD and LD-E groups. However, three statistically significant differences were evident between the LDS and LDS-E groups. The LDS-E group had 20% lower levels of HVA and 5-HIAA in the cerebral cortex and 21% higher content of NE in the pons-medulla compared to the LDS group.

DISCUSSION

One hour after the injection of 2.5 g ethanol/kg body weight, DOPAC and HVA levels were elevated in both the striatum and cerebral cortex of the P rats (Table 1). These increases are most likely due to increased metabolism of specific dopaminergic neurons as opposed to decreased efflux of acid metabolites, since elevated contents of DOPAC and HVA were not observed in other regions of the CNS nor was the content of 5-HIAA increased in any of the eight CNS regions. Elevated levels of HVA and DOPAC in whole brain [15] and of DOPAC in the striatum [4,7] have been reported following the acute administration of ethanol to animals not selected for alcohol-drinking preference. Therefore, it would seem that the P rats respond to alcohol in a manner similar to that of randomly-bred stock rats in these respects. On the other hand, a previous study found no effect of acute ethanol on DOPAC and HVA levels in the frontal cortex of randomly-bred rats [7] but elevated DOPAC and HVA levels were observed in the cerebral cortex of P rats in the present study (Table 1). Whether or not this difference is unique for the P line of rats requires further exploration. The acute administration of ethanol also decreased the NE content of the cerebral cortex in the P rats (Table 1). Similar decreases of NE content in whole brain [11,32] and telencephalon [5] have been reported following the acute administration of ethanol to randomly-bred rats.

While it is clear that sedative-hypnotic doses of ethanol

TABLE 3

LEVELS OF NE, DA, DOPAC, HVA, 5-HT AND 5-HIAA IN EIGHT CNS REGIONS OF THE P LINE OF RATS ALLOWED 15 WEEKS OF FREE ACCESS TO ETHANOL OR WITHDRAWN FROM EtOH FOR 24 HR

	nmole/g tissue (mean ± S.E.M.)					
Group	NE	DA	DOPAC	HVA	5-HT	5-HIAA
Cerebral Cortex						
Control	2.10 ± 0.13	4.94 ± 0.33	0.56 ± 0.02	0.23 ± 0.02	3.50 ± 0.08	1.70 ± 0.05
Ethanol	2.02 ± 0.10	5.29 ± 0.35	0.60 ± 0.03	0.23 ± 0.05	3.55 ± 0.07	1.78 ± 0.05
Withdrawn	2.06 ± 0.16	5.60 ± 0.48	0.58 ± 0.04	0.19 ± 0.02	3.51 ± 0.12	1.64 ± 0.07
Striatum						
Control	N.D.	50.6 ± 2.0	4.52 ± 0.23	2.44 ± 0.17	3.75 ± 0.12	2.50 ± 0.08
Ethanol	—	52.1 ± 2.7	4.28 ± 0.26	2.06 ± 0.17	3.36 ± 0.16	2.57 ± 0.09
Withdrawn	—	54.0 ± 2.7	4.60 ± 0.31	2.18 ± 0.22	3.95 ± 0.16	2.56 ± 0.12
Hypothalamus						
Control	10.30 ± 0.26	2.85 ± 0.12	0.48 ± 0.02	N.D.	6.09 ± 0.11	2.76 ± 0.08
Ethanol	10.81 ± 0.73	2.77 ± 0.16	0.48 ± 0.03	—	5.86 ± 0.20	2.77 ± 0.11
Withdrawn	11.31 ± 0.35	2.86 ± 0.13	0.47 ± 0.02	—	6.29 ± 0.12	2.64 ± 0.10
Thalamus						
Control	2.28 ± 0.06	1.28 ± 0.10	0.28 ± 0.02	0.10 ± 0.01	4.92 ± 0.09	3.18 ± 0.05
Ethanol	2.42 ± 0.06	1.39 ± 0.19	0.27 ± 0.01	0.10 ± 0.01	4.78 ± 0.06	3.07 ± 0.05
Withdrawn	2.27 ± 0.08	1.63 ± 0.16	0.28 ± 0.02	0.09 ± 0.01	4.74 ± 0.06	3.04 ± 0.07
Hippocampus						
Control	1.32 ± 0.19	0.20 ± 0.02	N.D.	N.D.	2.60 ± 0.05	1.54 ± 0.03
Ethanol	1.32 ± 0.22	0.21 ± 0.02	—	—	2.60 ± 0.04	1.57 ± 0.06
Withdrawn	1.36 ± 0.25	0.19 ± 0.03	—	—	2.70 ± 0.09	1.53 ± 0.09
Midbrain						
Control	3.59 ± 0.06	1.11 ± 0.05	0.21 ± 0.02	0.13 ± 0.01	5.90 ± 0.09	3.98 ± 0.09
Ethanol	3.68 ± 0.05	1.24 ± 0.09	0.23 ± 0.02	0.18 ± 0.02*	5.72 ± 0.10	4.02 ± 0.09
Withdrawn	3.72 ± 0.12	1.25 ± 0.06	0.25 ± 0.01	0.11 ± 0.01†	5.84 ± 0.05	3.79 ± 0.12
Pons-Medulla						
Control	2.54 ± 0.06	0.22 ± 0.01	0.13 ± 0.01	0.16 ± 0.01	3.80 ± 0.04	2.64 ± 0.05
Ethanol	2.51 ± 0.12	0.20 ± 0.02	0.11 ± 0.02	0.14 ± 0.03	3.77 ± 0.07	2.82 ± 0.06
Withdrawn	2.75 ± 0.06	0.24 ± 0.01	0.14 ± 0.03	0.21 ± 0.02	3.71 ± 0.08	2.54 ± 0.09†
Cerebellum						
Control	0.97 ± 0.06	0.05 ± 0.01	N.D.	0.10 ± 0.01	0.49 ± 0.02	0.31 ± 0.01
Ethanol	0.96 ± 0.13	0.06 ± 0.01	—	0.06 ± 0.01	0.51 ± 0.02	0.34 ± 0.01
Withdrawn	1.19 ± 0.06	0.05 ± 0.01	—	0.07 ± 0.01	0.49 ± 0.02	0.30 ± 0.02

*$p<0.05$ for ethanol vs. control group by ANOVA and Newman-Keuls test.
†$p<0.05$ for withdrawn vs. ethanol group by ANOVA and Newman-Keuls test.
The number of animals were 14, 7 and 7 for control, ethanol and withdrawn groups, respectively.

will result in some consistent effects on CNS catecholamine levels, the findings may not provide relevant information toward the understanding of the reinforcing actions of ethanol in drinking behavior. As observed in this study and previously [17,34], P rats allowed free-choice consumption of ethanol only occasionally exhibit BACs in excess of 100 mg%, but these BACs are voluntarily maintained on a daily basis over extended periods of time. The average BAC of 277 mg% obtained one hour after the 2.5 g/kg dose may far exceed a level that would normally be rewarding to a P rat. In fact, recent evidence suggests that IP doses of ethanol (0.06–0.5 g/kg) yielding BACs closer to the range obtained with free-choice consumption are behaviorally stimulating for P rats but not for the NP (nonpreferring) line of rats [35].

Previously we had found lower levels of 5-HT and 5-HIAA in discrete regions of the telencephalon and diencephalon of naive P rats compared to the NP rats [22], suggesting a potential neurochemical correlate of alcohol preference. The catecholamines are also of interest since these neurotransmitter systems have been implicated as possible CNS-reward mechanisms for the self-administration of drugs [3, 32, 36]. To determine if the chronic voluntary drinking of ethanol would be associated with alterations in any of the CNS monoamine systems, P rats allowed free-choice ethanol consumption were compared in two experiments with control animals given H_2O as the only drinking fluid. However, chronic free-choice alcohol drinking for eight or 15 weeks did not yield any consistent or substantially significant effects on the steady-state levels of any of the six compounds measured (Tables 2 and 3). A previous study on P rats from the S8 generation also found only minor alterations in monoamine content in two large CNS regions

TABLE 4

EFFECTS OF SEVEN WEEKS OF DIFFERENT DIETARY REGIMENS ON BODY WEIGHTS AND ALCOHOL CONSUMPTIONS OF ALCOHOL-PREFERRING P RATS

		Body Weight (g)		Ethanol intake (g/kg/day)	
	(N)	Start	After 7 Weeks	After Week 1	After Week 7
Normal Diet (Control)	(6)	208 ± 6	263 ± 9*	—	—
+ Ethanol(FC)	(6)	227 ± 12	274 ± 8*	8.5 ± 0.6	8.4 ± 0.3
Liquid Diet (LD)	(6)	205 ± 10	204 ± 7	—	—
+ Ethanol (LD-E)	(5)	208 ± 3	223 ± 5*	10.8 ± 0.5†	13.2 ± 0.2*†
+ Protein, Zinc (LDS)	(6)	209 ± 7	262 ± 4*	—	—
+ Protein, Zinc, Ethanol (LDS-E)	(6)	204 ± 6	270 ± 8*	11.7 ± 0.4†	9.5 ± 0.3*†‡

*$p<0.05$ by t-test for body weights and ethanol consumptions of start vs. seven weeks.
†$p<0.05$ by t-test for ethanol intake of LD-E and LDS-E vs. FC.
‡$p<0.01$ by t-test for ethanol intake of LDS-E vs. LD-E.

after 4 to 6 weeks of free-choice drinking [23]. Likewise, only a few, very small effects of chronic alcohol consumption on the CNS levels of catecholamines [2], 5-HT and 5-HIAA [1] have been observed in the Alko, alcohol-preferring (AA) rats. Thus, a significant involvement of CNS monoamine systems in the voluntary consumption of alcohol has yet to be identified.

Chronic free-choice ethanol consumption is known to produce signs of physical dependence in the P rat between 4 and 72 hours following withdrawal [33]. Therefore, the effects of withdrawal from alcohol following chronic, free-choice consumption were studied. To confirm that physical dependence occurred in the present study, withdrawn rats were observed 2 to 10 hours following ethanol removal. Withdrawal signs of Stage II–III in severity were evident. Previous studies by others have observed some effects on CNS monoamines of withdrawal from chronic alcohol for various times ranging from 8–48 hr [19, 24, 30]. Thus, a representative time point of 24 hr was selected to assess whether similar changes would occur in the P rat. Rats withdrawn for 24 hours after drinking 6–9 g ethanol/kg body weight/day for 15 weeks demonstrated little change in monoamine or metabolite levels with respect to the control group (Table 3). Gothoni and Ahtee [10] had reported only slightly elevated 5-HIAA levels in the CNS of Wistar rats treated with 8–11 g ethanol/kg daily for 7–10 days and then withdrawn for 16–18 hours. On the other hand, Tabakoff *et al.* [29] found no difference at all in the levels of either 5-HT or 5-HIAA in the CNS of withdrawn C57BL mice. However, some investigators have found time-dependent changes in the metabolism of monoamines after withdrawal from ethanol [19, 24, 30]. Conceivably, the steady-state levels of the monoamines and their metabolites might show changes at other time points after withdrawal in P rats.

Many studies on the chronic effects of alcohol have utilized methods that force animals to consume larger quantities of ethanol than obtained with free choice. Since some alterations in CNS monoamines have been reported following the chronic forced administration of ethanol [12, 25, 30], the present study compared the effects of free-choice and chronic forced consumption of ethanol. Forced administration of ethanol was accomplished with liquid diets. Since the LD and LD-E group given the commercial liquid diet (Bio-Mix No. 711) did not gain weight normally (Table 4), zinc and protein supplemented LDS and LDS-E groups were also examined to rule out any possible nutritional effects on the monoamine levels. No consistent changes were observed in the content of the monoamines or their metabolites in the CNS of the female P rats given free-choice ethanol or administered the ethanol-containing liquid diets (Table 5). Overall, therefore, the present study demonstrated in three separate experiments that chronic alcohol administration yields, at most, only a few minor, isolated changes in the CNS regional levels of the monoamines and metabolites. A number of conditions were varied in these experiments, including the sex of the animals, the degree of intoxication achieved, the duration of ethanol exposure, and the means of chronic administration of ethanol (i.e., by either free-choice drinking or forced consumption in liquid diets). The data would suggest that discrepant reports in the literature concerning the effects of chronic ethanol on CNS monoamine levels did not stem from the above variables, but may have arisen from methodological and procedural differences such as the species and strain of the animals employed, the methods of handling the animals, and the killing and processing of tissue for analysis. Our studies with P rats indicate that the effects of chronic ethanol administration and perhaps neurochemical correlates of alcohol-drinking behavior cannot be discerned with experiments measuring endogenous levels of the monoamines and their metabolites.

The findings of this study do not rule out, however, the possibility that chronic alcohol exposure may alter functional aspects of some monoamine systems that are not reflected in changes of steady-state contents. In fact, there is a body of literature indicating a variety of alterations in neurotransmitter turnover and release after chronic ethanol administration [11, 12, 19, 25, 30]. Some of the most consistently observed changes have been in NE metabolism. Since NE metabolites were not detectable with the assay procedure used in the present study, a selective change in the metabolism of this monoamine could not be determined.

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TABLE 5

EFFECTS OF CHRONIC FORCED ALCOHOL CONSUMPTION AND LIQUID DIETS FOR SEVEN WEEKS ON THE LEVELS OF NE, DA, DOPAC, HVA, 5-HT AND 5-HIAA IN EIGHT CNS REGIONS OF ALCOHOL-PREFERRING P RATS

	nmole/g tissue (mean ± S.E.M.)					
Region	NE	DA	DOPAC	HVA	5-HT	5-HIAA
Cerebral Cortex						
Control	1.37 ± 0.07	5.87 ± 0.32	N.D.	0.30 ± 0.04	4.64 ± 0.08	2.52 ± 0.04
LD	1.46 ± 0.13	5.72 ± 0.29	—	0.30 ± 0.04	4.70 ± 0.14	2.48 ± 0.11
LD-E	1.28 ± 0.09	5.67 ± 0.36	—	0.28 ± 0.03	4.45 ± 0.13	2.44 ± 0.16
LDS	1.44 ± 0.08	6.62 ± 0.50	—	0.33 ± 0.02	4.73 ± 0.15	2.75 ± 0.10
LDS-E	1.38 ± 0.10	5.48 ± 0.36	—	0.26 ± 0.01*	4.48 ± 0.07	2.25 ± 0.08*
Striatum						
Control	0.23 ± 0.09	55.61 ± 4.24	3.87 ± 0.35	2.85 ± 0.23	2.57 ± 0.11	2.20 ± 0.11
LD	0.27 ± 0.06	57.19 ± 4.29	4.29 ± 0.31	3.06 ± 0.17	2.56 ± 0.15	2.18 ± 0.08
LD-E	0.23 ± 0.07	55.68 ± 6.18	3.87 ± 0.53	3.05 ± 0.30	2.76 ± 0.15	2.16 ± 0.15
LDS	0.24 ± 0.06	62.42 ± 5.35	4.59 ± 0.50	3.57 ± 0.34	2.70 ± 0.14	2.35 ± 0.05
LDS-E	0.27 ± 0.06	56.35 ± 5.04	4.07 ± 0.35	3.01 ± 0.27	2.77 ± 0.10	2.16 ± 0.10
Hypothalamus						
Control	9.74 ± 0.47	2.25 ± 0.10	N.D.	N.D.	5.93 ± 0.22	2.62 ± 0.15
LD	10.40 ± 0.48	2.03 ± 0.15	—	—	5.55 ± 0.29	2.38 ± 0.15
LD-E	10.88 ± 0.23	2.17 ± 0.08	—	—	6.03 ± 0.32	2.56 ± 0.34
LDS	10.49 ± 0.43	2.32 ± 0.10	—	—	5.84 ± 0.16	2.75 ± 0.16
LDS-E	11.62 ± 0.51	2.40 ± 0.25	—	—	6.15 ± 0.15	2.72 ± 0.41
Thalamus						
Control	2.48 ± 0.14	1.61 ± 0.45	0.21 ± 0.02	N.D.	5.12 ± 0.22	3.46 ± 0.14
LD	2.51 ± 0.11	1.24 ± 0.10	0.16 ± 0.03	—	4.80 ± 0.20	3.21 ± 0.12
LD-E	2.68 ± 0.15	1.59 ± 0.31	0.22 ± 0.02	—	4.79 ± 0.22	3.23 ± 0.21
LDS	2.67 ± 0.12	1.07 ± 0.26	0.17 ± 0.04	—	4.88 ± 0.32	3.36 ± 0.11
LDS-E	2.58 ± 0.05	1.66 ± 0.26	0.25 ± 0.05	—	5.07 ± 0.18	3.16 ± 0.08
Hippocampus						
Control	1.22 ± 0.04	N.D.	N.D.	N.D.	2.16 ± 0.12	1.53 ± 0.10
LD	1.21 ± 0.03	—	—	—	2.18 ± 0.14	1.56 ± 0.07
LD-E	1.14 ± 0.08	—	—	—	2.36 ± 0.10	1.48 ± 0.08
LDS	1.21 ± 0.07	—	—	—	2.36 ± 0.12	1.64 ± 0.07
LDS-E	1.12 ± 0.10	—	—	—	2.13 ± 0.16	1.46 ± 0.13
Midbrain						
Control	2.99 ± 0.12	1.15 ± 0.09	0.20 ± 0.02	N.D.	5.43 ± 0.23	4.50 ± 0.14
LD	3.15 ± 0.16	1.25 ± 0.06	0.19 ± 0.02	—	5.23 ± 0.20	4.04 ± 0.17
LD-E	3.37 ± 0.08	1.33 ± 0.06	0.24 ± 0.01	—	5.76 ± 0.21	4.25 ± 0.20
LDS	3.15 ± 0.13	1.29 ± 0.06	0.23 ± 0.02	—	5.69 ± 0.25	4.54 ± 0.13
LDS-E	3.25 ± 0.07	1.31 ± 0.14	0.25 ± 0.03	—	5.93 ± 0.49	4.40 ± 0.22
Pons-Medulla						
Control	2.60 ± 0.16	0.38 ± 0.06	N.D.	N.D.	4.23 ± 0.13	2.56 ± 0.08
LD	2.99 ± 0.11	0.41 ± 0.05	—	—	4.47 ± 0.16	2.63 ± 0.15
LD-E	3.08 ± 0.16	0.48 ± 0.07	—	—	4.41 ± 0.09	2.52 ± 0.10
LDS	2.81 ± 0.14	0.46 ± 0.06	—	—	4.43 ± 0.09	2.56 ± 0.07
LDS-E	3.40 ± 0.15*	0.50 ± 0.06	—	—	4.50 ± 0.14	2.50 ± 0.15
Cerebellum						
Control	1.28 ± 0.23	N.D.	N.D.	0.19 ± 0.02	0.57 ± 0.08	0.38 ± 0.02
LD	1.33 ± 0.36	—	—	0.18 ± 0.02	0.59 ± 0.04	0.35 ± 0.01
LD-E	1.04 ± 0.32	—	—	0.22 ± 0.02	0.71 ± 0.05	0.38 ± 0.02
LDS	1.14 ± 0.27	—	—	0.18 ± 0.03	0.66 ± 0.04	0.40 ± 0.02
LDS-E	0.68 ± 0.11	—	—	0.17 ± 0.03	0.68 ± 0.01	0.39 ± 0.01

There were 6 animals in each of the groups except for the LD-E group which had 5 animals.
*$p<0.05$ by t-test for LDS vs. LDS-E.
N.D. indicates that the compound was not detected with the instrument sensitivity and HPLC conditions used.

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