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Neurochemical and Operant Behavioral Studies of a Strain of Alcohol-Preferring Rats[1]

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PENN, P. E., W. J. MCBRIDE, L. LUMENG, T. M. GAFF AND T.-K. LI. *Neurochemical and operant behavioral studies of a strain of alcohol-preferring rats.* PHARMAC. BIOCHEM. BEHAV. 8(4) 475–481, 1978. – The levels of serotonin (5-HT), 5-hydroxyindoleacetic acid, tyrosine (TYR), norepinephrine (NE), acetylcholine, GABA, glutamate (GLU), aspartate (ASP), alanine, glycine (GLY) and taurine were measured in the CNS of adult male rats selectively inbred to the F8 generation for alcohol preference (P) and non-preference (NP). With respect to the values found in the NP group, higher levels of 5-HT, GABA, GLU and GLY and lower levels of ASP were found in the diencephalon-mesencephalon (D-M) and higher levels of NE were found in the telencephalon (TEL) of the P group. The animals in the P and NP strains were further subdivided into two additional groups, one given only H_2O (W) and the second given 10% ethanol (A) during a one week period, thereby producing four groups (NP-W, NP-A, P-W and P-A). With these conditions, the level of (a) TYR in the D-M was higher in the P-A and NP-A animals than in the P-W and NP-W groups, respectively; (b) 5-HT in the TEL was higher in the NP-A group than NP-W group; and (c) GABA in the TEL was higher in the P-A than P-W animals. No differences were observed in the cerebellum between the two strains or between the subgroups within each strain. The present study also demonstrated that the P animals will work in an operant situation to obtain 10% ethanol, even when H_2O is freely available, and will voluntarily bar-press up to 6–7 times for each ethanol reinforcement.

Alcohol-preferring rats Serotonin Tyrosine Norepinephrine Acetylcholine GABA Glutamate Asparate Glycine Taurine Operant behavior Ethanol

THE ELUCIDATION of the biochemical determinants of alcohol-drinking behavior is a major objective of research on alcoholism. Direct investigations in humans are difficult because of the complex interaction between biological and environmental factors. An understanding of these processes may be gained through studies with animal models, which have been undertaken largely with rodents. However, rodents typically show an aversion to alcohol in concentrations greater than 5–7% [21], and the consumption of large quantities of alcohol must be induced experimentally by techniques such as stress avoidance, nutritional deprivation, psychogenic polydipsia or with the use of sweetners [9, 17, 33]. Induced drinking behavior is not ideal since alcohol consumption is volitional in man and, furthermore, most of these procedures are stressful to some degree. Therefore, studies with animals which exhibit innate preference for alcohol offer a more promising approach toward understanding the biochemical correlates of alcohol drinking behavior. In fact, voluntary drinking has been considered by some investigators to be a necessary criterion for animal models of alcoholism [17].

Work to date on natural alcohol preference and aversion has been done principally with the C57BL and DBA strains of mice [11, 13, 23, 28, 29] and, in Finland, with the ALKO, Alcohol (AA) and ALKO, Non-Alcohol (ANA) strains of rats [1, 2, 7, 8]. Recently, two new strains of rats have been selectively bred in our laboratory, one with a natural preference for alcohol and the other with an aversion to drinking alcohol [18,20]. It is important to compare the behavioral and biochemical characteristics of these new strains with those of animals derived from different stocks and by different selection criteria in order to discern mechanistic factors from coincidental correlates. This is particularly pertinent since studies of the neurochemical effects of alcohol in rodents not behaviorally selected for alcohol preference or nonpreference have yielded inconsistent results (for reviews see [6, 10, 14, 16]).

The objectives of this initial study were to determine: (a) if there are differences in the steady-state levels of certain neurotransmitters in the CNS between the alcohol-preferring and the nonpreferring rats; (b) if consumption of alcohol has any effect on the steady-state levels of these neurotransmitters in the CNS of the two groups of animals; and (c) if the animals preferring alcohol would work in an operant situation to obtain alcohol.

EXPERIMENTAL PROCEDURES

At 4 to 6 weeks of age, male rats of the preferring (P) and nonpreferring (NP) strains selectively inbred to the F8 generation [18,20] were housed in separate cages and

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TABLE 1

LEVELS OF 5-HT, 5-HIAA, TYROSINE, NE, ACETYLCHOLINE, GLUTAMATE, ASPARTATE, GABA, GLYCINE, ALANINE AND TAURINE IN THE DIENCEPHALON-MESENCEPHALON OF NONPREFERRERS AND PREFERRERS DRINKING WATER OR 10% ETHANOL

Compound	Nonpreferrers		Preferrers	
	Water	10% Ethanol	Water	10% Ethanol
	nmoles/g tissue wet wt.			
5-HT	3.90 ± 0.20 (11)	4.11 ± 0.14 (9)	4.06 ± 0.43 (5)	4.89 ± 0.29 (7)†‡
5-HIAA	2.52 ± 0.25 (12)	2.56 ± 0.23 (10)	3.06 ± 0.48 (5)	2.91 ± 0.11 (12)
Tyrosine	51.5 ± 1.7 (14)	62.6 ± 3.3 (13)*	53.2 ± 1.5 (5)	64.7 ± 3.2 (10)*†
NE	3.20 ± 0.38 (7)	2.94 ± 0.27 (6)	3.10 ± 0.23 (6)	3.13 ± 0.15 (8)
Acetylcholine	31.4 ± 0.6 (10)	32.8 ± 0.8 (8)	31.6 ± 0.6 (6)	33.1 ± 0.6 (10)†
	μmoles/g tissue wet wt.			
Glutamate	11.0 ± 0.7 (11)	11.9 ± 0.4 (8)	13.0 ± 0.8 (5)	13.0 ± 0.6 (11)†
Aspartate	2.56 ± 0.16 (10)	2.69 ± 0.14 (8)	1.98 ± 0.28 (5)†‡	2.30 ± 0.10 (11)‡
GABA	2.63 ± 0.15 (11)	2.86 ± 0.15 (8)	3.42 ± 0.26 (5)†‡	3.43 ± 0.17 (11)†‡
Glycine	1.32 ± 0.13 (11)	1.35 ± 0.09 (8)	2.47 ± 0.29 (5)†‡	1.89 ± 0.24(6)†‡
Alanine	0.204 ± 0.016(11)	0.235 ± 0.020(7)	0.250 ± 0.029(4)	0.345 ± 0.033(11)†‡
Taurine	3.63 ± 0.30 (6)	3.71 ± 0.30 (5)	§	§

Data represent the means ± SEM of number of determinations given in parentheses. Statistically significant differences ($p<0.05$) are indicated as follows: (a) *NP-A vs NP-W and P-A vs P-W; (b) †P-W vs NP-W and P-A vs NP-W; and (c) ‡P-W vs NP-A and P-A vs NP-A. NP are nonpreferrers, P are preferrers, W refers to drinking water for one week, and A refers to drinking 10% ethanol. The Student *t* test was used to determine significant differences.

§Due to unforseen problems we were not able to obtain data for taurine in the P group.

tested for alcohol preference. Food was available ad lib and a 12 hr day–night cycle was maintained throughout the experiments. The animals were initially given a solution of 10% ethanol (v/v) as the sole drinking fluid for 4 days. After this period, they were given a choice between 10% ethanol (A) and distilled water (W) in a 2-bottle preference test. The positions of the two Richter tubes were randomly rotated. Ethanol and water intake was monitored for 2 to 3 weeks. The criteria of selection for the P strain were >5 g ethanol/kg body weight/day and an A/W drinking ratio of >2:1 (v/v). For the NP strain, the criteria were <1.5 g ethanol/kg/day and an A/W drinking ratio of <0.2:1 (v/v). The NP animals were used as the control group since the alcohol-drinking behavior of these animals is similar to that normally found for rodents [21].

Rats meeting the above criteria were randomly subdivided into two groups: one to receive 10% ethanol and the other water for one week, producing four groups NP-W, NP-A, P-W and P-A.

Neurochemical

After receiving 10% ethanol or water for 1 week, the rats were killed within the same time period each day (11:00 a.m. to 1:00 p.m.) by the near-freezing method [32]. The rats had been previously handled and adapted to the killing apparatus. Although the rats had usually stopped feeding before the day–night cycle had changed to light, food was removed from their cage approximately 5 hr before killing in order to minimize the effects of food consumption on amino acid levels at the time of killing. The brains were dissected at −4° C and the telencephalon, diencephalon plus mesencephalon, and cerebellum were stored at −70° C until assayed.

The levels of serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), tyrosine, norepinephrine (NE), acetylcholine (ACh), GABA, glutamate, aspartate, glycine and alanine were assayed by the procedures described by Smith *et al.* [30]. Taurine was determined by the procedure of Orr *et al.* [22].

Behavioral

Six pretested alcohol-preferring male rats were trained (initially using sweetened milk as the reward) to bar press on a continuous reinforcement (CRF) schedule. Food was available ad lib. Using a counterbalanced design, the animals were subsequently allowed to bar press for 10% ethanol or water. The fluid not in the dipper was available from a bottle. After 2 to 4 days, the positions of the two fluids were reversed; this rotation was repeated at least twice more. The number of operant responses made and the volumes of 10% ethanol and water consumed were recorded daily. Corrections were made for volume loss due to evaporation in the dipper reservoir. This amounted to 12 ml/day for water and 14 ml/day for 10% ethanol.

The response/reinforcement ratio was raised on four male alcohol-preferring rats which had been previously trained to bar press for 10% ethanol on CRF. Using a fixed-ratio (FR) schedule, the ratio was increased 1 or 2 every 1 to 4 days until water consumption significantly increased. Number of responses, reinforcements and volumes of water and 10% ethanol consumed were monitored and recorded daily.

RESULTS

The levels of 11 different compounds were measured in the diencephalon-mesencephalon (Table 1), telencephalon (Table 2) and cerebellum (Table 3) of the nonpreferrers on water (NP-W) or 10% ethanol (NP-A) and of the preferrers on water (P-W) or 10% ethanol (P-A). During this 1 week of

TABLE 2

LEVELS OF 5-HT, 5-HIAA, TYROSINE, NE, ACETYLCHOLINE, GLUTAMATE, ASPARTATE, GABA, GLYCINE, ALANINE AND TAURINE IN THE TELENCEPHALON OF NONPREFERRERS AND PREFERRERS DRINKING WATER OR 10% ETHANOL

Compound	Nonpreferrers		Preferrers	
	Water	10% Ethanol	Water	10% Ethanol
	nmoles/g tissue wet wt.			
5-HT	3.00 ± 0.06 (16)	3.27 ± 0.12 (11)*	2.88 ± 0.18 (7)	2.70 ± 0.06 (10)‡
5-HIAA	1.51 ± 0.08 (16)	1.67 ± 0.07 (11)	1.45 ± 0.10 (7)	1.53 ± 0.07 (12)
Tyrosine	58.7 ± 2.2 (13)	63.3 ± 2.5 (12)	54.7 ± 1.4 (5)	56.6 ± 2.3 (11)
NE	1.46 ± 0.07 (15)	1.57 ± 0.09 (12)	1.78 ± 0.24 (5)†	1.84 ± 0.08 (11)†‡
Acetylcholine	21.3 ± 1.1 (7)	22.6 ± 1.2 (5)	24.2 ± 1.1 (7)	22.2 ± 0.7 (5)
	μmoles/g tissue wet wt.			
Glutamate	13.8 ± 0.5 (6)	14.7 ± 0.6 (6)	14.0 ± 0.6 (7)	14.8 ± 0.4 (7)
Aspartate	2.85 ± 0.08 (14)	2.97 ± 0.05 (12)	2.78 ± 0.04 (6)‡	2.90 ± 0.04 (11)
GABA	1.58 ± 0.05 (15)	1.67 ± 0.05 (13)	1.43 ± 0.08 (7)‡	1.66 ± 0.04 (12)*
Glycine	0.807 ± 0.037(15)	0.827 ± 0.036(13)	0.772 ± 0.044(7)	0.853 ± 0.038(11)
Alanine	0.498 ± 0.022(15)	0.546 ± 0.020(13)	0.493 ± 0.034(7)	0.539 ± 0.019(11)
Taurine	7.59 ± 0.16 (13)	7.61 ± 0.32 (11)	7.35 ± 0.46 (5)	7.35 ± 0.28 (12)

Data represent the means ± SEM of number of determinations given in parentheses. Consult footnote of Table 1 for additional information.

TABLE 3

LEVELS OF GLUTAMATE, ASPARTATE, GABA,ALANINE AND TAURINE IN THE CEREBELLUM OF NONPREFERRERS AND PREFERRERS DRINKING WATER OR 10% ETHANOL

Compound	Nonpreferrers		Preferrers	
	Water	10% Ethanol	Water	10% Ethanol
	μmoles/g tissue wet wt.			
Glutamate	12.5 ± 0.6 (5)	13.8 ± 0.8 (5)	13.0 ± 0.6 (5)	13.6 ± 0.2 (6)†
Aspartate	2.52 ± 0.10 (7)	2.63 ± 0.05 (7)	2.74 ± 0.20 (6)	2.69 ± 0.06 (6)
GABA	1.01 ± 0.03 (7)	1.00 ± 0.05 (7)	0.911 ± 0.070(6)	1.01 ± 0.05 (7)
Alanine	0.347 ± 0.017(7)	0.364 ± 0.013(7)	0.369 ± 0.017(6)	0.373 ± 0.020(7)
Taurine	6.86 ± 0.29 (7)	6.47 ± 0.19 (7)	6.48 ± 0.28 (5)	6.21 ± 0.20 (7)

Data represent the means ± SEM for number of determinations given in parentheses. Consult footnote for Table 1 for additional information. Because of technical difficulties we were unable to measure glycine. Assays for 5-HT, 5-HIAA, tyrosine, NE and acetylcholine were not carried out with the cerebellum because of certain limitations existing in the laboratory at the time of initial tissue preparation.

a no choice situation, the mean daily fluid intakes were 27, 22, 26, and 28 ml for the NP-W, NP-A, P-W and P-A groups, respectively. The rats in the NP-A group consumed an average of 6.6 g ethanol/kg/d while those in the P-A group had a value of 7.4 g ethanol/kg/d.

In the diencephalon-mesencephalon of both the P and NP groups, the levels of tyrosine were approximately 20% greater when 10% ethanol was consumed than when H_2O was consumed (Table 1). In the telencephalon, the level of 5-HT was 10% higher in the NP-A group than in the NP-W animals and the content of GABA was 15% greater in the P-A group than in the P-W group (Table 2). Of the compounds measured in the cerebellum, none appeared to be altered by alcohol consumption (Table 3).

In the diencephalon-mesencephalon, the content of glycine and GABA was significantly higher and that of aspartate was lower in the P-W animals than in either the NP-W or NP-A group (Table 1). The levels of 5-HT, GABA, glycine and alanine were greater in the diencephalon-mesencephalon of the P-A group than in either the NP-W or NP-A group (Table 1). In the telencephalon, the content of NE was higher in the P-W and P-A group with respect to the NP-W animals; the level of NE in the P-A group was also greater than the value found for the NP-A animals.

If the data for the animals drinking water are combined with the data for animals drinking 10% ethanol (providing there was no statistically significant difference between the W and A groups) then it would appear that in the diencephalon-mesencephalon of the preferrers, the levels of 5-HT, GABA, glutamate and glycine were higher and the content of aspartate lower than that found for the nonpreferrers (Fig. 1). In the telencephalon, only the content of NE was different between the P and NP groups. In the cerebellum, no differences were observed.

Upon operant testing, the preferrers bar pressed at a significantly greater rate (mean value approximately 4 times

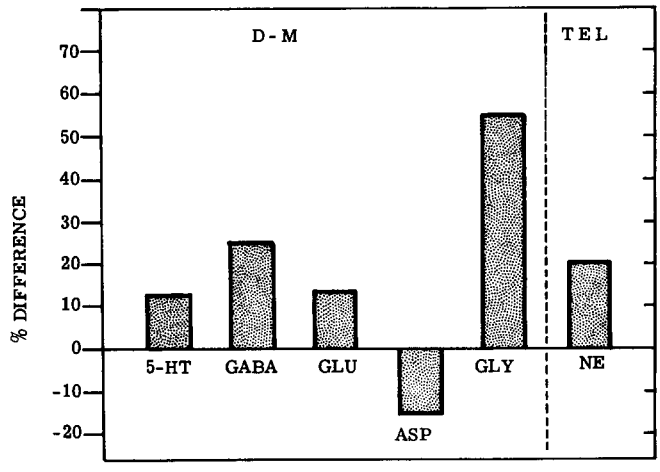

FIG. 1. Summary of significant differences ($p<0.05$) found in levels of several compounds in the diencephalon-mesencephalon (D-M) and telencephalon (TEL) of the preferrers (P) with respect to values found for the nonpreferrers (NP). Values were computed from data given in Tables 1–3.

higher) to obtain 10% ethanol than they did to obtain water (Figs. 2 and 3). The total daily fluid intake remained fairly constant regardless of which fluid was in the dipper (Fig. 3), although about half the animals would occasionally drink large amounts of alcohol on a given day (e.g., rat P-359 in Fig. 2). Alcohol and water consumption before and after this day was not abnormal. This occasional high increase in alcohol intake has also been observed for some animals in their home cages when drinking from Richter tubes in a free choice situation. The mean volume ratio of 10% ethanol: water consumed by the 6 animals during the days when ethanol was in the dipper was 15.2:1 and when water was in the dipper the value was 6.2:1. The mean value for all days during operant testing was 10.7:1 and was greater than that obtained during routine testing for preference with Richter tubes (4.7:1). The mean amount of ethanol consumed under these conditions was 7.5 g ethanol/kg/d for all days. The solution first offered from the dipper did not appear to alter the behavioral response (Fig. 2).

It was possible to increase the number of required responses to 6 or 7 before a reinforcement of 10% ethanol was given (Fig. 4). Even when the animals had to respond on a fixed-ratio schedule (FR 6 or 7) to obtain 10% ethanol, the ethanol: water ratios remained high (6.5:1). The mean amount of ethanol consumed under these conditions was 7.1 g ethanol/kg/d.

DISCUSSION

These preliminary attempts to characterize neurochemically and behaviorally the alcohol-preferring strain of rats have yielded interesting findings. Higher levels of 5-HT, GABA, glutamate and glycine and lower levels of aspartate were found in the diencephalon-mesencephalon of the P group as compared with the NP group (Fig. 1). The latter three amino acids, as well as 5-HT and GABA, are considered to be neurotransmitters in certain regions of the CNS (for a review of amino acid neurotransmitters see [5]). In addition, significantly higher levels of NE were found in the telencephalon of the P group (Fig. 1). The differences in the levels of GABA, glutamate, aspartate, glycine and NE between the P and NP groups may be due to general metabolic effects, differences in functional activity of specific neuronal pathways, and/or differences in the relative proportion of different populations of neurons. The fact that these changes were not observed in all regions of the CNS could be taken as evidence against a general metabolic effect. However, it is not possible, at this time, to state with certainty whether the differences in the levels of GABA, glutamate, aspartate, glycine and NE are innate or are due to the prior exposure to ethanol during the 2–3 weeks of free choice testing for preference, during which time the P animals were consuming larger quantities of ethanol than the NP animals.

Comparison of the data for 5-HT, GABA, glutamate, aspartate and glycine in the diencephalon-mesencephalon (Table 1) and for NE in the telencephalon (Table 2) of the P and NP groups with data previously published by our laboratory [30] for common stock male Wistar rats shows the following differences: (a) the value for 5-HT is 30% higher in the P strain; (b) the content of GABA is 15% lower in the NP group; (c) the level of aspartate is 20% lower in the P strain; (d) the level of glycine is 15% lower in the NP animals and 30% higher in the P group; and (e) the level of NE is 30% lower in the NP strain. In the case of glutamate, the mean value found for the common stock fell between the values obtained for the P and NP strains and it was not markedly different from either. Therefore, on this basis, it would appear that the best neurochemical correlates of alcohol-drinking behavior in the P strain may be the high levels of 5-HT and glycine and low levels of aspartate in the diencephalon-mesencephalon. However, because of the limitations of the experiments and the complex nature of behavior, it is not possible, at this early stage, to make definitive statements regarding neurochemical correlates of alcohol-drinking behavior in rodents.

Our data demonstrating a higher level of 5-HT in the diencephalon-mesencephalon of the P group is in agreement with the data reported by Ahtee and Eriksson [2] for their alcohol-preferring and nonpreferring strain of rats. Our data indicating that the levels of 5-HIAA were the same in the CNS of both strains is also in agreement with the data of Ahtee and Eriksson [2]. The finding that the steady state level of 5-HT was greater in the preferring strain than in the nonpreferring strain in the two separate studies lends support to the idea that certain serotonergic pathways may be involved in this behavior in rats [1]. On the other hand, Pickett and Collins [23] and Ho *et al.* [13] found no difference in the brain 5-HT levels of the C57BL (preferrers) and DBA (nonpreferrers) strains of mice. However, these investigators measured whole brain samples, a procedure which may have obscured any differences that may exist in specific regions of the CNS.

The levels of GABA, glutamate, glycine and aspartate in the diencephalon-mesencephalon and NE in the telencephalon have not been reported for the Finnish strains of rats. However, Ahtee and Eriksson [3] did measure the levels of NE in whole brain samples of their alcohol preferring and nonpreferring strains of rats and found no difference between the two groups. Kiianmaa *et al.* [15] found that destruction of the coerulo-cortical NE pathway by direct injections of 6-hydroxydopamine increased ethanol drinking in the alcohol preferring strain of rats, suggesting a possible involvement of NE neurons in alcohol drinking behavior of rodents. On the other hand, Ho *et al.* [13] found no difference in the whole brain content of NE, and

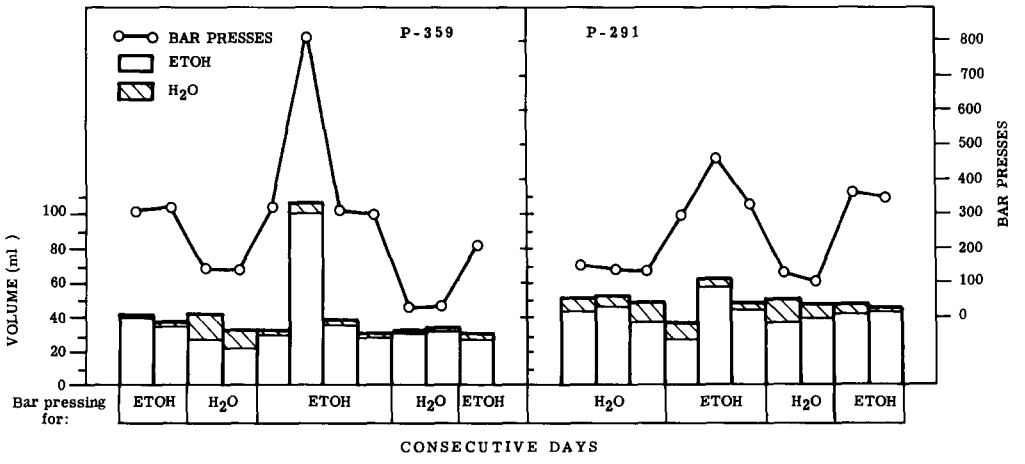

FIG. 2. Representative daily records of two animals (Preferrers) on continuous reinforcement (CRF) schedules. Each point represents data accumulated during one 24 hr period. The animals had to alternately bar press for 10% ethanol (ETOH) or H_2O. The solution not in the dipper reservoir was freely accessible in a drinking bottle.

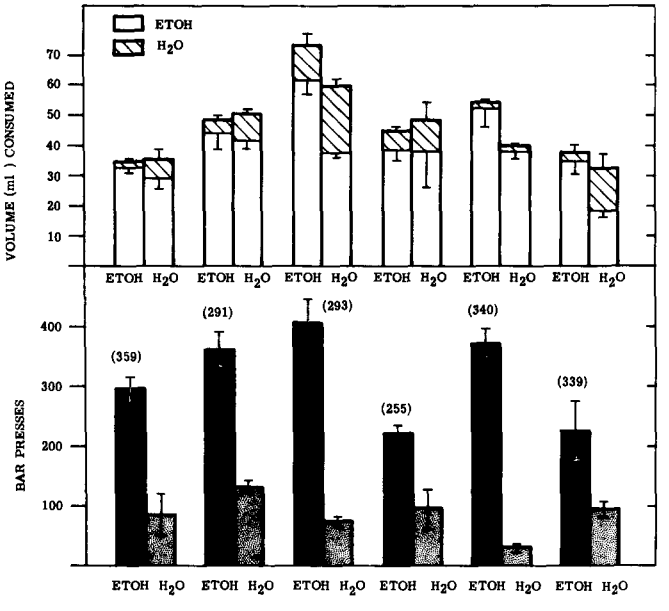

FIG. 3. Mean response rates and mean volumes of 10% ethanol and water consumed per 24 hr period over a 10–12 day span by preferrers on a CRF schedule in which the animals had to bar press for ethanol or H_2O (see Fig. 2).

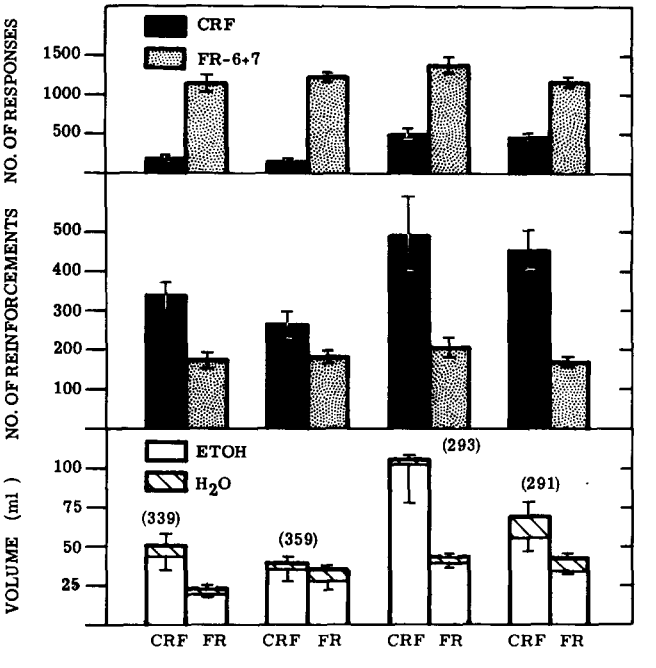

FIG. 4. Comparison of mean response rates and volumes of H_2O and 10% ethanol consumed during a 24 hr period by preferrers bar pressing for 10% ethanol on continuous reinforcement (CRF) and fixed-ratio (FR) schedules of reinforcement.

Chan [4] reported no difference in the levels of GABA in the cerebrum, cerebellum, thalamus-hypothalamus and medulla between the C57BL and DBA strains of mice.

Ho *et al.* [13] have suggested a direct involvement of central cholinergic mechanisms in alcohol preference. They found higher levels of ACh in whole brain of the C57BL preferring strains of mice in comparison to the DBA nonpreferring strain. In the present study, no difference in the content of ACh was found in 2 brain regions between the P and NP animals. This apparent disagreement might be a result of a combination of factors, such as species differences and methodological approaches. For example, the apparent lower content of ACh found in the DBA strain may be due to its higher acetylcholinesterase activity in the CNS [13]. In the experiments of Ho *et al.* [13], the animals were killed at room temperature, instead of by freezing in liquid N_2 or by microwave fixation which minimizes the postmortem breakdown of ACh by acetylcholinesterase [27,32].

Under the experimental conditions of a no choice situation for one week on either H_2O or 10% ethanol: (a) the levels of tyrosine in the diencephalon-mesencephalon were higher in the NP-A and P-A groups than in the NP-W and P-W groups (Table 1); (b) the levels of 5-HT in the telencephalon were higher in the NP-A group than in the NP-W animals (Table 2); and (c) the levels of GABA in the telencephalon were higher in the P-A group than in the P-W group (Table 2). In this regard, Ahtee and Eriksson

[1,2] did not find a change in 5-HT levels in whole brain in their nonpreferring strain following one month of forced ethanol administration. Pohorecky *et al.* [25], however, did find a 20% increase in 5-HT levels in several brain parts of rats that were not selected for ethanol preference but were given ethanol in their diet over a 16-day period.

The difference in the content of GABA between the P-A and P-W groups (Table 2) may be a result of removal of alcohol from the P-W group or, alternatively, that the content of GABA is effected more by alcohol consumption in the P group than in the NP group. These data are consistent with the findings of Chan [4], who reported that IP injections of ethanol slightly raised GABA levels in the CNS of the C57BL as well as in the DBA strains of mice. In addition, Rawat [26] reported that both acute and chronic administration of ethanol in the diet increased GABA levels in the whole brain of mice and that GABA levels returned to normal within 2 days after withdrawal. On the other hand, Littleton [19] found a decrease in GABA levels in whole brain of mice forced to inhale ethanol vapors.

Alcohol seemed to increase the levels of tyrosine in the diencephalon-mesencephalon of both the P and NP strains (Table 1); this effect was not found in the telencephalon (Table 2). Pohorecky [24] did not find any change in the levels of tyrosine in the CNS of rats which had alcohol in their diet for 2 weeks. However, Littleton [19] found an increase in tyrosine levels in mouse brain following chronic administration of alcohol. Tyrosine is the blood precursor of dopamine and NE, as well as being used for protein synthesis. Its higher levels in the NP-A and P-A groups may reflect greater uptake or decreased utilization for catecholamine and/or protein synthesis. Additional experiments are required to resolve this question.

The literature concerning the neurochemical effect of alcohol intake is inconsistent for every compound studied (for reviews see [6, 10, 14, 16]). These apparent contradictory results are most likely a result of a combination of factors such as, the route, dosage and duration of ethanol administration, the brain regions studied, the effects of stress, species differences, etc. Therefore, comparison of the results of other investigators with those obtained in the present study, which were done under experimental conditions different from those of others and with a unique strain of rats, may not necessarily be valid. However, the finding of a possible involvement of 5-HT pathways independently reported by two separate laboratories working with different strains of alcohol-preferring rats gives great encouragement to future studies concerned with determining neurochemical correlates of alcohol-drinking behavior.

The results of the present study also indicate that the alcohol-preferring rats will work in an operant situation to obtain 10% ethanol when water is freely available from a bottle (Figs. 2–4). It had been previously reported that male Sprague-Dawley rats, which were not genetically selected for alcohol preference, could be trained to bar press for 8% ethanol if motivated by food deprivation [12] and could be trained to bar press for intragastric or intravenous ethanol [31]. The fact that the preferrers will, in a free choice situation, voluntarily bar press up to 6 or 7 times for one ethanol reinforcement is taken as evidence for a strong consumatory drive for ethanol. Furthermore, it suggests that perhaps olfactory cues may not be playing a large determining role in alcohol drinking behavior of the preferrers, since such cues are not present at the lever.

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