Pharmacology Biochemistry & Behavior, Vol. 27, pp. 317–322. © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

Reexamination of the Relationship Between Alcohol Preference and Brain Monoamines in Inbred Strains of Mice Including Senescence-Accelerated Mice

KANJI YOSHIMOTO AND SETSUO KOMURA

Department of Legal Medicine, Kyoto Prefectural University of Medicine
Kawaramachi, Kamigyo-ku, Kyoto 602, Japan

Received 1 October 1986

YOSHIMOTO, K. AND S. KOMURA. *Reexamination of the relationship between alcohol preference and brain monoamines in inbred strains of mice including senescence-accelerated mice.* PHARMACOL BIOCHEM BEHAV 27(2) 317–322, 1987.—The relationship between voluntary alcohol consumption and brain monoamine levels was studied in the inbred strains of C57BL/6N, C57BL/6J, A/J, BALB/cA, CBA/N, C3H/He and DBA/2cr mice; the congeneric mouse strain, B10.Br/Sg, and the senescence accelerated mouse (SAM P1, SAM P2). The C57BL strains exhibited a high alcohol preference whereas the other strains exhibited a low alcohol preference. A clear positive relationship was found between alcohol intake (g/kg/day) and brain norepinephrine level ($r=0.683$, $p<0.05$), and a clear negative relationship between alcohol intake and brain serotonin level ($r=-0.628$, $p<0.05$). The content of brain dopamine was not clearly correlated with alcohol intake ($r=-0.206$, $p>0.05$). These findings suggest that in mice voluntary alcohol preference is influenced by brain norepinephrine and serotonin levels genetically.

Alcohol preference Norepinephrine Dopamine Serotonin Inbred strains of mice Senescence-accelerated mice

SEVERAL strains of rats and mice are known to exhibit different degrees of alcohol preference. McClearn *et al.* [15] found that inbred mouse strains had markedly different levels of voluntary alcohol consumption. They noted that the C57BL strain has a high preference for alcohol, and other strains, such as the C3H or DBA, show intermediate or low degrees of preference when given a choice between alcohol and water.

The strain differences in alcohol preference have been assumed to be determined by metabolic differences in enzyme activites. In inbred mice and rats, high liver alcohol dehydrogenase activity has been reported to be related to high voluntary alcohol consumption [10,21]. We also found C57BL mice to have significantly higher liver alcohol dehydrogenase activity than DBA/2 mice and found a clear positive relationship between alcohol preference and alcohol dehydrogenase activity [27].

Some experimental findings suggest that various neurotransmitters, e.g., norepinephrine (NE), dopamine (DA) and serotonin (5-HT), may be related to alcohol-drinking behavior and alcohol preference [1,7]. We previously reported a clear positive relationship between alcohol preference and brain NE and a clear negative relationship between alcohol preference and 5-HT or DA levels in five inbred strains of mice [26]. In contrast, Ahtee *et al.* [1] reported that the 5-HT level in the brain of an alcohol-preferring line was slightly higher than that in the alcohol-nonpreferring line. However, a clear role for neurotransmitters in the explanation of alcohol preference has not yet been found.

We examined the relationship between alcohol preference and the contents of NE, DA and 5-HT assayed simultaneously in many inbred strains of mice, including a congeneric mouse strain (B10.Br/Sg) and senescence accelerated mouse strains (SAM P1, SAM P2).

METHOD

Animals

Male mice from the following strains, maintained by brother-sister breeding, were used: C57BL/6N, A/J, AKR/J, BALB/cA, B10.Br/Sg, CBA/N, C3H/He and DBA/2cr (Japan CLEA Co., Osaka, Japan), C57BL/6J (Kyoto Prefectural University of Medicine, Department of Legal Medicine, Kyoto, Japan) and Senescence Accelerated Mouse (SAM P1, SAM P2) (Kyoto University, Faculty of Medicine, Department of Pathology, Chest Disease Research Institute, Kyoto, Japan). The animals were individually housed; food (CE-2, Japan CLEA, Osaka, Ja-

TABLE 1

VOLUNTARY ALCOHOL DRINKING BEHAVIOR OF INBRED MOUSE STRAINS AND THE SENESCENCE ACCELERATED MOUSE

Mouse Strain	n	Average Body Weight (g)	Water (ml/day)	Ethanol Consumption (ml/day, 10%)	Ethanol Consumption (g/kg/day)	Alcohol Preference (% total fluids)
C57BL/6N	15	24.7 ± 0.8[a]	3.6 ± 0.3[a]	1.4 ± 0.3[a]	4.6 ± 0.8[a]	29.4 ± 5.2[a]
C57BL/6J	10	22.5 ± 0.9[b]	3.0 ± 0.4[a]	0.9 ± 0.1[b]	6.0 ± 0.3[b]	39.6 ± 5.4[b]
A/J	6	26.0 ± 1.2[a,d,e]	6.2 ± 0.6[b,c]	0.3 ± 0.1[c]	0.9 ± 0.3[c]	4.0 ± 1.1[c]
AKR/J	6	31.1 ± 2.0[c]	7.3 ± 0.5[c]	0.3 ± 0.1[c]	0.8 ± 0.2[c]	4.2 ± 1.3[c]
BALB/cA	6	30.9 ± 2.1[c]	6.5 ± 0.8[b,c]	0.3 ± 0.1[c]	0.7 ± 0.2[c]	3.3 ± 0.5[c]
B10.Br/Sg	6	26.9 ± 1.0[d,e]	3.7 ± 1.3[a]	0.9 ± 0.8[d]	2.6 ± 2.3[d]	22.0 ± 19.8[d]
CBA/N	6	25.8 ± 1.0[a,d,e]	5.4 ± 0.5[b]	0.3 ± 0.1[c]	0.9 ± 0.3[c]	4.9 ± 1.2[c]
C3H/He	6	25.1 ± 1.2[a,d]	6.2 ± 0.5[b,c]	0.3 ± 0.1[c]	0.9 ± 0.1[c]	5.0 ± 1.0[c]
DBA/2cr	6	26.0 ± 1.4[a,d,e]	6.1 ± 0.5[b,c]	0.2 ± 0.1[c]	0.7 ± 0.1[c]	3.7 ± 0.8[c]
SAM P1	6	22.9 ± 3.0[b]	8.8 ± 2.0[d]	0.4 ± 0.2[c]	1.0 ± 0.3[c]	5.0 ± 3.1[c]
SAM P2	6	27.3 ± 1.7[e]	11.8 ± 2.0[e]	0.3 ± 0.1[c]	0.9 ± 0.2[c]	2.7 ± 0.7[c]

Values expressed as mean ± SD. After a significant strain difference was noted by ANOVA, differences in individual mouse strain means were assessed using Duncan's multiple range test. In each column, values with the same letter are not significantly different at the $p<0.05$ level.

pan) and water were provided ad lib except during the period of acclimation to 10% (v/v) ethanol and a normal 12-hr light/12-hr dark cycle was maintained beginning at 08:00. All animals were 40–42 days old exactly when alcohol drinking behavior testing was begun. Maintenance on 10% (v/v) ethanol as the only fluid was performed for the first 5 days, the acclimation period, before alcohol preference testing was done.

Alcohol Drinking Behavior Test

The mice were given 5 days of exposure to 10% ethanol contained in a measured glass bottle as the sole source of fluid. Thereafter, the animals were given access to both 10% (v/v) ethanol and tap water in measured glass bottles (20 ml) for 4 weeks, the locations of the bottle being changed daily on a random basis. The daily intakes of water and 10% ethanol during the 4 weeks of testing were measured to evaluate alcohol preference by means of an ethanol consumption score (g/kg of body weight/day) for each animal.

The baseline alcohol preferences of all mice were measured individually before the analysis of the brain monoamines. Alcohol preference was defined as the alcohol intake expressed as percentage of the total fluid intake of each mouse individually.

Brain Monoamine Analysis

Two days after the alcohol preference experiments, all mice were killed within the same time period each day (0900–1000 hr) by cervical dislocation, performed quickly in order to minimize stress. The mouse brain was quickly removed and placed on a cold paraffin plate. The mouse cerebrum was dissected and then transported in liquid nitrogen for weighing.

All experiments were performed using a high performance liquid chromatograph (HPLC) (a model L-4000S, Yanagimoto Mfg. Co., Ltd., Japan) with an electrochemical detector (VMD-101A, Yanagimoto Mfg. Co., Ltd., Japan). The electrode potential was set at 0.8 V for the catecholamines (vs. Ag/AgCl reference electrode). The column used was a TSK gel ODS-120T C18 reversed-phase column (5 μm particle size, 250×4.6 mm i.d., Toyo Soda MFG. Co., Ltd., Yamaguchi, Japan). To protect the column, a Shodex DT filter (ED-03, Showa Denko, K.K., Japan) was used before performance of HPLC.

The mobile phase was prepared with a 0.1 M K-phosphate buffer, (potassium dihydrogen phosphate, KH2PO4), adjusted precisely to pH 3.1 with phosphoric acid, containing 1% acetonitrile and 372 mg ethylenediaminetetra-acetic acid disodium salt (EDTA-2Na) per liter of phosphate buffer.

The brain tissue was assayed for the content of norepinephrine (NE), dopamine (DA) and serotonin (5-HT) by HPLC with an electrochemical detector. These samples were prepared using the method of Sasa and Blank [20] with modifications by us [25]. The compounds were extracted from the frozen tissue by ultrasonic homogenization containing 500 μl 0.025 N HCl, 0.1 M EDTA-2Na 100 μl and 100 ng dihydroxybenzylamine (DHBA) as an internal standard. The concentration of each of the compounds in the brain tissue was calculated from the chromatographic peak heights and recorded by a recorder (Model R-111, Shimadzu Co., Ltd., Japan). Several standards containing equal amounts of NE, DA, 5-HT and DHBA were analyzed in parallel. These standards were used to calculate the calibration factor for each component. The contents of NE, DA and 5-HT were quantitated from the calibration factor.

Drugs

Norepinephrine hydrochloride, dopamine (3-hydroxytryptamine) hydrochloride, serotonin (5-hydroxytryptamine) creatinine sulfate and 3,4-dihydroxybenzylamine hydrochloride were purchased from Sigma, St. Louis. Other specially prepared reagents were purchased from Wako Chemical Co., Japan.

Data Analysis

Means and their standard deviations were determined.

TABLE 2

BRAIN MONOAMINES IN INBRED MOUSE STRAINS AND THE SENESCENCE ACCELERATED MOUSE (SAM)

Mouse Strain	n	NE (ng/g)	DA (ng/g)	5-HT (ng/g)
C57BL/6N	15	700.1 ± 39.4[a]	1614.1 ± 109.0[a,b]	494.0 ± 124.1[a]
C57BL/6J	10	696.3 ± 33.1[a]	1643.9 ± 103.8[b,d]	391.6 ± 31.9[b]
A/J	6	473.4 ± 25.2[b,g]	1372.5 ± 54.6[c]	524.1 ± 28.3[a]
AKR/J	6	471.4 ± 26.1[b,g]	1759.1 ± 61.8[d]	496.4 ± 10.7[a]
BALB/cA	6	537.1 ± 38.6[c,f]	1652.4 ± 51.7[b,d]	522.3 ± 78.0[a]
B10.Br/Sg	6	508.4 ± 71.4[c,g]	1507.7 ± 129.4[a,c]	396.4 ± 58.7[b]
CBA/N	6	539.0 ± 21.8[c,f]	1710.0 ± 84.9[d]	558.7 ± 49.3[a]
C3H/He	6	602.8 ± 45.0[d,e]	1917.8 ± 85.7[e]	666.2 ± 70.3[c]
DBA/2cr	6	635.1 ± 39.7[e]	1988.0 ± 201.0[e]	543.6 ± 54.3[a]
SAM P1	6	584.4 ± 18.8[d,f]	1474.9 ± 62.6[c]	478.4 ± 25.5[a,b]
SAM P2	6	461.1 ± 32.5[b]	1709.5 ± 107.8[d]	543.5 ± 113.4[a]

Values expressed as mean ± SD. NE=norepinephrine, DA=dopamine and 5-HT=serotonin. After a significant strain difference was found by ANOVA, differences in individual mouse strain means were assessed using Duncan's multiple range test. In each column, values with the same letter are not significantly different at the $p<0.05$ level.

Data were analyzed by a one-way analysis of variance (ANOVA) as appropriate [24]. Comparisons between individual strains of mice were made by Duncan's multiple range test [3]. Correlation coefficients between alcohol intake (g/kg/day) or alcohol preference (%) and brain monoamines were calculated by the method of least squares. Differences were assesed with Student's two-tailed t-test.

RESULTS

Table 1 presents the body weight, water intake, ethanol consumption and alcohol preference in all the strains of mice. The amounts of water (ml/day) and ethanol (ml/day and g/kg/day) shown are the means for the 4 week alcohol preference test period. The absolute alcohol intake (g/kg/day) was calculated from the daily consumption of 10% alcohol (ml).

Body Weight

Analyses of variance revealed a significant strain difference in weight gain, $F(10,72)=30.45$, $p<0.001$. Among all strains of mice, the AKR/J and BALB/cA mice had the maximum weight gain. Of the C57BL strains, C57BL/6N had a significantly larger weight gain (24.7 g) than the C57BL/6J strains (22.5 g) (Duncan's multiple range test, $p<0.05$). As compared with the AKR/J and SAM strains, SAM P1 and SAM P2 had a significantly smaller weight gain.

Water Intake

Analyses of variance of water intake revealed significant strain differences, $F(10,72)=87.77$, $p<0.001$. SAM P2 and C57BL/6J had the maximum and minimum water intakes, respectively. There were no significant differences among the C57BL strains, but analyses of variance revealed a significant difference among the AKR/J, SAM P1 and SAM P2 strains (Duncan's mutliple range test, $p<0.05$). The congeneric strain, B10.Br/Sg, exhibited a small water intake similar to that of the C57BL strains.

Voluntary Alcohol Consumption

Analyses of variance revealed significant and characteristic differences according to strain in the intake of the 10% alcohol solution in ml/day, the alcohol intake in g/kg/day, and the alcohol preference in %, ANOVA $F(10,72)=49.38$, $p<0.001$, ANOVA $F(10,72)=362.63$, $p<0.001$ and ANOVA $F(10,72)=71.03$, $p<0.001$, respectively.

The C57BL/6N and C57BL/6J strains exhibited moderate to high degrees of ethanol preference, with intake averaging 4.6–6.0 g of ethanol/kg/day. The C57BL/6J strain liked the alcohol solution the best, as measured both by the alcohol preference ratio and by daily alcohol intake. On the other hand, the BALB/cA strain liked alcohol the least. The range of intake for the BALB/cA strains was 0.31–1.04 g/kg/day.

The mean alcohol intake and alcohol preference for the B10.Br/Sg strains was roughly intermediate among the other inbred strains, but the largest variation was seen in this strain, with an intake of 0.71–5.62 g of alcohol/kg/day. The B10.Br/Sg mice may be useful in future selection studies for alcohol-drinking behavior.

Except for the B10.Br/Sg strain, the inbred strains of mice did not show any clear differences in alcohol intake. The C57BL strains had a high alcohol preference, whereas most of the other inbred strains of mice exhibited low alcohol preference or low voluntary ethanol consumption.

The body weight of the SAM P1 and SAM P2, the senescence-prone strains, was significantly different from the original AKR strain. However, there were no differences in alcohol consumption or alcohol preference. The factor accelerating senescence had no direct influence on the alcohol drinking behavior in the early stage of senescence.

Table 2 shows the levels of brain norepinephrine (NE), dopamine (DA) and serotonin (5-HT) in all the strains of mice. After the alcohol preference test, no gross behavioral alteration such as sedation or increased psychomotor activity was observed.

One-way ANOVA revealed significant and characteristic differences in the levels of NE, $F(10,72)=153.08$, $p<0.001$,

DA, $F(10,72)=3.93$, $p<0.001$, and 5-HT, $F(10,72)=6.31$, $p<0.001$. This indicates that brain monoamines, NE, DA and 5-HT, do vary significantly among the different strains of mice. The highest content of NE was found in the C57BL strains of mice. There were no differences in the levels of NE between C57BL/6N and C57BL/6J mice. As compared with AKR mice, SAM P1 mice revealed a significantly higher content of NE (Duncan's multiple range test, $p<0.05$).

The C3H/He and C57BL/6J mice had the highest and lowest contents of 5-HT, respectively. The B10 strains of mice also had a low content of 5-HT. These contents were significantly different from those of the C57BL/6N and C57BL/6J mice. There was no significant difference in the levels of 5-HT between AKR and SAM mice. However, the content of 5-HT in the C57BL/6J subline that originated from a homozygous Jackson Laboratory stock was significantly different from that of the C57BL/6N subline that originated from an NIH stock.

Among the SAM which had been obtained by continuous brother-sister breeding from original litters of AKR mice, SAM P1 had significantly different noradrenergic and dopaminergic systems, as compared to the AKR mice.

The correlation between alcohol intake (g/kg/day) and brain monoamines for all strains of mice calculated from the results shown in Table 1 and Table 2 were: NE, $r=0.683$, $p<0.05$; DA, $r=-0.206$, n.s.; 5-HT, $r=-0.628$, $p<0.05$. Furthermore, the correlations between alcohol preference (%) and these brain monoamines for all strains of mice were: NE, $r=0.651$, $p<0.05$; DA, $r=-0.220$, n.s.; 5-HT, $r=-0.678$, $p<0.05$.

This study, therefore, showed a clear positive relationship between alcohol intake and the levels of NE. In contrast, a clear negative relationship was found between alcohol intake and the levels of 5-HT. These data strongly suggest that static levels of brain NE and 5-HT directly influence alcohol preference in mice. This indicates that mouse alcohol preference is dependent upon genetic factors.

DISCUSSION

Genetically characteristic variations exist with respect to alcohol drinking behavior among rodents [5, 11, 26]. McClearn and Roger [15] noted that C57BL mice consumed 60–90% of their fluid intake as the 10% alcohol solution whereas the DBA and BALB mice drank only 5–10%. In our study using eleven strains, C57BL/6N, C57BL/6J, A/J, AKR/J, BALB/cA, B10.Br/Sg, CBA/He, DBA/2cr, SAM P1 and SAM P2, we also found that the C57BL/6N and C57BL/6J mice have a high alcohol preference and high voluntary alcohol intake, whereas the other strains exhibit a low alcohol preference.

Li and Lumeng [12] noted that the ACI/N animal has a low alcohol preference or low voluntary alcohol consumption, whereas the MR/N animals have a high alcohol preference among eight inbred strains of rats. In male MR/N strains, the average alcohol intake was 3.6 g/kg/day (g of ethanol/kg of body weight/day). In this study, the C57BL/6N and C57BL/6J mice had an even higher degree of alcohol preference, with an intake averaging 4.6 and 6.0 g of ethanol/kg/day, respectively. Selective breeding for divergent voluntary alcohol-drinking behavior in rats led to the production of alcohol-preferring and alcohol-nonpreferring strains. Those developed by Alko are called the AA and ANA strains, respectively [6,7], and those maintained at Indiana University School of Medicine are called the P and NP lines, respectively [12,14]. In the male AA and P lines, the average ethanol intake was 4.8 and 5.3 g/kg/day, respectively [12]. Furthermore, Li and Lumeng [12] noted that the range of average intake for the males was 0.2–3.6 g/kg/day and that that for the females was 0.4–7.8 g/kg/day, in eight inbred strains of rats, and that there was a large within-strain variability in alcohol preference and voluntary consumption in both sexes. In this study for male mice of many strains, including congeneric strains, the B10.Br/Sg and C57BL strains exhibited a high alcohol preference, whereas the other strains showed low alcohol preference; there was no intermediate alcohol preference strain. In rodents, especially rats and mice, there are more variable sublines of rats than of mice. The variation in alcohol preference among the different strains may support the theory that drinking behavior is probably influenced by genetic factors.

We investigated the relationship between alcohol intake or alcohol preference and the contents of the brain monoamines among eleven strains of mice. Among these strains of mice, alcohol preference (%), alcohol intake (g/kg/day) and the contents of NE, DA and 5-HT were significantly different (one-way ANOVA). We found a clear positive relationship between alcohol intake and NE level, and a clear negative relationship between alcohol intake and 5-HT level. In Table 2, the levels of NE in the B10.Br/Sg strain and two C57BL strains show significant differences, and the ethanol intake (g/kg/day) and the alcohol preference (%) in these strains also show significant differences. In these three strains, those with a higher alcohol preference tend to have the higher levels of NE. However, B10.Br/Sg mice have a large deviation in both alcohol intake per day and alcohol preference. This strain, therefore, is interesting and available for use in the alcohol drinking behavior test. We previously reported a negative relationship between alcohol preference and DA level among five inbred strains of mice [26]. However, in this study, there was no significant relationship between alcohol intake and brain DA content. Our studies suggest that mice exhibiting a higher alcohol preference tend to have a lower content of DA.

Alcohol preference is a complex behavior. There are some methodological problems in research on alcohol preference. These may be due to differences in the percent of ethanol used, the chronology of ethanol administration, variations in brain ethanol levels at the time biochemical measurements are made, species differences, the effects of ethanol on the nutritional status of the animal, and the methods used for evaluating neurotransmitter levels.

Our results are somewhat obscured by the fact that alcohol preference was measured for a period prior to the assay of the brain monoamines. Thus, the results could be interpreted as reflecting changes in brain monoamine levels which resulted from the amount of ethanol consumed during the voluntary alcohol preference testing period.

When 10% (v/v) ethanol is the only fluid available for 5 days, mouse brain monoamines may be changed. This exposure to 5 days of involuntary ethanol ingestion may change the subsequent voluntary alcohol consumption level.

This inconsistency has been previously illustrated in the report by Perhach *et al.* [18], who found that the levels of NE, DA and 5-HT in the brains of alcohol-naive C57BL/6J, BALB/c and DBA mice revealed no differences among strains, but exposure to a free choice between a 10% (v/v) ethanol solution and water for 14 days resulted in a signifi-

cant increase (16%) in the 5-HT level in only the C57BL mice. The reason why voluntary alcohol consumption increases the level of 5-HT in only C57BL/6J mice is unclear. Forced ethanol consumption alters a number of parameters including the brain monoamines. However, it is also true that normal laboratory mice do not usually show alcoholic damage or intoxication with voluntary alcohol consumption.

To minimize the effect of alcohol, the neurochemical determination was performed 2 days after the alcohol preference test in this study. However, at the time of the brain monoamine assays, the mice already had consumed a variable amount of ethanol; therefore, it is difficult to separate the initial monoamine levels from subsequent induced levels. The endogenous levels of brain monoamines and the levels of these transmitters following forced and/or voluntary alcohol consumption should be determined.

The relationship between alcohol intake and brain monoamine levels in mice has been somewhat obscure. Little is known concerning the basis for the observed strain differences in alcohol preference. Perhach *et al.* [18] found no differences among alcohol-naive C57BL/6, BALB/c, and DBA/2 mice in NE, DA or 5-HT levels. Ho *et al.* [8], also comparing C57BL/6 and DBA/2 mice, found no differences by strain in serotonin or in uptake of 3H-norepinephrine or 3H-dopamine but found higher brain acetyl-cholinesterase (cholinetransferase) levels in DBA/2 mice. This was felt to implicate a central cholinergic mechanism in alcohol preference. Furthermore, Boismare *et al.* [2] found that calcium acetylhomotaurine significantly reduces the voluntary intake of alcohol and that this is inhibited by GABA antagonist, bicuculline. This suggests that the gabaergic system also may be implicated in voluntary intake of alcohol.

In P and NP rats, significantly higher contents of 5-HT, GABA, glutamate and glycine and a lower content of asparate were found in the alcohol-preferring animals (P lines) than in the alcohol-nonpreferring animals (NP lines) [11,22]. Furthermore, Ahtee and Eriksson [1] reported a higher serotonin content in AA than ANA animals, and a higher content of DA, but not NE, in the AA rats; we found the reverse, a significantly higher content of NE being seen in the higher alcohol intake animals. Though the correlation between the content of DA and the alcohol intake was not significant, a lower content of DA and the alcohol intake was not significant, a lower content of DA was found in the higher alcohol intake animals.

The finding of a higher level of 5-HT in the lower alcohol preference strains is in agreement with Murphy's finding [16] that rats with a higher alcohol preference have lower metabolic rates, lower functional activity, and/or lower density of serotonergic brain areas than do alcohol-nonpreferring rats. These data strongly suggest that the static level of brain serotonin directly influences alcohol preference. Myer and Veale [17] found that alcohol preference was significantly reduced in rats orally give p-chlorophenylalanine, a tryptophan hydroxylase inhibitor that selectively depletes brain serotonin, and that administration of alpha-methyl-p-tyrosine, a tyrosine hydroxylase inhibitor that depletes brain catecholamines, slightly reduced the selection of alcohol. Alcohol preference may be influenced by the naive level of brain monoamines. Our comparison of mouse strains provided strong evidence that static norepinephrine and serotonin levels control alcohol preference.

Li *et al.* [11] and Elmer *et al.* [14] showed that more important than the behavioral differences between alcohol-preferring and alcohol-nonpreferring rats is the possibility that preference and non-preference is related to innate differences in the sensitivity of the central nervous system to the effects of alcohol. Kiiammaa *et al.* [9] found that ethanol stimulated dopaminergic synthesis and that C57BL/6 mice were less sensitive to this effect of ethanol than were the BALB/c and DBA/2 strains. These findings also suggest that genetically determined differences exist in the sensitivity of the CNS of mice to ethanol. Furthermore, Littleton *et al.* [13] suggested that differences in sensitivity to or tolerance of ethanol is due to an altered phospholipid composition of the synaptic membrane at the GABA-containing synapses in the mouse brain.

Finally, we studied the relationship between aging and alcohol preference. Takeda *et al.* [23] obtained a senescence-prone series (P1) (P2) by continuous brother-sister breeding from original litters of AKR strain of mice with severe deterioration. The P series was named "SAM (Senescence Accelerated Mouse"). The life span in the P series was shortened to about 26% of the senescence-resistant series. Popp [19] reported accelerated aging in B10 strains of mice in which premature graying of the hair and shortening of the mean life span were observed. These mice exhibited a low alcohol preference. Particularly when compared with SAM P1, SAM P2 and AKR/J mice, although there were no differences in alcohol preference or alcohol intake among these strains, SAM P1 mice exhibited significant differences in brain NE and DA. At the time of our final brain monoamine assays, all mice were 75 days old. As compared with the SAM mice and the original strains of mice, AKR/J, at the same ages, there were no significant differences in alcohol intake (g/kg/day) or alcohol preference. These results strongly suggest that the accelerated senescence factor does not directly influence the alcohol preference. Further detailed investigations are needed to determine alcohol preference in SAM.

We have found innate differences in the brain monoamine content between alcohol-preferring and alcohol-nonpreferring animals, which is of interest in view of the attention recently focused on ethanol-induced changes in neuronal membrane structure in the investigations of the genetic basis of alcohol drinking behavior.

ACKNOWLEDGEMENTS

This investigation was supported in part by a Grant-in-Aid No. 61770421 from the Ministry of Education, Science and Culture, Japan. The authors wish to express their appreciation to Dr. T. Takeda, Kyoto University, Faculty of Medicine, Department of Pathology, Chest Disease Research Institute, for supplying senescence accelerated model mice (SAM P1, SAM P2) and for his helpful criticism.

REFERENCES

1. Ahtee, L. and K. Eriksson. Dopamine and noradrenaline content in the brain of rat strains selected for their alcohol intake. *Acta Physiol Scand* **93:** 563–565, 1975.
2. Boismare, F., M. Dacust, N. Moore, C. Caligaut, J. P. Lhuintre, P. Chretien and J. Durlach. A homotaurine derivative reduces the voluntary intake of ethanol by rats: Are cerebral GABA receptors involved? *Pharmcol Biochem Behav* **21:** 787–789, 1984.
3. Duncan, D. B. Multiple range and multiple F tests. *Biometrics* **11:** 1–42, 1955.
4. Elmer, G. I., R. A. Meisch and F. R. George. Oral ethanol reinforced behavior in inbred mice. *Pharmacol Biochem Behav* **24:** 1417–1421, 1985.
5. Elston, S. F. A., K. Blum, L. Delallio and A. M. Briggs. Ethanol intoxication as a function of genotype dependent responses in three inbred mice strains. *Pharmacol Biochem Behav* **16:** 13–15, 1982.
6. Eriksson, K. Genetic selection for voluntary alcohol consumption in the albino rat. *Science* **159:** 739–741, 1968.
7. Eriksson, K. Inherited metabolism and behavior towards alcohol: Critical evaluation of human and animal research. In: *Animal Models in Alcohol Research,* edited by K. Eriksson and K. Kiianmaa. New York: Academic Press, 1980, pp. 3–20.
8. Ho, A. K. S., C. S. Tsai and B. Kissin. Neurochemical correlates of alcohol preference in inbred strains of mice. *Pharmacol Biochem Behav* **3:** 1073–1076, 1975.
9. Kiianmaa, K. and B. Tabakoff. Neurochemical correlates of tolerance and strain differences in the neurochemical effects of ethanol. *Pharmacol Biochem Behav* **18:** Suppl 1, 383–388, 1983.
10. Komura, S., Y. Irizawa and H. Funazu. Alcohol preference and liver alcohol dehydrogenase activity in several animal species. *Jpn J Stud Alcohol* **8:** 51–57, 1973.
11. Li, T.-K., W. Lumeng, W. J. McBride, M. B. Waller and T. D. Hawkins. Progress toward a voluntary oral consumption model of alcoholism. *Drug Alcohol Depend* **4:** 45–60, 1979.
12. Li, T.-K. and L. Lumeng. Alcohol preference and voluntary alcohol intakes of inbred rat strains and the national institutes of health heterogenous stock of rats. *Alcoholism: Clin Exp Res* **8:** 485–486, 1984.
13. Littleton, J. M., G. R. John, P. A. Jones and S. J. Grieve. The rapid onset of functional tolerance to ethanol—role of different neurotransmitters and synaptosomal membrane lipids. *Acta Psychiatr Scand* **62:** Suppl 286, 137–151, 1980.
14. Lumeng, L., M. B. Waller, W. J. McBride and T.-K. Li. Different sensitivities to ethanol in alcohol-preferring and nonpreferring rats. *Pharmacol Biochem Behav* **16:** 125–130, 1982.
15. McClearn, G. E. and D. A. Rogers. Differences in alcohol preference among inbred strains of mice. *Q J Stud Alcohol* **20:** 691–695, 1959.
16. Murphy, J. M., W. J. McBride, L. Lumeng and T.-K. Li Regional brain levels of monoamines in alcohol-preferring and -nonpreferring lines of rats. *Pharmacol Biochem Behav* **16:** 145–149, 1982.
17. Myers, R. D. and W. L. Veale. Alcohol preference in the rat: reduction following depletion of brain serotonin. *Science* **160:** 1469–1471, 1968.
18. Perhach, J. L., Jr., R. H. Cox, Jr. and H. C. Ferguson. Possible role of serotonin in the voluntary selection of ethanol by mice. *Fed Proc* **32:** No. 2728, 1973.
19. Popp, D. M. Use of congenic mice to study the genetic basis of degenerative disease. *Birth Defects* **14:** 261–279, 1978.
20. Sasa, S. and C. L. Blank. Determination of serotonin and dopamine in mouse brain tissue by high performance liquid chromatography with electrochemical detection. *Anal Chem* **49:** 354–359, 1977.
21. Schlesinger, K. Genetic and biochemical correlates of alcohol preference in mice. *Am J Psychiatry* **122:** 767–773, 1966.
22. Tabakoff, B. and L. Hoffman. Neurochemical aspects of tolerance to and physical dependence on alcohol. In: *The Biology Alcoholism, The Pathogenesis of Alcoholism Biological Factors, vol 7,* edited by B. Kissin and H. Begleiter. New York: Plenum Press, 1983, pp. 199–252.
23. Takeda, T., M. Hosokawa, S. Takeshita, M. Irino, K. Higuchi, T. Matsusita, Y. Tomita, K. Yasuhira, H. Hamamoto, K. Shimizu, M. Ishii and T. Yamamuro. A new murine model of accelerated senescence. *Mech Ageing Dev* **17:** 183–194, 1981.
24. Wardlaw, A. C. *Practical Statistics for Experimental Biologists.* New York: John Wiley and Sons, 1985, pp. 163–187.
25. Yoshimoto, K., Y. Irizawa, N. Itoh, Y. Hashimoto and S. Komura. Central monoamines and the death process time (Antemortem Time) during asphyxia: An experimental study in the mouse brain. *Z Rechtsmed* **93:** 211–218, 1984.
26. Yoshimoto, K., S. Komura, A. Kano and K. Mizohata. Alcohol preference and brain monoamines in five inbred strains of mice. *IRCA Med Sci* **13:** 1192–1193, 1985.
27. Yoshimoto, K., Y. Yamamoto, Y. Irizawa and S. Komura. Alcohol selection and liver alcohol dehydrogenase activity in several animal species (Japanese). *Jpn J Stud Alcohol* **13:** 56–57, 1978.