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Neurochemical Correlates of Alcohol Preference in Inbred Strains of Mice

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HO, A. K. S., C. S. TSAI AND B. KISSIN. *Neurochemical correlates of alcohol preference in inbred strains of mice.* PHARMAC. BIOCHEM. BEHAV. 3(6) 1073–1076, 1975. – C_{57}B1/6J, a specific inbred strain of mice with high alcohol preference and DBA/2J, a specific inbred strain with poor preference for alcohol were studied. Brain content of acetylcholine, uptake of ^{14}C-Choline by whole brain homogenate were significantly higher in the C_{57}B1/6J mice whereas brain acetylcholinesterase was higher in the DBA/2J mice. No significant difference was found for the level of brain serotonin, uptake of ^{3}H-norepinephrine or ^{3}H-dopamine. Treatment with a specific inhibitor of choline transferase, 4-(1-napthylvinyl) pyridine salt (10 mg/kg, twice daily) shifted the selection of alcohol to water in the C_{57}B1/6J mice. These findings suggest a direct involvement of central cholinergic mechanism in alcohol preference.

Ethanol preference Strain differences Central cholinergic mechanism Brain monoamines
4-(1-napthylvinyl) pyridium salt (NVP)

RECENT interest in the neurochemical correlates of alcohol preference has been focused on the role of various putative neurotransmitters, particularly, serotonin (5-HT). Myers and co-workers [12,15] reported a decrease in the volitional consumption of ethanol in rats after chronic treatment with DL-parachlorophenylalanine (PCPA), an inhibitor of tryptophan hydroxylase, whereas alpha-methyl-para-tyrosine (α-MT), an inhibitor of tyrosine hydroxylase, had no effect. However, the decrease in ethanol consumption produced by PCPA was not observed by Geller [5] who showed that PCPA increased ethanol selection. Recent studies in our laboratory using 5, 6-dihydroxytryptamine (5,6-DHT), a long-acting depletor of brain 5-HT, [3] showed an increase in the volitional consumption of ethanol. In addition, following 5,6-DHT treatment there was a significant increase in the brain level of acetylcholine (ACh) parallel to the appearance of the increase in alcohol preference [8]. It was suggested that the 5,6-DHT induced alcohol preference might be attributed to an increase in central cholinergic activities.

In order to further elucidate the role of central cholinergic system(s) in the selection of ethanol, we studied the neurochemical correlates in two specific inbred strains of mice with markedly different alcohol preference status, viz C_{57}B1/6J, an alcohol preferring strain, and DBA, an alcohol non-preferring strain. Thus far, the major biochemical differentiating factor between the two strain has been in the rate of acetaldehyde metabolism, where the DBA strain has been shown to metabolize ethanol less rapidly and thus shows a greater accumulation of acetaldehyde after ethanol ingestion with subsequent greater associated signs of toxicity [16]. In addition, the effect of 4-(1-napthylvinyl) pyridium salt (NVP), a choline acetyltransferase inhibitor [17], on ethanol selection in the C_{57}B1 mice was studied.

METHOD

Measurement of Alcohol Preference

Adult male C_{57}B1/6J and DBA/2J mice, aged 6–8 weeks and weighing between 20 to 25 g were used (supplied by Jackson Laboratory, Bar Harbor, Maine). The animals were housed individually in wire mesh cages in a constant temperature room (70°F) with ad lib access to food and water. To test for alcohol preference, two small graduated 25 ml glass drinking tubes (Richter type) were used. One was filled with water and the other with ethanol (diluted fresh each day from 95 percent ethanol with tap water on a volume to volume basis to the required concentration). The tubes were rotated randomly each day to prevent the development of a position habit [13]. Measurements of water and ethanol consumption were taken at 10 a.m. each

day. Alcohol preference was tested in each animal for 7 days and the base-line consumption was established using data obtained during the last 4 days. In a subgroup of 32 mice, NVP was injected IP twice daily at 10 a.m. and 10 p.m. using 2 mg/kg and 10 mg/kg doses respectively, and the daily selections of either 5 or 10 percent ethanol were recorded.

Neurochemical Studies

To determine whether there were significant differences in the brain chemistry of these two strains, naive animals were used. Various parameters were compared, including the levels of brain ACh, norepinephrine (NE), dopamine (DA) and 5-HT; the activities of acetylcholinesterase (AChE), choline acetyltransferase (ChAc) and the in vitro uptake of ^{14}C-Choline, ^{3}H-NE and ^{3}H-DA by whole brain homogenates. The mice were killed by decapitation at about 3 to 5 p.m. and the brains were removed on ice. The brains were dissected into two halves along the mid-line; one half was used for ACh and the other half for the monoamine assays. ACh was extracted according to the method described by Hebb [6]. The brains were weighed and homogenized in 4 volumes of 10 percent trichloroacetic acid, centrifuged, washed and the combined supernatant was further extracted with ether to remove the lipids. The last trace of ether was removed by aspiration. ACh was assayed using the guinea pig ileum preparation in the presence of morphine (2 mg/l) and diphenhydramine (2 mg/l) [4]. For catecholamines and 5-HT assays, the brains were weighed, homogenized in 4 volumes of chilled 0.4 N perchloric acid, centrifuged and the supernatants were decanted into stoppered tubes containing washed alumina. NE, DA and 5-HT were assayed fluorometrically according to the method of Ansell and Beeson [1].

In another subgroup of mice, whole brain homogenates were prepared and used for both enzyme and uptake experiments. In vitro uptake of ^{14}C-Choline, ^{3}H-NE and ^{3}H-DA, whole brain homogenates were used. The brains were homogenized in 8 volumes of ice-cold 0.32 M sucrose as described by Whittaker [18]. After centrifugation of the homogenate for 10 min at 1000 × g and 4°C, the precipitate which consisted of nuclei and unbroken cells (P_1) was discarded. The supernatant fraction (S_1) was used for uptake experiments. Into 4.4 ml of an incubation medium containing 118 mM sodium chloride, 4.7 mM potassium chloride, 2.2 mM calcium chloride, 1.18 mM magnesium sulfate, 11 mM glucose and 25 mM sodium phosphate at pH 7.0, 0.25 ml of the brain homogenate was added. One tenth ml of incubation medium containing 50× concentrated solution of ^{14}C-Choline (specific activity 61 mCi/mM; final incubation concentration 0.01 mM), ^{3}H-NE (specific activity, 3.7 Ci/mM; final concentration, 0.1 μM) or ^{3}H-DA (specific activity, 11.0 Ci/mM; final concentration, 0.1 μM) was added and incubated at 37°C for 20 min (All three isotopes were supplied by New England Nuclear Corp., Boston, Mass.). The incubated samples were centrifuged at 17,000 G and the pellets resuspended in 400 μl of 0.4 N perchloric acid in 50 percent ethanol. A 200 μl aliquot was then transferred into counting vials containing a 12 ml of phosphor ethanol counting mixture and the radioactivity was measured by liquid scintillation spectrometry.

Crude extracts containing brain AChE and ChAc were prepared by the method previously described [7]. AChE was determined by a modified radiometric method according to McCamen and Hunt [11] using acetyl-l-^{14}C-Choline iodide as substrate. The incubation mixture consisted of 0.2 ml potassium buffer, pH 7.2, 0.085 M NaCl, 0.15 M and 0.04 M $MgCl_2$; 0.1 ml of ^{14}C-ACh, 0.1 mM (containing 0.02 μCi acetyl-l-^{14}-C-Choline diluted with acetylcholine iodide) and 0.1 ml of tissue extract containing 8–10 mg tissue wet weight. The incubations were carried out for 10 min in a Dubnoff shaking incubator at 37°C. At the end of the incubation, the tubes were removed and immersed in ice. The reaction was stopped by addition of 1 g of Dowex 50 resin, filtered, and 100 μl aliquot of the filtrate, containing hydrolysed acetic-1-^{14}C acid, was transferred to counting vials containing 15 ml of Bray's PPO-POPOP liquid scintillation cocktail. Radioactivity was measured by liquid scintillation spectrometry in a LS-Beckman 100 counter and corrections were made for quenching. ChAc activity was determined using 1-(^{14}C)-acetylcoenzyme A as substrate according to the method described previously [7]. Protein was determined by the method of Lowry et al [10].

RESULTS

Data obtained on the volitional consumption of ethanol showed that the C_{57}B1/6J mice consumed significantly greater amounts of ethanol than the DBA/2J strain. In groups of 20 C_{57}B1/6J mice, mean daily values of consumption were 182 ± 16 ml/kg and 230 ± 18 ml/kg for 5 and 10 percent ethanol respectively. Mean value of consumption for DBA/2J mice was 59 ± 6 ml/kg for 5 percent ethanol. The consumption of 10 percent ethanol was less than 10 ml/kg and in most mice, no consumption was recorded. Mean daily values for water and food consumption prior to testing for alcohol preference for C_{57}B1/6J mice were 253 ± 3 ml/kg and 189 ± 11 g/kg respectively; for the DBA/2J, the values were 213 ± 20 ml/kg and 176 ± 19 g/kg respectively. The mean level of brain ACh was significantly higher in the C_{57}B1/6J than in the DBA/2J mice ($p<0.01$). Mean values of 3.30 ± 0.18 μg/g and 2.20 ± 0.12 μg/g were obtained for the C_{57}B1/6J and the DBA/2J mice respectively, representing a difference of approximately 45 percent. Mean values of AChE activities were 0.8 ± 0.03 μM/mg/hr and 1.08 ± 0.08 μM/mg/hr of ACh hydrolysized for the C_{57}B1/6J mice respectively. The difference was small but significant ($p<0.05$). Mean values for choline acetyltransferase were 0.55 μM/g/hr and 0.54 μM/g/hr of ACh synthesized for C_{57}B1/6J and DBA/2J respectively; there was no significant difference in ChAc activity. However, ^{14}C-Choline uptake was significantly higher ($p<0.05$) in the C_{57}B1/6J than in the DBA/2J mice. Mean values obtained were 6.0 ± 0.35 × 10^6 dpm/g/hr and 5.0 ± 0.37 × 10^6 dpm/g/hr respectively (Fig. 1). There was no significant difference in the brain levels of NE, DA, and 5-HT between the two strains (Table 1). In addition, there was no significant difference between the two strains in the uptake either of ^{3}H-NE or ^{3}H-DA in whoe brain homogenate. Mean values of 5.82 ± 0.29 × 10^4 dpm/mg/20 min (n = 5), and 5.74 ± 0.28 × 10^4 dpm/mg/20 min (n = 5) were obtained for ^{3}H-NE in the C_{57}B1/6J and DBA/2J mice respectively. Mean values of 4.75 ± 0.10 × 10^4 dpm/mg/20 min (n = 5), and 4.75 ± 0.28 × 10^4 dpm/mg/20 min (n = 5) were obtained for ^{3}H-DA in the C_{57}B1/6J and DBA/2J mice respectively.

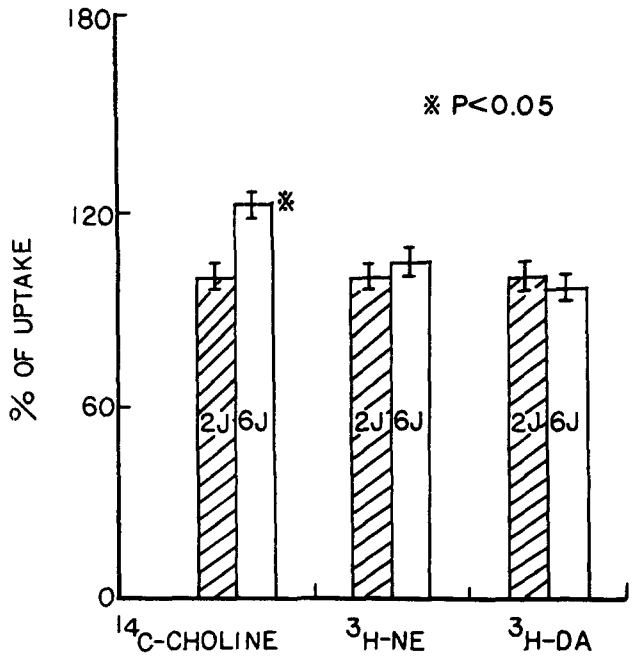

FIG. 1. Histograms showing the comparative uptake of ^{14}C-Choline, ^{3}H-NE and ^{3}H-DA by whole brain homogenates in DBA/2J and C_{57}Bl/6J mice. Data were expressed as percent uptake using the DBA/2J values as 100 mean ± S.E.M. (n = 5) significance, $p<0.05$.

TABLE 1

COMPARISONS BETWEEN THE NORMAL DBA/2J AND C_{57}Bl/6J MICE IN THE WHOLE BRAIN CONCENTRATIONS OF PUTATIVE NEUROTRANSMITTERS

Neurotransmitters	No. of Animals	DBA/2J (μg/g±S.E.)	C_{57}Bl/6J (μg/g±S.E.)
ACh	6	2.20±0.12	3.30±0.18*
NE	6	0.43±0.02	0.44±0.02
DA	6	1.13±0.09	1.05±0.05
5-HT	6	0.44±0.02	0.46±0.02

*$p<0.01$

Results obtained on the effect of NVP (10 mg/kg) showed that the selection of 5 percent ethanol by the C_{57}Bl/6J mice was significantly reduced. At the low dose (2 mg/kg), only a small reduction in ethanol intake was obtained (Fig. 2). The selection of 10 percent ethanol was reduced to a lesser degree compared with 5 percent ethanol. There was no apparent change in the total fluid intake. In addition, the NVP inhibited intake of ethanol was only transient and the animals resumed their selection for ethanol after 3 days. Chronic administration with NVP using the same dose for periods up to 6 days produced no further reduction in ethanol selection.

The level of brain ACh in mice treated with NVP (5 mg/kg, twice daily), for two days, showed a significant reduction whereas there was no significant change in ACh

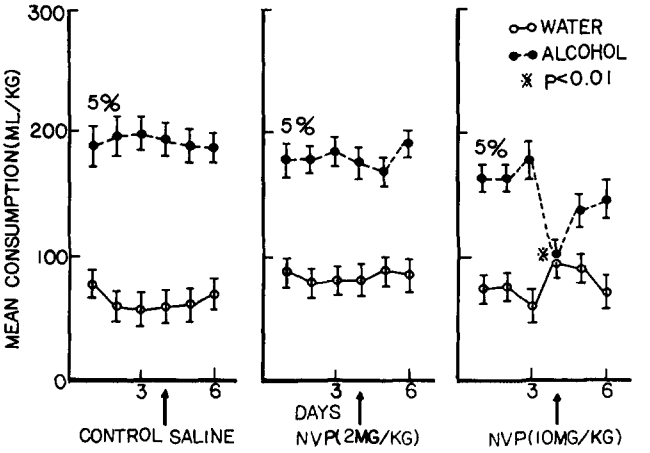

FIG. 2. Effects of 4-(1-napthylvinyl) pyridine (NVP) on the selection of ethanol (5%) in C_{57}Bl/6J mice. Consumptions of ethanol and water were recorded daily from groups of 6 mice each. Values were expressed as ml/kg in mean ± S.E.M. Drug was given on the days indicated by (↑).

TABLE 2

THE EFFECTS OF NVP ON ACh LEVEL IN C_{57}Bl/6J MICE

Drugs	No. of Animals	Dose (mg/kg)	Time (hours)	ACh (μg/g±S.E.)	Changes (%)
Control	11	–	–	2.88±0.16	–
NVP	6	5	7	2.83±0.14	–
NVP	6	20	7	2.68±0.15	7
NVP	6	5*	48	2.27±0.13	27†

*Twice a day.
†$p<0.001$

level seven hours after treatment of NVP with either 5 or 20 mg/kg IP doses (Table 2). Following NVP treatment, no gross behavioral alterations such as sedation or psychomotor activity were detected. Since only the preference for ethanol solutions was examined in this study, the effects of NVP on preference for solutions other than ethanol is not discussed; however, such possibilities should not be excluded.

DISCUSSION

The C_{57}Bl/6J mice showed a significantly greater volitional consumption of ethanol than the DBA/2J variety, and the present findings confirm the well-documented alcohol preference status of these two inbred strains of mice [16]. The neurochemical findings show that only the processes involved in the central cholinergic system(s) were significantly different between these two strains of mice. The fact that the C_{57}Bl/6J mice showed a significantly higher level of brain ACh and uptake of ^{14}C-Choline than the DBA/2J mice indicates that the synthesis may be greater in the C_{57}Bl/6J strain, whereas the metabolism of ACh may be higher in the DBA/2J variety as shown by the small but significantly higher AChE activity. Since ChAc activity is only one of the factors regulating the synthesis

and utilization of ACh, the higher ACh content in the $C_{57}B1/6J$ mice may be attributed to the differences in ^{14}C-Choline uptake and AChE activities.

Treatment with repeated doses of NVP, an inhibitor of ChAc, lowered brain ACh and also reduced the volitional consumption of ethanol in the $C_{57}B1/6J$ mice. These findings indicate that there is a correlation between the level of central cholinergic activity in the brain and preference of alcohol in $C_{57}B1/6J$ mice as compared with the DBA/2J strain. As of this time, there is no available data on the possibility that NVP interferes with the metabolism of ethanol by inhibiting liver alcohol dehydrogenase. The lack of apparent differences in the levels of NE, DA and 5-HT, and in the uptake of labelled catecholamines appears to suggest that in these two strains of mice, catecholaminergic system(s) may play a minor role in alcohol selection. Our previous reports [8,9] on the induced alcohol preference by 5,6-DHT and 6-OHDA and the corresponding increase in brain ACh in rats treated with these compounds alone are in good agreement with the data presented in the alcohol preference and nonpreference strains. However, the problem still remains as to the possible relationship between the central cholinergic and serotonergic mechanisms. It is paradoxical that the selection of ethanol can be either increased or decreased by drugs such as PCPA and 5-hydroxytryptophan [5,14], which either increase or decrease alcohol preference and the levels of brain 5-HT. One possible explanation for these apparent anomalies is that 5-HT may play an indirect role by modulating cholinergic activities [2,8].

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