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The Influence of Mouse Genotype on the Changes in Brain Cyclic Nucleotide Levels Induced by Acute Alcohol Administration

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CHURCH, A. C. AND D. FELLER. *The influence of mouse genotype on the changes in brain cyclic nucleotide levels induced by acute alcohol administration.* PHARMAC. BIOCHEM. BEHAV. **10**(3) 335–338, 1979.—Two mouse strains (C57BL/6By and BALB/cByJ) were found to differ widely in their sleep-time response to ethanol (3 g/kg), but showed no difference in their hypothermic response to the same ethanol dose. Comparison of brain cyclic nucleotide levels in the two strains revealed a strain difference in the brain cyclic AMP response to ethanol but no strain difference in the magnitude of the brain cyclic GMP change. Alcohol produced a significant drop in cerebellar cyclic GMP in both strains, and a decrease in cerebellar cyclic AMP in C57BL/6By mice. The cyclic AMP/cyclic GMP ratio increased following alcohol in the cerebellum of BALB/cByJ mice but not C57BL/6By mice. The results are discussed in terms of possible relationships between alcohol-induced changes in neurochemistry and behavior.

Ethanol narcosis Ethanol hypothermia Cyclic nucleotides Cyclic AMP Psychopharmacogenetics

SINCE it was noted that different genetic strains of mice displayed differing degrees of central nervous system (CNS) sensitivity to ethanol [10], a number of studies have supported the idea that gene actions substantially influence an animal's response to acute alcohol administration [1, 3, 12, 13, 15]. The information generated by these studies can be used to provide alcohol researchers with experimental preparations which allow tests of certain hypotheses concerning ethanol's action on the CNS. In this regard, a number of studies have examined the influence of ethanol on brain cyclic nucleotide levels. One group [21,22] has reported that rats administered large doses of alcohol show reductions in cerebellar adenosine 3′:5′-monophosphate (cyclic AMP) and generalized reductions in brain guanosine 3′:5′-monophosphate (cyclic GMP). Another group of investigators [9,16] found that, in rats, alcohol produced marked drops in cerebellar cyclic GMP but had no effect on brain cyclic AMP. In contrast to both groups, alcohol has been reported to increase both cerebellar and subcortical cyclic AMP in mice [14]. It seems apparent that little consensus exists with respect to ethanol's action on cyclic AMP levels, while on the other hand, cyclic GMP decreases after acute ethanol. In an effort to determine whether genetic differences could plausibly account for the inconsistencies obtained between different laboratories with respect to cyclic AMP changes following ethanol, a study of brain cyclic nucleotide changes following alcohol was conducted using two inbred strains of mice which differ in alcohol sensitivity [13]. In addition, alcohol-induced sleep-time and hypothermia were measured in order to determine possible cyclic nucleotide-behavior relationships.

METHOD

Male C57BL/6By and BALB/cByJ mice ranging in age from 2-3 months were obtained from the Animal Resources Department of the Jackson Laboratory. The mice were group-housed (5 per cage) in transparent plastic cages containing pine shavings in a vivarium maintained at 22 ± 2°C with 12 hr/12 hr light-dark cycle. A total of 70 mice were used in the experiments. Sensitivity to alcohol was measured by injecting animals IP with a 20% ethanol solution (w/v) at an alcohol dose of 3.0 g/kg, and recording the duration of the loss of righting reflex (sleep-time). Animals were considered to have regained the righting reflex when they could right themselves twice within 30 sec from a V-shaped polyacrylate trough.

An additional measure of CNS sensitivity to alcohol is the hypothermic response which follows acute ethanol administration [17]. Body temperature was measured using a lubricated rectal thermistor probe and a Tele-Thermometer (Yellow Springs Instrument Co.) at an environmental temperature of 22 ± 2°C. The lubricated probe was inserted 2.5 cm into the rectum and the temperature was recorded after a stable reading was noted. Temperatures were recorded prior to injection and 30 min following injection.

Cyclic nucleotide levels were measured in mice that were injected IP with either saline solution (0.9%), or with 20% alcohol (w/v) at a dose of 3 g/kg. Following a 30 min post-injection period, animals were sacrificed by a 4 sec exposure to head-on microwave radiation (Sanyo-EM8003). Brain temperatures following decapitation were found to be at least 51°C. Brains were rapidly removed and dissected on an ice

TABLE 1

MEAN RECTAL TEMPERATURES (C°) ± SEM TAKEN BEFORE AND 30 MIN AFTER IP ADMINISTRATION OF 3.0 G/KG OF ETHANOL

Strain	N	Preinjection	Postinjection
C57BL/6By	10	37.22 ± 0.08	34.35 ± 0.17
BALB/cByJ	10	37.29 ± 0.13	34.00 ± 0.15

cold platform. The hypothalamus and cerebellum were then immersed in liquid nitrogen and stored at −25°C until assay. The hypothalamic region was obtained by making shallow cuts on the lateral edges of the hypothalamus, along the caudal border of the mammillary bodies and at the optic chiasm. The average hypothalamic sample contained approximately 410 micrograms of protein.

Tissues were homogenized in 6% trichloroacetic acid, centrifuged at about 2500 g for 15 min, and the supernatant drawn off and neutralized [20]. Cyclic AMP and GMP were measured using radioimmunoassay kits (New England Nuclear). Protein content of the pellet was determined by resuspending the precipitate in 0.5 N NaOH and then measuring the binding of Coomassie Brilliant Blue G-250 (Sigma) [2].

Since the cyclic nucleotide experiment was run in two parts, the influence of alcohol, within a strain and a particular region, was evaluated using a two-factor (drug condition, replication) analysis of variance. Determination of genotype effects was made by converting data derived from alcohol-treated animals into percent change from the appropriate saline mean. In this fashion data could be pooled across replications allowing a two-factor (genotype, region) analysis of variance to be used. In all figures, independent standard errors for each mean were calculated.

With respect to the hypnotic effects of ethanol, C57BL/6By mice showed shorter alcohol-induced sleep-times than BALB/cByJ mice (13.8 and 82.6 min respectively), $F(1,8)=33.9$, $p<0.001$. However, as shown in Table 1, body temperature measured before and 30 min after ethanol injection did not differ significantly between the two strains. The mean drop in body temperature was 2.87 ± 0.19 for C57BL/6By compared to 3.29 ± 0.19 for BALB/cByJ. Thus, alcohol-induced sleep-time and hypothermia seem to represent two independent CNS responses to ethanol.

The results of radioimmunoassay of brain cyclic nucleotides in alcohol and saline treated mice are presented in Table 2.

Statistical examination of cyclic AMP measures in the cerebellum revealed that, although cyclic AMP levels in the BALB/cByJ strain did not change substantially following alcohol, the alcohol treated C57BL/6By mice had significantly lower cerebellar cyclic AMP concentrations than their saline treated controls, $F(1,16)=8.72$, $p<0.01$. Moreover, as illustrated in Fig. 1, alcohol treatment produced changes in brain cyclic AMP that were strain dependent, C57 vs BALB; $F(1,36)=13.5$, $p<0.001$. In addition, the cyclic AMP response to ethanol differed in the hypothalamus compared to the cerebellum, $F(1,36)=14.6$, $p<0.001$.

Analysis of cyclic GMP measures demonstrated that both strains showed significant drops in cyclic GMP levels in the cerebellum following alcohol, C57:$F(1,16)=32.1$, $p<0.001$; BALB:$F(1,16)=30.0$, $p<0.001$.

As shown in Fig. 2, the change in cyclic GMP produced by ethanol was restricted to the cerebellum, $F(1,36)=38.3$, $p<0.001$. Genotypic differences did not influence the cyclic GMP response to ethanol.

An alternative way to express the results of the neurochemical experiment is to compute a ratio of the two nucleotides. Analysis of the cyclic AMP/cyclic GMP ratio indicated that in the cerebellum, alcohol produced a significant increase in the cyclic AMP/cyclic GMP measure in BALB/cByJ mice (saline: 5.48; alcohol: 10.61) but not in C57BL/6By mice (saline: 5.71; alcohol: 6.60).

DISCUSSION

The results of the alcohol-induced sleep-time study suggest that BALB/cByJ mice are more sensitive to the depressant effects of ethanol. An alternative explanation would

TABLE 2

MEAN CONCENTRATION OF CYCLIC AMP AND GMP (PMOLES/MG PROTEIN) IN C57BL/6By AND BALB/cByJ MICE 30 MIN FOLLOWING EITHER SALINE OR ETHANOL (3G/KG) ADMINISTRATION

Strain	Nucleotide	Treatment	Cerebellum	Hypothalamus
C57BL/6By	Cyclic AMP	Saline	43.54	48.44*
		Alcohol	24.77	43.66
	Cyclic GMP	Saline	7.84	25.56*
		Alcohol	3.91	23.78
BALB/cByJ	Cyclic AMP	Saline	48.08	35.85*
		Alcohol	41.76	55.85
	Cyclic GMP	Saline	10.46	25.11*
		Alcohol	3.97	25.36

All values were derived from 10 animals each except those which contained 9 (*).

Mice were sacrificed by 4.0 sec of head-on microwave irradiation.

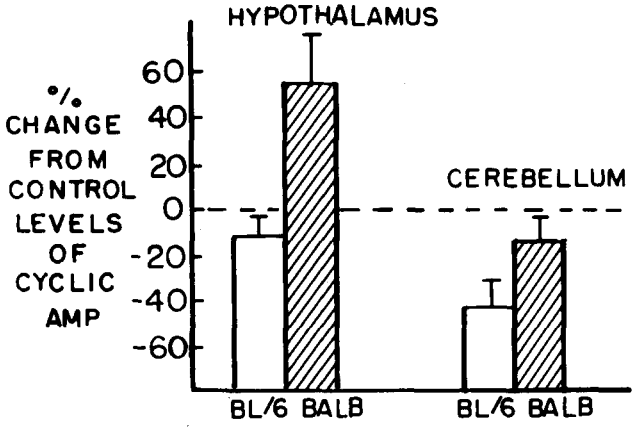

FIG. 1. The influence of ethanol (3 g/kg) on hypothalamic and cerebellar levels of cyclic AMP in C57BL/6By (BL/6) and BALB/cByJ (BALB) mice.

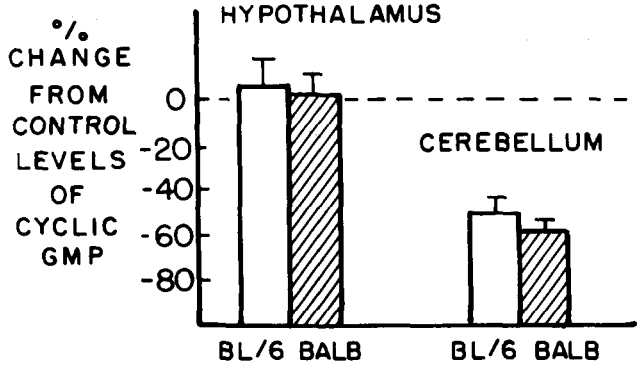

FIG. 2. The influence of ethanol (3 g/kg) on hypothalamic and cerebellar levels of cyclic GMP in C57BL/6By (BL/6) and BALB/cByJ (BALB) mice.

be that the sleep-time measure is merely reflecting differences in alcohol metabolism rates between the two strains. Although ethanol clearance rates were not measured in the present study, previous work has demonstrated that the BALB and C57 strains show no differences in hepatic alcohol dehydrogenase activities [1] and minor differences in blood alcohol clearance at the ages presently tested [4,5].

In similarity to the strain differences in the behavioral index of alcohol sensitivity, alcohol produces a greater change in the cerebellar cyclic AMP/cyclic GMP ratio in the alcohol sensitive BALB/cByJ strain than in the less sensitive C57BL/6By strain. Since this measure may compare the hyperpolarizing effects of cyclic AMP [8,18] to the depolarizing actions associated with cyclic GMP [7,19], it may better reflect the activity level or metabolic state [6] of the neuronal tissue under study.

When the cyclic nucleotide levels obtained in the present experiment were compared with those obtained by others (in rats and mice), it was noted that cyclic AMP levels were about twice as high as those reported by investigators using more powerful microwave devices [11]. Cerebellar cyclic GMP levels were comparable to previously determined values, while hypothalimic levels were much higher. It is likely, therefore, that limited postmortem increases in cyclic nucleotide levels are reflected in our data. However, such increases were not sufficiently large to obscure the strain-dependent differences in the cyclic nucleotide response to ethanol.

Recently it has been found that C57BL/6By mice show greater decreases in activity following low doses (0.5–2.0 g/kg) of ethanol than do BALB/cBy mice [13]. Similar results have been previously found in the closely related strains, C57BL/6J and BALB/cJ [15]. In the present study and others [1,10], C57BL/6 mice show shorter alcohol-induced sleep-time than BALB/c mice. The present results suggest that the strain differences observed in the neurochemical response to ethanol are more related to measures of alcohol-induced sleep-time than of alcohol-induced hypothermia. However, the alcohol-induced strain activity differences reported by other investigators may also be related to the observed strain differences in neurochemical response to ethanol. Pharmacological manipulation of cyclic nucleotides with concomitant measures of these alcohol-induced changes in behavior may determine whether any of these measures of CNS sensitivity to ethanol are related to changes in the cyclic nucleotide levels.

In contrast, no strain differences were evident in either alcohol-induced hypothermia or in brain cyclic GMP changes consequent to ethanol administration. Further studies are needed to determine what mechanisms mediate the hypothermic response to ethanol.

The present results agree in part with those of both Redos and colleagues [16] and Volicer and coworkers [21,22]. In the former, cerebellar cyclic GMP levels were depressed with no decrease in cyclic AMP levels as in the BALB/cByJ strain, while in the latter both cerebellar cyclic GMP and AMP levels were depressed following ethanol as in the C57BL/By strain. The inconsistencies found between laboratories with respect to acute alcohol and cyclic AMP may be due to genetic differences in the animals employed. Thus, while both groups used Sprague-Dawley rats, they were from different animal suppliers, and thus may be derived from differing gene pools. Such findings emphasize the importance of the genetic background of an animal in determining both its behavioral and neurochemical response to ethanol. In addition, the use of the psychopharmacogenetic design has generated potentially testable hypotheses concerning the diverse actions of ethanol on the central nervous system. Further research will determine the validity of these hypotheses.

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