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Effect of Hypnotics on Mice Genetically Selected for Sensitivity to Ethanol

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ERWIN, V G., W. D W. HESTON, G. MCCLEARN AND R. A. DEITRICH. *Effect of hypnotics on mice genetically selected for sensitivity to ethanol.* PHARMAC. BIOCHEM. BEHAV. 4(6) 679–683, 1976. – It was previously shown that the rate of disappearance of blood ethanol was identical for 2 lines of mice selectively bred for differences in sleep-time after ethanol administration The ED_{50} values for the loss of righting response with ethanol were significantly different at 3 64 g per kg for the SS line and 1.65 g per kg for the LS line In the present study the mean sleep time is 367 sec for SS mice and 9342 sec for LS mice The ED_{50} values remain essentially the same as previously reported. Unchanged LD_{50} values for ethanol, however, are not different at 4 8 g per kg for the SS and 4 5 g per kg for the LS line of mice The ED_{50} value for loss for righting response following administration of methanol, butanol and *t*-butanol is approximately 2 fold greater for the SS line of mice than for the LS line The ED_{50} values for sodium pentobarbital or ether in the 2 lines of mice for loss of righting response are virtually identical In addition, the sleep-time values obtained after the administration of pentobarbital, chloral hydrate, trichloroethanol and paraldehyde are not significantly different. These data indicate that while the SS and LS lines of mice differ in central nervous system sensitivity to ethanol, methanol, butanol and *t*-butanol it is implied that they do not differ in central nervous system sensitivity to other hypnotic agents tested. Proof of this latter suggestion awaits determination of metabolic rates, and brain levels of these other depressants

Ethanol Central Nervous System Sensitivity Genetics Alcohols Hypnotics

RECENT studies on the actions of ethanol, [4,5] have reported on the utilization of 2 lines of mice which were derived from a genetically heterogeneous HS stock by means of a selective breeding program The heterogeneous stock was established by intercrossing of 8 inbred strains of mice (A, AKR, BALB/c, C3H/2, C57BL, DBA/2, Is/Bi and RIII) and subsequently maintained by a random mating procedure. Details of this procedure were given previously [11]. After determining the sleep-time (following 3.4 g ethanol per Kg body weight) of a large number of mice from the heterogeneous stock, those animals with the shortest sleep-time were mated, and their progeny was the first generation of the short-sleep (SS) line of mice. The first generation of long-sleep (LS) mice was produced in a comparable manner by mating those animals with the longest sleep-time. In subsequent generations of the short-sleep line, selection pressure was maintained by only breeding animals with the shortest sleeping times. Similarly, the long-sleep line was produced by utilizing as parents in each successive generation only those animals with the longest sleeping time. Previously, we reported that the activities of liver alcohol dehydrogenase and the rates of blood ethanol disappearance were virtually identical for samples of the 2 lines of mice [5]. The ED_{50} values for loss of righting response with ethanol were approximately 2 fold greater in the SS animals than in the LS mice. It was suggested that the 2 lines of mice differ in their central nervous system sensitivity to ethanol.

Prior investigations [14,15] of the enzymes responsible for ethanol metabolism demonstrated differences in the activity of liver enzymes responsible for ethanol metabolism in various inbred strains of mice. Other investigators have shown that mice from various inbred strains differ in sleep-time induced by barbiturates [6,17]. Liver microsomal fractions isolated from these inbred strains of mice differed in rates of hexobarbital metabolism However, studies of possible differences in brain sensitivity to barbiturates were not reported. It has been noted that

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barbiturates and general anesthetics as well as aliphatic alcohols depress the reticular activating system which subserves wakefulness [8,13]. Consequently it appeared that the LS and SS lines of mice which differ in brain sensitivity to ethanol might be of value in comparing the mechanisms of action of various central nervous system depressants. In the present study we compared the hypnotic potencies and duration of response of various central nervous system depressants in the LS and SS lines of mice. Some of these data have appeared in abstract form [4].

MATERIALS AND METHOD

Mice of the long-sleep (LS) and the short-sleep (SS) line of the 14th generation were obtained from the Institute for Behavioral Genetics (IBG) and after at least a week of adaptation to the testing room in an adjacent laboratory, were tested between 90–200 days of age HS animals were also tested in 1 experiment. Equal numbers of male and female mice were tested at each dose. Doses of ethanol of 0.008–0.012 ml/g were injected IP (25% v/v in saline). The mice weighed between 18–34 g.

Response to the alcohols and the various sedative hypnotic agents was measured by determination of sleeping time. Because awakening from alcohol is not as clear cut as awakening from other sedative hypnotics, the animal was not considered awake until it had righted itself 3 times within 30 sec. For the ED_{50} studies, the animal was considered to have lost its righting response if it remained on its back for 1 min or longer. For the LD_{50} acute toxicity study, the animals were examined after 24 hr had elapsed and the number of deaths at that dose recorded [2]. The ED_{50} doses were determined by the method of Litchfield and Wilcoxon [9].

To obtain ED_{50} values for ether, animals were placed in 4 l jars with air tight lids and ether was injected through a rubber septum. Animals tested without ether administration showed no signs of hypoxia during the time necessary for testing.

RESULTS

Table 1 illustrates the sleep-time scores for the present sample of animals from the 14th generation of the sleep-time selection lines. A mean sleep-time score of 367 sec for the SS line and 9342 sec for the LS line is obtained following a dose of 3.75 g/kg of ethanol IP. These results can be compared with times after administration of various hypnotic drugs. Both methanol and butanol cause animals of the LS line to sleep significantly longer than those of the SS line. However, there is no statistically significant difference among the lines in pentobarbital, chloral hydrate, paraldehyde or trichloroethanol induced sleep-time.

The ED_{50} for loss of righting response should be a more definitive measure of CNS sensitivity for these 2 lines of animals, since any metabolic changes will be less important in the few min required to produce loss of righting response in the animals than during the course of sleep-time determination. Such experiments were carried out and the results presented in Fig. 1. The dose response lines are essentially parallel. In Table 2 are listed the ED_{50} values with the 95% confidence limits for these alcohols and for sodium pentobarbital and ether It can be seen from the data that there is approximately a 2 fold difference in ED_{50} for the various alcohols between LS and SS animals. However, there is no difference between HS, LS and SS lines in ED_{50} for Na pentobarbital or between the SS and LS mice in ED_{50} for ether.

Previously we had found no difference in the rate of metabolism of ethanol in the 2 lines, yet the LS line sleeps much longer. Obviously then, the SS animals must awaken at higher blood alcohol levels than do the LS animals. This prediction is fulfilled as illustrated in Table 3.

Table 4 lists the LD_{50} doses for ethanol. The LS animals

TABLE 1

LONG SLEEP (LS), SHORT SLEEP (SS) AND HETEROGENEOUS STOCK (HS) SLEEP TIMES WITH VARIOUS HYPNOTIC AGENTS*

Animal Line	Drug	(n)	Dose†	Sleep Time (sec)	p Value
SS	Ethanol	10	3.75	367 (±547)‡	<0 01
LS	Ethanol	10	3 75	9,342 (±5,969)	
SS	Methanol	10	4 5	52 (±77)	<0 01
LS	Methanol	10	4 5	18,456 (±10,750)	
SS	n-Butanol	10	0 567	280 (±384)	<0 01
LS	n-Butanol	10	0 567	2,169 (±890)	
SS	Pentobarbital	70	0 060	2,107 (±1,678)	ns
LS	Pentobarbital	56	0 060	1,414 (±873)	
HS	Pentobarbital	10	0 060	2,500 (±1,035)	ns
SS	Chloral Hydrate	19	0 45	5,502 (±1,842)	ns
LS	Chloral Hydrate	20	0 45	7,203 (±1,662)	
SS	Paraldehyde	20	1 0	2,766 (±1,172)	ns
LS	Paraldehyde	19	1 0	3,720 (±846)	
SS	Trichloroethanol	10	0 225	1,502 (±1,263)	ns
LS	Trichloroethanol	10	0 225	2,542 (±844)	

*Sleep times were taken as the time from the loss of righting reflex to the time the animal was able to right itself three times within 30 sec.

†IP dose in g/kg

‡Represents ± the standard deviation

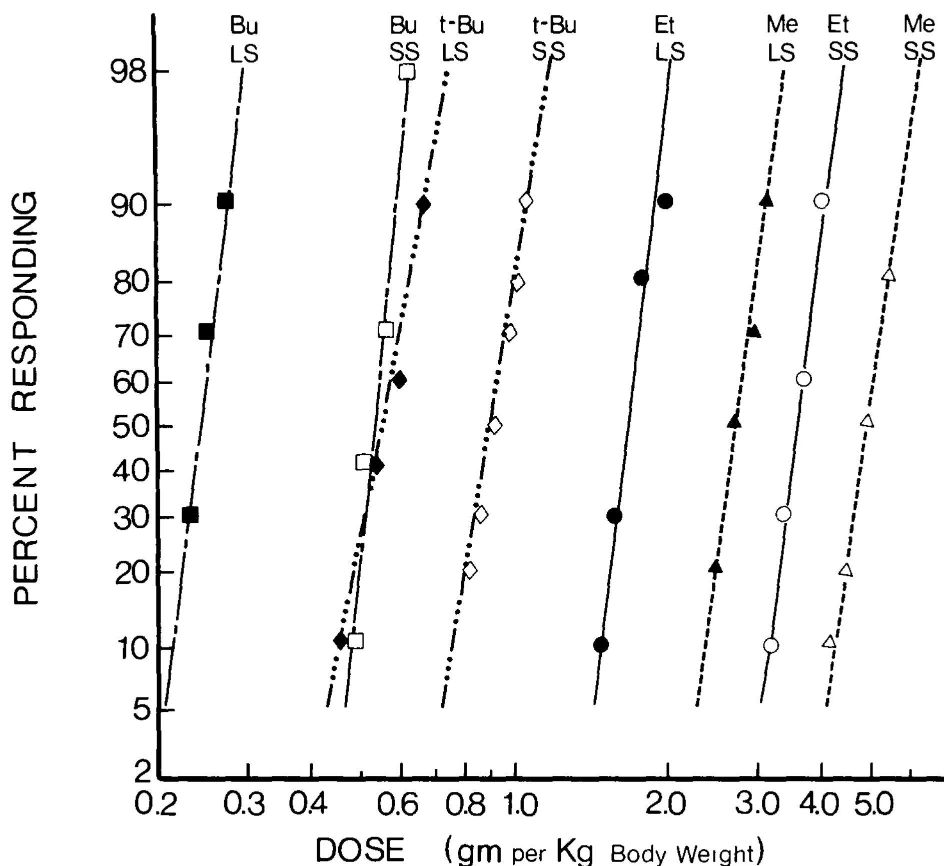

FIG 1. Dose response curve for alcohol in SS and LS mice Methanol; (Me) LS mice (▲- - - - - -▲), SS mice (△- - - - - -△) Ethanol (Et) LS mice (●————●); SS mice (○————○) Butanol (Bu) LS mice (■- ————-■), SS mice (□- ————-□) t-Butanol (tBu) LS mice (◆——···——◆), SS mice (◇——···——◇)

TABLE 2

ED50 VALUES FOR LOSS OF RIGHTING REFLEX WITH VARIOUS HYPNOTIC AGENTS*

Animal Line	Drug	ED50 (g/kg, IP)	ED50 Ratio SS/LS
SS	Ethanol	3 7 (±0.11)†	2 24
LS	Ethanol	1 65 (±0 09)	
SS	Methanol	5 0 (±0 24)	1 82
LS	Methanol	2 75 (±0 11)	
SS	Butanol	0 535 (±0.026)	2 18
LS	Butanol	0 245 (±0 011)	
SS	t-Butanol	1 39 (±0 065)§	1 65
LS	t-Butanol	0 84 (±0 078)§	
SS	Na Pentobarbital	0.039 (±0.0028)	
LS	Na Pentobarbital	0 038 (±0 0026)	
HS	Na Pentobarbital	0 040 (±0.0027)§	
SS	Ether	1 04 (±0 26)‡§	
LS	Ether	1 04 (±0.32)‡§	

*ED50 values determined by the method of Litchfield and Wilcoxon (n=10 animals each at four different doses)
†Represents 95% confidence limits
‡mM/l by inhalation
§Tested in the 18th generation or contemporaneous HS stock

TABLE 3

BLOOD ALCOHOL CONCENTRATIONS IN SS AND LS MICE AT TIME OF AWAKENING

Animal Line	Sleep Time sec ± SD (n)	Blood Alcohol mg% ± SD (n)
SS	3937 ± 2502 (10)	380 2 ± 52 6 (10)
LS	16069 ± 5443 (12)	230 0 ± 43.5 (12)
	$p<<0$ 001	$p<<0$ 001

Mice of each line were given 4 1 g/kg ethanol as outlined in the text This dose was necessary to insure loss of righting response in all SS mice At the time of regaining the righting reflex, blood was taken from the retro-orbital sinus and blood alcohol determined by gas chromatography as previously described [5].

are only slightly more sensitive to the acute toxic effects of ethanol. It should be noted that at the higher doses of methanol and t-butanol animals were often found dead the following day, even though they had not always acutely lost the righting response.

DISCUSSION

In certain generations, a sample of HS mice is tested for ethanol sleep time contemporaneously with the LS and SS

TABLE 4

LD_{50} VALUES FOR ETHANOL IN SS AND LS SELECTED LINES OF MICE*

Animal Line	LD_{50}†
	g/kg
SS	4 8 (±0 2)‡
LS	4.5 (±0 2)

*Lethality was determined by the number of deaths occurring within 24 hr following IP injections of various doses of ethanol (n=8 at each of 5 doses)

†According to the method of Litchfield and Wilcoxon [9]

‡Represents 95% confidence limits

mice These samples show the expected gaussian distribution of sleep-times that was present in the foundation stock HS animals. The SS and LS mice are mated in such a way that mating pairs never have common grandparents. Thus, in distinction to inbred mouse strains that show differences in sleep times similar to those observed in this report [7,12], the genetic variability present initially in the HS stock with respect to phenotypes unrelated to ethanol sleep time is preserved as much as possible. This preservation of heterogenity is shown by the fact that the responses of HS, LS and SS mice to pentobarbital do not differ. There is a significant difference in ED_{50} between lines for loss of righting response for methanol, ethanol, butanol and *t*-butanol.

Previously we had demonstrated that there was no change in the rate of alcohol metabolism between the 2 lines [5]. We have not made similar determinations for the other alcohols tested here However, each alcohol tested is metabolized via a different enzymatic system. The metabolic route for methanol in the rodent is primarily via the catalase system [10]. Ethanol is metabolized primarily via the alcohol dehydrogenase system. *t*-Butanol is only slowly metabolized if at all via conjugation with glucoronide [18], although other possible routes of metabolism have not been studied. It would seem unlikely that we have selected for similar changes in several diverse metabolic pathways, and we conclude, therefore, that the selective breeding program has developed lines that differ in brain sensitivity to alcohols in general, not just to ethanol

It appears that we have not selected for a general CNS sensitivity to depressants because none of the other CNS depressants show differential effects in the two lines. This observation is somewhat surprising since all the tested compounds (pentobarbital, ether, chloral hydrate and paraldehyde) are at least additive with ethanol in their effects on the CNS. In particular ether, chloral hydrate and paraldehyde have long been thought to possess a similar if not identical mechanism to that of ethanol [13]. These results permit a conclusion that the mechanism of these compounds all differ in some respect from that of ethanol even though the final common result is the same. Firm conclusions concerning the non-alcohol drugs must await analysis of their metabolism in the 2 lines of mice, but again it is unlikely selection has been change simultaneously in a large, diverse group of disposition pathways.

It is also of interest that the LD_{50} values for ethanol do not differ in the two lines indicating that the mechanisms responsible for sleep are not the same as those responsible for eventual death from overdosage

Sleep time determinations with these animals are subject to a number of variations from experiment to experiment as evident in Table 3 and our previous results [5]. The values of 3937 sec vs 1250 sec for the SS line and 16069 vs 6250 sec for the LS line shows the consistency in the line differences but also illustrate variability in the absolute numbers. The reasons for the variability are unknown but certainly different injection techniques and different laboratory environments contribute. In any given experiment the age of all of the animals was approximately the same. From experiment to experiment there were age differences and this may account for some of the variations [1]. It is also critical to point out that the absolute difference in sleep times between SS and LS mice is dependent upon the blood level of alcohol achieved. This is best illustrated by considering the case where the level is so low that few SS mice lose the righting response but the LS mice sleep a substantial time. From Table 3 it is seen that such a level would be approximately 327 mg% Given that the LS mice must reach 230 mg% for half of the mice to awake and that the rate of metabolism is about 19 mg% per 1000 sec [5] the LS mice would sleep about 5000 sec on the average. These calculations are possible because of the zero order rate of ethanol elimination

Siemens and Chan [16] reported that pentobarbital produced a significantly longer loss of righting reflex in SS mice than in LS mice but that this was due to a different apparent volume of distribution of the drug in the two lines. In the present study we did not observe a statistically significant difference in the sleep times but did find a slightly longer loss of righting response in the SS mice.

Goldstein and Kakihana [3] tested the SS and LS lines for severity of withdrawal after 3 days of intoxication. They find that the SS mice have more severe withdrawal reactions than do the LS mice and these differences are not casually related to the line differences in acute alcohol effects.

Recently Collins (personal communication) found that the influence of ethanol on the turnover of dopamine differed by about 2 fold in the SS and LS mice although no differences in norepinephrine or serotonin turnover were observed Analysis of the major synthetic and degradative enzymes of the dopamine pathways revealed no differences. Fuller (personal communication) has observed a difference in the sleep time induced by salsolinol (the condensation product of dopamine and acetaldehyde) when injected intracranially. From these data then it would appear that study of the dopaminergic system might prove fruitful in the search for a biochemical explanation of results reported in this paper.

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