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Thiopental, Phenobarbital, and Chlordiazepoxide Induce the Same Differences in Narcotic Reaction as Ethanol in Long-Sleep and Short-Sleep Selectively-Bred Mice

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McINTYRE, T D AND H P ALPERN *Thiopental, phenobarbital, and chlordiazepoxide induce the same differences in narcotic reaction as ethanol in Long-Sleep and Short-Sleep selectively-bred mice* PHARMACOL BIOCHEM BEHAV 24(4) 895–898, 1986 —Hypnotic effects following administration of thiopental, phenobarbital or chlordiazepoxide were evaluated in mice selectively-bred for differential hypnotic sensitivity to ethanol For every dose employed, except one which had no effect, all three agents induced greater sedation in the ethanol-sensitive Long-Sleep (LS) line than in the ethanol-insensitive Short-Sleep (SS) line Such findings with regard to the LS and SS lines suggest that the differences in sedative response to ethanol, as well as some barbiturates and benzodiazepines, may be mediated, in part, by a common mechanism The second experiment showed that age of the subjects can be an important variable influencing hypnotic-induced sleep time For thiopental, significant line differences occurred only with 150 day old mice, whereas chlordiazepoxide produced differences in 50, 75, 100 and 150 day old mice

Pharmacogenetics LS/SS Alcohol Barbiturates Benzodiazepines

TWO lines of mice that have been selectively-bred for different hypnotic reactions to a sedative dose of ethanol may be interesting models for elucidating mechanisms affected by CNS depressants, because there is a growing body of evidence indicating that the LS and SS lines can also be differentiated by butanol, methanol [8], chloral hydrate, trichloroethanol, paraldehyde [1,8], pentobarbital [1], barbital [1,12], adenosine [7], and nitrous oxide, enflurane, and isoflurane [13] A clear picture does not emerge, however, with respect to the above findings if one wishes to generalize about CNS depressants. Although it appears that there may be a general response pattern to agents classified as alcohols or perhaps general anesthetics, a similar conclusion cannot be made about barbiturates For instance, several investigators have reported similar line differences for barbital-induced sleep time [1,12], but the reports regarding pentobarbital are not as consistent. Certain studies report that the SS line is more sensitive to the soporific effects of pentobarbital in comparison to the LS line [5,17], while other studies report the opposite results [1]. It has been suggested that the pentobarbital findings are related to lipid solubility [11], or procedural variables such as dose, age, and time of day testing took place [1,16].

In order to clarify the nature of the response pattern to barbiturates, we examined the response pattern in LS and SS for a barbiturate with higher lipid solubility than pentobarbital (thiopental), and one with a lower lipid solubility (phenobarbital) than pentobarbital. Further, benzodiazepines are a major class of depressants for which the hypnotic response pattern in LS and SS mice has not been ascertained. Finding similar response patterns in these mice for benzodiazepines and barbiturates would be important in view of the recent evidence implicating the GABA receptor in the depressant actions of both of these agents [18] Consequently, we examined the response pattern for chlordiazepoxide in the LS and SS mice.

In addition, a salient feature of reports dealing with these lines is that there is no consistency in the ages of animals employed (e g , 45–70 [6]; 50–130 [3], 60–90 [12], 150, vide infra). In the second experiment, therefore, the influence of age on comparative hypnotic responses of the two lines to thiopental and chlordiazepoxide was investigated

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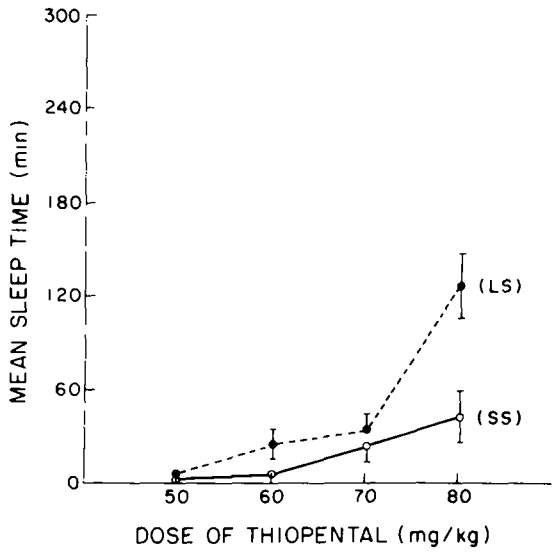

FIG 1 Mean sleeptime±S E M for each of the independent groups of LS and SS mice administered doses of thiopental sodium

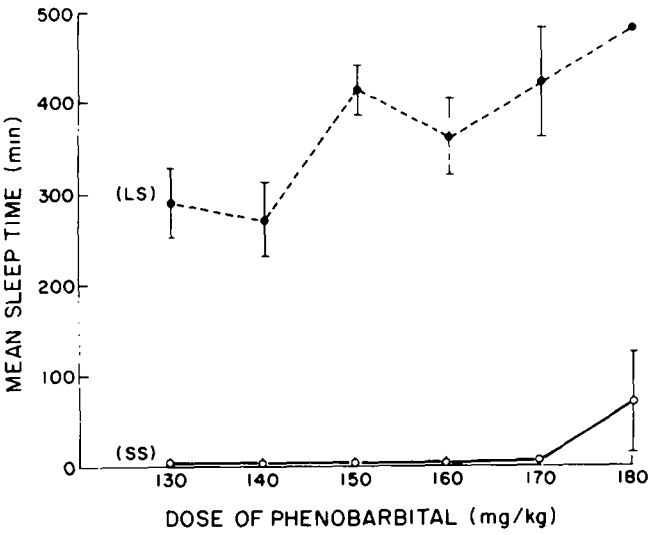

FIG 2 Mean sleeptime±S E M for each of the independent groups of LS and SS mice administered doses of phenobarbital sodium

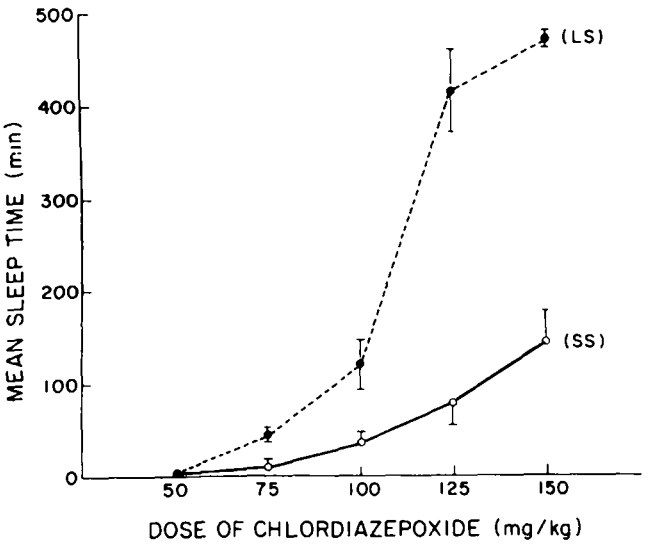

FIG 3 Mean sleeptime±S E M for each of the independent groups of LS and SS mice administered doses of chlordiazepoxide hydrochloride

TABLE 1

MEAN SLEEP TIME (MIN) ±S E FOR THIOPENTAL SODIUM (80 mg/kg) AND CHLORDIAZEPOXIDE HYDROCHLORIDE (100 mg/kg) ADMINISTERED TO LONG-SLEEP AND SHORT-SLEEP MICE AT 50, 75, 100 AND 150 DAYS OF AGE

Age	Thiopental		Chlordiazepoxide	
	LS	SS	LS	SS
50	73 5 ± 18 6	75 1 ± 23 3	178 2 ± 71 1†	2 9 ± 2 9
75	79 3 ± 15 4	72 3 ± 30 8	212 4 ± 64 3†	34 4 ± 14 6
100	83 1 ± 32 8	32 3 ± 10 9	282 5 ± 74 7†	33 5 ± 27 2
150	122 0 ± 20 6*	39 1 ± 15 8	121 7 ± 29 2†	36 4 ± 12 2

*$p<0.01$ for t-test comparison between LS and SS
†$p<0.001$ for t-test comparison between LS and SS

EXPERIMENT 1

Method

A total of 240 male LS and SS mice 150 days old from the 36th production generation were obtained from the Institute for Behavioral Genetics, University of Colorado, Boulder 80309. Littermates were housed together, but randomly distributed across experimental groups Animals were maintained on a 12 hr light/dark cycle with food and water continuously available Thiopental sodium dissolved in 0 9% saline was administered intraperitoneally in an injection volume of 0 1 ml/gram body weight to four independent groups (50, 60, 70 and 80 mg/kg) each of LS and SS mice containing 8 animals each Using the same procedure, phenobarbital sodium was administered in doses of 130, 140, 150, 160, 170 and 180 mg/kg, while chlordiazepoxide hydrochloride was administered in doses of 50, 75, 100, 125 and 150 mg/kg All injections were made between 0730 and 1130 hr and animals were allowed to sleep up to 8 hr before being returned to their home cages. Sleep time was assessed in the following manner. After injection, an animal was placed on its back in a V-shaped (90° angle) Plexiglas sleep trough until it was unable to right itself four times within 60 seconds, at which time it was considered to have lost its righting reflex An animal was considered to have regained its righting reflex when it could right itself four times within 60 seconds, unless it slept 8 hr and that was considered its sleep time It should be noted that no animal succumbed to the drug treatment

Results

The results of this experiment were unequivocal For every dose of thiopental, phenobarbital, and with the exception of the 50 mg/kg dose of chlordiazepoxide, the LS mice slept longer than SS mice (Figs 1, 2 and 3) For every dose of the drugs employed, the Rank Sum test, which is the non-parametric equivalent of the *t*-test for two independent groups, was used to statistically verify these results (see Dixon and Massey, 1957). A non-parametric statistical test was chosen because many of the groups lacked homogeneity of variance, which could not be corrected by recommended mathematical transformations (i e., within many of the groups the scores were identical)

For thiopental, differences between lines were significant for all doses (50 mg/kg $p<0.001$, 60 mg/kg $p<0.05$; 70 mg/kg $p<0.05$; 80 mg/kg $p<0.001$). For phenobarbital, differences between lines were significant for all doses ($p<0.001$). With the exception of 50 mg/kg, for chlordiazepoxide, line differences for all doses was highly significant ($p<0.001$).

EXPERIMENT 2

Method

Sixty-four mice, 32 from each line, were administered 80 mg/kg of thiopental sodium and 64 mice, 32 from each line, were administered 100 mg/kg of chlordiazepoxide hydrochloride. For each drug and each line there were 4 independent groups (n=8) of animals tested at 50, 75, 100 and 150 days of age. It should be noted that two animals administered thiopental were omitted from the experiment because they had sleep-times that were over 3 standard deviations from the means of their respective groups With the above exceptions the methods were identical to those outlined in Experiment 1 The doses of thiopental and chlordiazepoxide were selected because in Experiment 1 they had produced nearly identical sleep times within a given line

Results

Upon inspecting Table 1 it is quite clear that thiopental produced significant line differences only in mice of 150 days of age, whereas chlordiazepoxide produced significant line differences for all of the ages tested. For each drug and age tested *t*-tests were used to verify the differences between the two lines (see Table 1 for significant differences).

DISCUSSION

The results of these experiments indicate that the ethanol sensitive LS line is significantly more sensitive than the ethanol insensitive SS line to the benzodiazepine chlordiazepoxide, and to the barbiturates thiopental and phenobarbital. The exception to this finding appears to be for thiopental in animals up to 100 days of age, where line differences did not occur With that caveat, our results seem to support those reports which have demonstrated that the LS and SS lines show similar hypnotic sensitivities to a wide range of sedative-hypnotics and anesthetics (vide supra). We have suggested elsewhere [16] that, contrary to earlier reports, it is probably the case that these lines were not selectively-bred for just alcohol sensitivity.

For instance, we have recently reported [1] that the paper most often cited to support the alcohol-specificity hypothesis for the LS and SS lines actually supports a different interpretation. Reanalysis of these data showed that paraldehyde, chloral hydrate and trichloroethanol induced a significantly greater hypnotic effect in the LS line than in the SS line, while the opposite was found for pentobarbital. This finding with pentobarbital, as well as several other reports [5,17], is not in agreement with reports showing LS mice more sensitive than SS mice to pentobarbital [1], as well as barbital [1,12] It has been suggested that either lipid solubility [11] or procedural variables [1,16] may be responsible for these dissimilar findings. Although it seems plausible that an agent's lipid solubility should affect sleep time in these two lines of mice, the evidence to date seems equivocal For example, it has been shown that lipid solubility of long-chain alcohols influences narcosis in these lines [11]. However, it has also been shown that although the SS mice are less sensitive than LS mice to nitrous oxide, isoflurane and enflurane, no demonstrable relationship was discovered between sleep time and membrane phospholipid, fatty acid and cholesterol compositions [13]. Furthermore, our present findings with thiopental and phenobarbital, as well as previous results concerning barbital [1,12], indicate that lipid solubility is not a critical factor influencing barbiturate-induced hypnosis in these two lines.

With respect to procedural variables, the second experiment was designed to evaluate one of these factors, and, indeed, age was an important factor in determining the response to thiopental Thus, one should exercise caution before concluding that a particular depressant does not significantly distinguish these lines, unless age, and perhaps other design factors are fully explored.

It has been suggested that the data on CNS depressant-induced hypnosis in these lines, as well as those concerning susceptibility to several convulsant protocols, indicates that a possible candidate mechanism for the mediation of the differences between LS and SS mice may be the GABAergic system [14,16]

The majority of the previous neurochemical analyses of these lines have focused on the catecholamines at baseline and in response to alcohol. However, we have suggested that since the two behavioral responses which most clearly distinguish these lines (depressant-induced anesthesia and analeptic susceptibility) are predominantly mediated by GABAergic mechanisms in other circumstances, that a similar mechanism may be responsible for these distinctions in the LS and SS lines Additionally, GABA has been shown to influence dopaminergic activity [9] In further support of such a GABA hypothesis, it has been shown that GABA mimetics enhance the incoordinating and soporific effects of ethanol in LS and SS, while GABA antagonists had the opposite effect [14]

Using LS and SS mice Chan [2] did not discover any significant line differences in whole brain GABA content in several general regions, even though ethanol does cause a marked elevation of GABA levels for both lines The lack of significant line differences does not rule out a role for GABAergic mechanisms, however, since others have reported equivocal results [10, 19, 20] when ascertaining GABA's response to ethanol in other non-selected animals, and thus, this procedure may not reflect subtle cellular dynamics. It has also been reported recently that ICV picrotoxin reduces ethanol-induced sleep time in both lines, but since only one dose was employed any possible differential sensitivity was not fully evaluated [15] Further, if the particular site responsible for these differences is the GABA receptor itself, rather than the allosteric regulatory site where barbiturates and picrotoxin bind, then this procedure

might not reveal the relevant mechanism Alternatively, differences in receptor number, turnover, affinity, coupling dynamics or regulation may be the key factor(s) which distinguish these lines.

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REFERENCES

1 Alpern, H P and T D McIntyre Evidence that the selectively-bred long- and short-sleep mouse lines display common narcotic reactions to many depressants *Psychopharmacology (Berlin)* **85**: 456–459, 1985
2 Chan, A W K Gamma aminobutyric acid in different strains of mice Effect of ethanol *Life Sci* **19**: 597–604, 1976
3 Church, A C , J L Fuller and B C Dudek Salsolinol differentially affects mice selected for sensitivity to alcohol *Psychopharmacology (Berlin)* **47**: 49–52, 1976
4 Dixon, W J and F J Massey, Jr *Introduction to Statistical Analysis* New York McGraw-Hill Co , 1957, p 289
5 Dudek, B C , M E Abbott and T J Phillips Stimulant and depressant properties of sedative-hypnotics in mice selectively bred for differential sensitivity to ethanol *Psychopharmacology (Berlin)* **68**: 89–97, 1984
6 Dudek, B C and M E Abbott A biometrical genetical analysis of ethanol response in selectively bred Long-Sleep and Short-Sleep mice *Behav Genet* **14**: 1–19, 1984
7 Dunwiddie, T V and W R Proctor Behavioral sensitivity to purinergic drugs parallels ethanol sensitivity in selectively bred mice *Science* **224**: 519–521, 1984
8 Erwin, V G , W D W Heston, G E McClearn and R A Deitrich Effect of hypnotics on mice genetically selected for sensitivity to alcohol *Pharmacol Biochem Behav* **4**: 679–683, 1976
9 Fuxe, K , C Kohler, L F Agnati, K Anderson, S -O Ogren, P Eneroth, M Perez de La Mora, M Karobath and P Krogsgaard-Larsen GABA and benzodiazepine receptors Studies on their localization in the hippocampus and their interaction with central dopamine neurons in the rat brain In *GABA and Benzodiazepine Receptors*, edited by E Costa New York Raven Press, 1981, pp 61–76
10 Gordon, E R The effect of ethanol on the concentration of γ-aminobutyric acid in the rat brain *Can J Physiol Pharmacol* **45**: 915–918, 1967
11 Howerton, T C , M E O'Connor and A C Collins Differential effects of long-chain alcohols in long- and short-sleep mice *Psychopharmacology (Berlin)* **79**: 313–317, 1983
12 Howerton, T C , J B Burch, M F O'Connor, L L Miner and A C Collins A genetic analysis of ethanol, pentobarbital, and methyprylon sleep-time response *Alcoholism Clin Exp Res* **8**: 546–550, 1984
13 Koblin, D D and J E Deady Anaesthetic requirement in mice selectively bred for differences in ethanol sensitivity *Br J Anaesth* **53**: 5–10, 1981
14 Martz, A , R A Deitrich and R A Harris Behavioral evidence for the involvement of γ-aminobutyric acid in the actions of ethanol *Eur J Pharmacol* **89**: 53–62, 1983
15 Masserano, J M and N Weiner Investigations into the neurochemical mechanisms mediating differences in ethanol sensitivity in two lines of mice *J Pharmacol Exp Ther* **221**, 404–409, 1982
16 McIntyre, T D and H P Alpern A reinterpretation of the literature indicates differential sensitivities of Long-Sleep and Short-Sleep mice are not specific to alcohol *Psychopharmacology (Berlin)* **87**: 379–389, 1985
17 O'Connor, M F , T C Howerton and A C Collins Effects of pentobarbital in mice selected for differential sensitivity to ethanol *Pharmacol Biochem Behav* **17**: 245–248, 1982
18 Olsen, R W Drug interactions at the GABA receptor-ionophone complex *Annu Rev Pharmacol Toxicol* **22**: 245–277, 1982
19 Roach, M K and W N Reese Effect of ethanol on glucose and amino acid metabolism in brain *Biochem Pharmacol* **20**: 2805–2812, 1971
20 Sytinsky, I A , B M Guzikov, M V Gomanko, V P Erenien and N N Konovalova The gamma-aminobutyric acid (GABA) system in brain during acute and chronic ethanol intoxication *J Neurochem* **25**: 43–48, 1975