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Hypothermic Effect of Ethanol in Mice Selected for Differential Sleep-Time Response to Pentobarbital

KEMMY M. MIZINGA, FARID K. R. STINO, SABER S. SAMAAN, KARAM F. A. SOLIMAN AND MALAK G. KOLTA[1]

College of Pharmacy and Pharmaceutical Sciences, Florida A&M University, Tallahassee, FL 32307

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MIZINGA, K. M., F. K. R. STINO, S. S. SAMAAN, K. F. A. SOLIMAN AND M. G. KOLTA. *Hypothermic effect of ethanol in mice selected for differential sleep-time response to pentobarbital.* PHARMACOL BIOCHEM BEHAV 51(2/3) 525–528, 1995.—The hypothermic action of ethanol was investigated in genetically distinct lines of mice selected for sleep-time response to pentobarbital for six generations. Ethanol (3 g/kg, intraperitoneally) was administered to alcohol-naive males and females from each of the unselected control, long-, and short-sleep mouse lines. Rectal temperatures were measured immediately before, and at 15, 30, 60, 90, 120, and 240 min after ethanol injection. Eight female and eight male mice from each line were sacrificed at each time point, and trunk blood was collected for plasma ethanol analysis. The results show that short-sleep mice were less hypothermic ($p < 0.05$) compared to long-sleep mice at 15 and 30 min after ethanol administration. However, plasma ethanol concentrations were not significantly different between the mouse lines at any time point. Therefore, the line-dependent differential ethanol-induced hypothermia observed may be a result of differences in "brain sensitivity" rather than in the rates of ethanol metabolism among the mouse lines.

Ethanol Pentobarbital Hypothermia Long-sleep Short-sleep Mice

MOUSE lines selectively bred for differential sensitivity to hypnotic doses of ethanol (8) were used previously to study pharmacologic actions of ethanol and barbiturates (9). Cross-tolerance to barbital accompanied the development of tolerance to ethanol-induced hypothermia (EIH), ataxia, and narcosis, whereas only a marginal degree of cross-tolerance to pentobarbital was observed (5). These reported differences in the degree of cross-tolerance between ethanol and various barbiturates may indicate the existence of a degree of specificity in the sites of action of these drugs.

In mice bred for differential sensitivity to ethanol-induced loss of righting reflex (LORR), long-sleep (LS) mice were consistently more sensitive compared to short-sleep (SS) mouse lines to other effects of ethanol. These effects included depression of body temperature (2), increase in liver plasma membrane Na^+, K^+-ATPase (18), elevation of plasma corticosterone concentration (17), and depression of cerebellar Purkinje cell firing (10). However, reports on the relative sensitivity of LS and SS mice to pentobarbital-induced LORR are discrepant. Greater (14), similar, or less (1) sensitivity to pentobarbital-induced LORR was reported in short-sleep (SS) compared to long-sleep (LS) mice selectively bred for differential sensitivity to hypnotic doses of ethanol, depending on the quantity of pentobarbital administered. Results from these studies suggested that factors which govern brain sensitivity to ethanol and pentobarbital were not equivalent. Therefore, selection for long-sleep time (LST) or short-sleep time (SST) based on differential responsiveness to hypnotic doses of pentobarbital instead of ethanol may result in mouse lines with substantially different pharmacologic responses to ethanol. The present experiment was designed to investigate the hypothermic action of ethanol in two distinct lines of mice selected for LST or SST response to pentobarbital.

METHOD

Random-bred Swiss mice were obtained from the National Institutes of Health. Two lines were selected for differences in pentobarbital-induced sleep time, defined as the duration of the pentobarbital [50 mg/kg, intraperitoneally (IP)]-induced

[1] To whom requests for reprints should be addressed.

loss of righting reflex (16). Animals were maintained at 21 ± 1°C under a 12 L : 12 D cycle with water and food provided ad lib. The first line was selected for LST, the second for SST. A third line was maintained as an unselected control line. Figure 1 presents the generation-to-generation progression in divergence in sleep times for LST and SST mice.

To study ethanol-induced hypothermia in LST, SST, and controls, 112 sixth-generation mice (56 males and 56 females) from each line were used. All experiments were conducted between 1000 and 1430 h with ambient temperature maintained at 21 ± 1°C. Ethanol (20% solution prepared from absolute ethanol with saline) was administered to mice (3 g/kg, IP). Rectal temperatures were measured using a Tele-Thermometer (YSI Model 431; Yellow Springs Instrument Company, Yellow Springs, OH) before and at 15, 30, 60, 90, 120, and 240 min after ethanol administration. Immediately after each time point, eight female and eight male mice from each line were sacrificed and trunk blood was collected for plasma ethanol analysis (11). The sensitivity of the enzymatic ethanol assay used in the present study was 0.94 mg/dl with an intra- and interassay coefficient of variation of 1.7 and 2.1%, respectively. Data were analyzed by least squares analysis of variance using the SAS system (12). Separation of means was performed according to the method described by Scheffé (13).

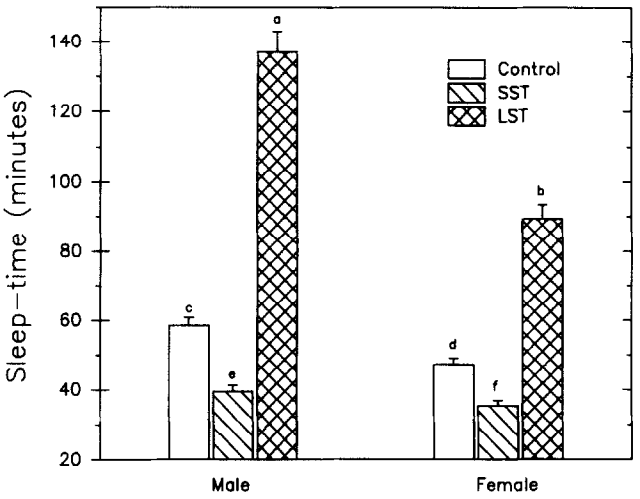

FIG. 2. Sleep-time response (mean ± SEM) to pentobarbital (50 mg/kg, IP) in unselected control mice and in mouse lines selected for six generations for differential sleep-time response to pentobarbital. SST, Short-sleep line; LST, long-sleep line. Bars without a common superscript (a–f) are significantly ($p < 0.0001$) different.

RESULTS

The sleep times in response to pentobarbital (50 mg/kg IP), for the littermates of the mice used in this study are presented in Fig. 2. Sleep times for the three mouse lines were significantly different from each other. The sleep times (mean ± SEM) for male LST, SST, and unselected control mice were 137.0 ± 5.9, 39.4 ± 1.8, and 58.5 ± 2.3 min, respectively. Sleep times for females of corresponding lineage were 89.4 ± 3.9, 35.3 ± 1.5, and 47.2 ± 1.8 min, respectively. Males from all mouse lines slept significantly [$F(2, 599) = 11.91$, $p < 0.0001$] longer compared to females of corresponding lineage.

Maximal hypothermia was observed in all mouse lines 30 min after ethanol administration (Fig. 3). Data from males and females of each mouse line at each time point were pooled because sex had no statistically significant influence on the hypothermic response. The magnitude of ethanol-induced hypothermia (mean ± SEM) was lower in SST compared to LST mice at 15 and 30 min posttreatment [2.26 ± 0.13 vs. 2.75 ± 0.16; $F(2, 282) = 9.25$, $p < 0.0001$, and 2.35 ± 0.14 vs. 2.99 ± 0.19; $F(2, 234) = 12.60$, $p < 0.0001$, respectively] but was not different for the remainder of the experiment (Fig. 3). There was no significant difference in hypothermia between controls and either LST or SST mice at any time point.

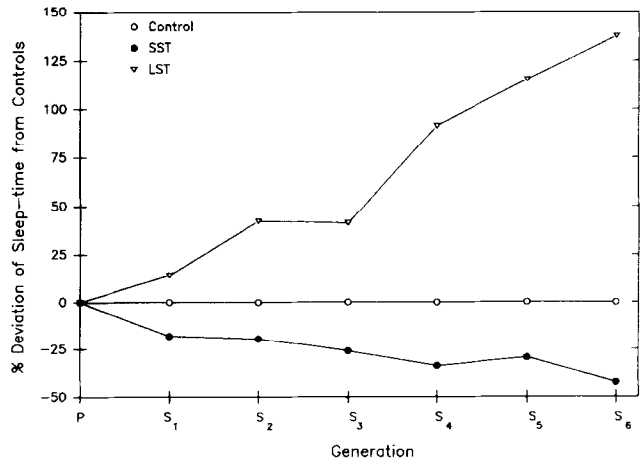

FIG. 1. Divergence of sleep time (percent deviation from respective contemporary controls) in successive generations of mice selected for differential responsiveness to a hypnotic dose (50 mg/kg, IP) of pentobarbital. P, Parents; S_i = ith selected generation; SST, short-sleep line; LST, long-sleep line.

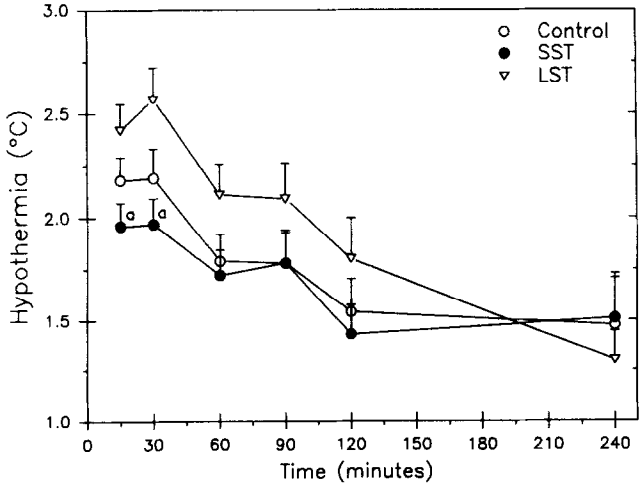

FIG. 3. Ethanol-induced hypothermia (mean ± SEM) in unselected control mice and mouse lines selected for six generations for differential sleep-time response to pentobarbital. SST, Short-sleep line; LST, long-sleep line. (a) SST mice significantly ($p < 0.0001$) less hypothermic than LST mice.

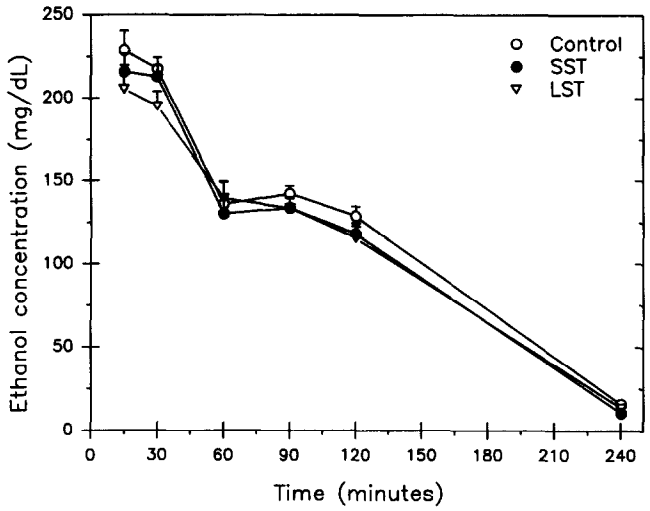

FIG. 4. Ethanol concentration-time profiles (mean ± SEM) in unselected mice and mouse lines selected for six generations for differential sleep-time response to pentobarbital. SST = Short-sleep line; LST, long-sleep line.

Plasma ethanol concentrations did not differ significantly among the mouse lines at any of the time points studied (Fig. 4). The data from males and females of each mouse line were pooled at each time point because sex had no statistically significant effect on plasma ethanol concentrations.

DISCUSSION

The mouse lines used in the present study were sufficiently divergent in their pentobarbital-induced LORR and were therefore suitable subjects for the experiment. After combining the data from male and female littermates of the animals used in this study, the difference between SST and LST mice represented a 188% disparity in pentobarbital-induced sleep time. The sleep time of SST mice was 44% shorter, and that of LST mice 144% longer compared to unselected controls. Previous reports of asymmetrical cross-tolerance to ethanol and pentobarbital suggested the existence of a degree of specificity in the site(s) of action of these drugs (5–7). Interestingly, the differential sleep-time response of LST and SST mice to ethanol in the present study is analogous to previously documented observations in LS and SS mice selectively bred for differential sensitivity to ethanol (8). Our results suggest that EIH in mice selected for differential sensitivity to hypnotic doses of pentobarbital follows similar response patterns to those observed in mice selected using ethanol. These results suggest that there may be overlaps in the effect of the selection using ethanol and pentobarbital when the differential responsiveness to the hypnotic effect of either agent is used as the selection criterion.

SST mice were less hypothermic compared to LST mice during the first 30 min following ethanol administration. However, the hypothermic responses of LST and SST mice were not significantly different from those observed in controls at any of the time points studied. Despite disparities in hypothermia, plasma ethanol concentrations were not significantly different between sexes or mouse lines selectively bred for differential response to hypnotic doses of pentobarbital. In previous studies, the magnitude of sleep times and EIH were greater in Roman Low Avoidance (RLA) compared to Roman High Avoidance (RHA) female rats (4). However, plasma ethanol concentrations in these two lines were not different at 15, 30, 60, 90, or 120 min after the administration of ethanol (3). Differential responses of RLA and RHA rats were ascribed to sensitivity differences in the CNS (4). Another study (15) revealed that differences in ethanol or acetylaldehyde metabolism did not contribute significantly to differential effects of ethanol between young LS and SS mice selectively bred for differential sensitivity to hypnotic doses of ethanol. In that study, differential hypnotic effects of ethanol in LS and SS mice were ascribed to differences in CNS sensitivity.

Our results obtained from mouse lines (LST and SST) selectively bred for differential response to hypnotic doses of pentobarbital are therefore in agreement with previous findings in which mice (LS and SS) or rats (RHA and RLA) selected for differential sensitivity to ethanol were used. We conclude from this study that differences in CNS sensitivity may have contributed to differential responsiveness to the hypothermic effects of ethanol in mouse lines selected using either ethanol or pentobarbital. Therefore, there may be overlaps in the overall effects of selection based on differential responsiveness to hypnotic doses of ethanol or pentobarbital.

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