Pharmacology Biochemistry and Behavior, Vol. 45, pp. 465–472, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Tolerance to Effects of High Doses of Ethanol: 1. Lethal Effects in Mice

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Received 28 July 1992

TSIBULSKY, V. L. AND Z. AMIT. *Tolerance to effects of high doses of ethanol: 1. Lethal effects in mice.* PHARMACOL BIOCHEM BEHAV 45(2) 465–472, 1993.—Male Swiss Webster mice were injected with ethanol doses ranging from 6.5–10.5 g/kg (20% w/v, IP). Survival time distribution revealed three waves of deaths with peaks around 5 min, 300 min, and 33 h. There were two windows with very low density of probability of death between 30–130 min and between 22–25 h following lethal injections. This time structure of the probability density function did not significantly depend upon ethanol overdose, novelty of the experimental environ ment, or prior injections of saline and/or 3.5 g/kg ethanol. Injections of high doses of ethanol in BALB/c mice showed that this strain of mice was more sensitive to ethanol-induced lethality (LD_{50} = 6.6 g/kg) and over 99% of deaths occurred between 5–200 min following injections of the doses from 5.5–7.5 g/kg. Preexposure to ethanol increased tolerance to ethanol-induced lethality. LD_{50} increased from 8.1 g/kg (at 24 h following lethal injections in ethanol-naive Swiss Webster mice) to 8.5 and 9.0 g/kg in mice following four and eight injections of 3.5 g/kg ethanol, respectively. In BALB/c mice, eight prior injections of 3.5 g/kg ethanol increased LD_{50} also slightly but significantly to 7.15 g/kg. The results suggest that: a) Ethanol-induced lethality is not a unitary phenomenon and that deaths thta occurred within distinct waves probably have different causes; b) mice strains have different susceptibility to different causes of ethanol-induced deaths; c) preexposure to 3.5 g/kg ethanol results in significant but small increase in tolerance to ethanol-induced lethality.

Tolerance Lethal effects Novelty Ethanol Mice strains

STUDIES on the behavioral effects of ethanol have been limited usually to administration of low and intermediate doses. There have been, however, a few attempts to investigate ethanol-induced lethality as a behavioral phenomenon. For example, Melchior and Tabakoff (12,13) have shown that the LD_{50} for ethanol was significantly higher in an environment previously associated with administration of the drug than in a novel environment. On a number of different dimensions, high-dose toxic effects, such as lethality, resemble the well-known behavioral effects of ethanol, such as the hypnotic and discoordinating effects. Sensitivity to the lethal effects increases when body temperature is increased (4,5). It was also reported that adult animals are more sensitive to the lethal effects of ethanol than young animals (16,18). Lethality was reduced by prior administration of the catalase inhibitor 3-amino-1,2,4-triazol (1), and ethanol was found to be more lethal during the dark phase as compared to the light phase (4). Nevertheless, the mechanisms underlying this similarity between toxic and behavioral effects of ethanol are still unknown because the phenomenon of ethanol-induced lethality has not been adequately studied altogether.

The present study was conducted to determine the basic pharmacodynamic characteristics of ethanol-induced lethality, such as cumulative distribution and probability density functions of survival time and dose-response relationship in Swiss Webster and BALB/c male mice. It was also designed to examine the effects of routine laboratory treatments, such as moving animals into different environments, as well as saline injections, on ethanol-induced lethality. Finally, changes in tolerance to the lethal effects produced by preexposure to a variety of doses of ethanol have also been investigated.

GENERAL METHOD

Swiss Webster male mice (19–24 g) and BALB/c male mice (18–23 g) (Charles River Canada, Inc.) were housed in groups of five in standard macrolon cages. For 6 days before the onset of the experiments, all animals were maintained on a 12 L : 12 D cycle (light 0800–2000) in the colony room, which was regulated for constant temperature (22 + 1°C) and humidity. Food and water were available ad lib.

Ethanol solution was prepared 2 days before the beginning of administrations by mixing 96% distilled (in the laboratory) ethanol and saline to make a 20% (w/v) concentration. Injec-

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tions were given IP and the lethal doses were administered between 0900–1200 h. Latency to loss and recovery of righting reflex (LORR and RORR, respectively) and time of death were measured. Death was defined as the absence of breathing and heartbeats for 30 s. Criterion for recovery of righting reflex was three successful rightings within a 30-s period. The integrity of the peritoneal cavity and the condition of the gastrointestinal tract were examined in dead animals.

STATISTICAL ANALYSIS

Survival time distributions were analysed by standard methods (9). The lethal effects of ethanol were expressed as a percent of dead animals at a given time (mortality). Dose-lethality curves based upon the cumulative mortality during 2 h, 24 h, or 10 days after injections of ethanol were analyzed by the method of Litchfield and Wilcoxon (10). All animals had latency to LORR determined but only some of them had all three variables evaluated because a number of them died before awakening or did not die at all. Therefore, multiple analysis of variance (MANOVA) could not be applied and ANOVA was conducted separately for latencies to LORR and RORR and for survival time. There is no suitable method to replace missed values for latency to RORR after injections of the LD_{50} or higher doses of ethanol, and these doses, therefore, were excluded from ANOVA for latency to RORR. Latency to RORR and survival time were taken as logarithms of actual minutes when the ANOVA and regression analysis were conducted because these variables showed a lognormal distribution. ANOVA was used to compare the effects of treatment conditions (e.g., novelty: noMOV-MOV; saline preexposure: NONE-8SAL-16SAL; ethanol preexposure: NONE-4EtOH-8EtOH-20EtOH; and ethanol doses ranging from 6.5–10.5 g/kg) followed by Tukey's posthoc test. Significance was set at the $p < 0.05$ level.

EXPERIMENT 1: SURVIVAL TIME IN SWISS WEBSTER MICE

The purpose of this experiment was to explore thoroughly the features of the probability of death at variable time points and establish the appropriate intervals for the assessment of dose–effect relationship. The second purpose was to determine the effects of novelty on survival time and mortality.

METHOD

Two-hundred and 6 male Swiss Webster mice were given injections of high ethanol doses ranging from 7.0–9.5 g/kg in the colony room (Rm-c) or in the experimental room (Rm-1; a small room, one floor below Rm-c, with ongoing noise from a radio).

RESULTS AND DISCUSSION

Cumulative mortality curves revealed at least three waves of deaths with intervals within which no death occurred. No mice died between 77–130 min and between 22–26 h after ethanol injections regardless of ethanol doses. This pattern of survival time distribution did not appear to depend upon the factor of novelty, as the F-test of survival analysis had shown, $F(132, 132) = 1.06$, $p = 0.37$, and the data were therefore collapsed for each dose (Fig. 1A). Following injection of the highest dose (9.5 g/kg), the largest number of animals died during the first 20 min. However, around half the mice survived past this first wave of deaths after injection of 8.5–9.0 g/kg but died within the next 22 h. Among the animals receiv-

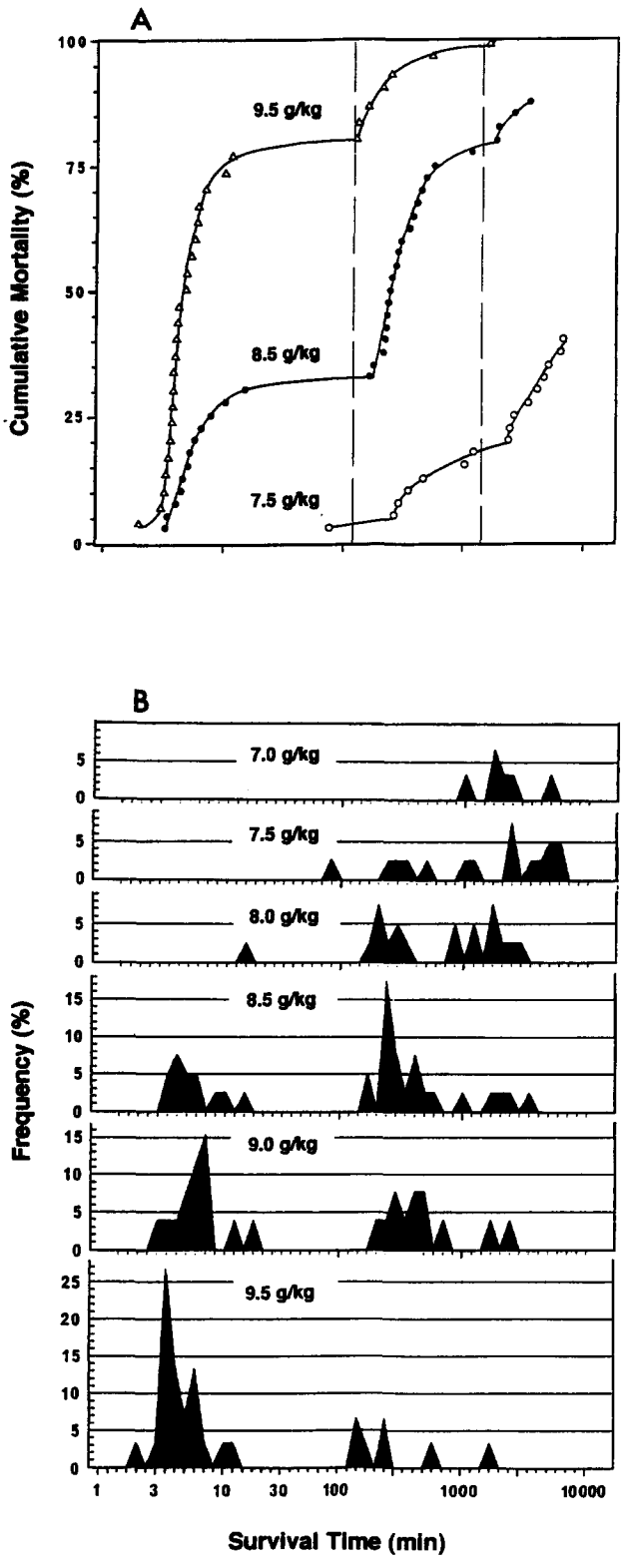

FIG. 1. (A) Cumulative mortality following injections of 7.5, 8.5, and 9.5 g/kg ethanol in naive Swiss Webster mice. Time points at 120 min, 24 h, and 7 days were selected for assessing the dose–response relationships. (B) Survival time distribution following injections of ethanol in doses ranging from 7.0–9.5 g/kg in naive Swiss Webster mice.

ing 7.0–8.0 g/kg ethanol, few deaths occurred during the first 77 min after injection. Most of these animals died within 2–22 h or 2–5 days following injections. Survival time distribution revealed three peaks around 5 min, 5 h, and 33 h after injections of ethanol overdoses (Fig. 1B).

The distribution of survival time was not normal or lognormal and was also not continuous; therefore, ANOVA and Kruskal–Wallis ANOVA by ranks could not be applied to the whole sample. However, survival time distribution within each single wave of deaths fitted lognormal distribution (κ_2 test, $p > 0.08$) and three waves of deaths were taken as three levels of an additional independent factor wave. Only one death occurred during the first wave of deaths after injections of 7.5 g/kg and none after 7.0 g/kg; therefore, these two doses were excluded from the analysis [current level: four doses × three waves, $F(3, 98) = 2.45$, $p = 0.07$]. For the same reason, the first wave was also excluded from the analysis of six doses × two waves, $F(5, 69) = 2.86$, $p = 0.02$. Posthoc analysis revealed significant difference only between the two lowest doses (7.0 and 7.5 g/kg) and the highest dose (9.5 g/kg). This result suggested that with increase in the lethal dose from 8.0 to 9.5 g/kg survival time did not seem to have slipped from higher to lower values but rather skipped from one wave of deaths to another (Fig. 1B).

Dose, $F(5, 95) = 22.62$, $p < 0.00001$, but not novelty, $F(1, 95) = 0.02$, $p > 0.86$, affected latency to RORR, which increased from 502 ± 12 min (7.0 g/kg) to 992 ± 103 min (8.5 g/kg). ANOVA did not reveal a significant effect of dose, $F(5, 193) = 1.04$, $p = 0.4$, or novelty, $F(1, 193) = 0.21$, $p > 0.65$, on latency to LORR. However, the interaction between these two factors was significant, $F(5, 193) = 3.27$, $p < 0.01$, and posthoc analysis showed that in a novel environment latency to LORR significantly increased after injections of 8.0 g/kg ($p < 0.005$) and decreased after 7.0, 7.5, and 8.5 g/kg ($p < 0.05$). Regression analysis showed no correlation between any pairs of dependent variables. This suggested that latency to LORR could not serve as a predictor for either latency to RORR or survival time and that perhaps the mechanisms controlling duration of LORR, RORR, and survival time are substantially different.

Two waves of ethanol-induced lethality were demonstrated earlier in dogs (8). Thus, oral administration of 9.5 g/kg ethanol led to deaths scattered around blood alcohol peaks between 4 and 9 h. No deaths occurred for the next 5 h and the second wave of deaths appeared between 14 and 24 h following ethanol administration (8). It seems likely that each separate wave of deaths has its own distinct cause. One could speculate that the highest levels of alcohol and acetaldehyde or the highest absorption rate of alcohol during the 20 min following injections caused a disorder of membrane function in respiratory neurons of the brain stem. It has been shown that dose-related increases in membrane fluidity can be observed at high concentrations of 4–16 mg/ml of ethanol for a variety of membranes except myelin (3). To the best of our knowledge, there are no studies on effects of ethanol on neuron activity of the respiratory center; however, it is clear that the direct effects of high doses of ethanol on spontaneous unit activity are primarily depressant (5). Klingman and Haag (8) showed that death during the second wave was characteristically preceded by a progressive decrease in blood pressure. This observation suggested a critical role for cardiovascular failure as a cause of death 22 h following injections of the highest doses of ethanol. In the present study, a relatively small number of deaths (less than 10%) occurred within 2–6 days after ethanol injections, when blood alcohol levels could obviously not be the cause of death. Postmortem investigation revealed a high frequency of gastrointestinal hemorrhages, ulcers, and peritonitis in these animals but not in animals that died within the first and second waves. It has been reported that deaths from peritonitis produced by 15–20% w/v ethanol did not occur within the first 48 h in rats (15). Thus, it is possible that respiratory, cardiovascular, and gastrointestinal malfunctioning contributed at least in part for the first, second, and third waves of deaths, respectively.

EXPERIMENT 2: THE DOSE RESPONSE CURVES

The choice of the time period for defining the LD_{50} is, of necessity, arbitrary. "Windows," that is, periods without any deaths, after injections of any dose of ethanol are naturally suitable time points for determination of the dose–response relationship because the results of any process should be complete when the process seems to be finished. This experiment was designed to determine dose–response relationships at 2 h, 24 h, and 10 days after ethanol injection, as well as the influence of routine laboratory procedures such as exposure to a novel experimental room and saline injections, on the lethal effects of ethanol.

METHOD

Four-hundred and 6 male Swiss Webster mice were divided into six groups. Naive mice of the noSAL/noMOV group were given ethanol overdoses ranging from 6.5–9.5 g/kg in the colony room. Mice of the noSAL/MOV group were moved to a small room with loud noise (Rm-1) and given the same doses of ethanol. Mice of the SAL groups were moved into Rm-1 twice daily (0900–1200 and 1500–1700 h) and there given saline injections for 4 or 8 days. Fifty minutes following injections, mice were returned to the colony room. After that, on day 5 mice were given ethanol injections (from 7.5–9.5 g/kg) in the same Rm-1 (8SAL/noMOV and 16SAL/noMOV groups) or moved into a different room (Rm-2; an adjacent room, no noise, lower illumination; 8SAL/MOV group).

RESULTS AND DISCUSSION

The Litchfield and Wilcoxon method (10) showed that dose–response data yielded at all three time points and plotted on logarithmic-probability paper were homogeneous in all cases and a straight line appeared to fit the data satisfactorily. Statistical analysis revealed strong dose effects on probability of deaths in all groups and at all three time points ($p < 0.0001$). There was no influence of saline injections or of moving animals into novel environment on mortality. This allowed the collapsing of the data for all mice groups (Table 1). The slope of the dose–response curves was extremely steep and the difference between the LD_{16} and the LD_{84} was in the range of 15–18%.

The distribution of survival time in saline-preexposed mice did not depend upon the factor of novelty [F-test, $F(60, 196) = 1.26$, $p > 0.1$] and did not differ from this distribution in naive animals presented in Fig. 1. To the best of our knowledge, this study is the first to have demonstrated that the probability density function has several peaks and that there are at least two periods (80–130 min and 22–26 h) with extremely low probability of death following ethanol overdose administration in Swiss Webster mice. Perhaps the most intriguing finding obtained in this experiment is that the time scale localization of these three waves of deaths was only weakly affected by the ethanol dose factor, saline preexposure, and

TABLE 1

EFFECTS OF NOVELTY, SALINE, AND ETHANOL INJECTIONS ON MORTALITY IN SWISS WEBSTER MICE

Groups	n	120 min			24 h			10 Days		
		LD_{16}	LD_{50}	LD_{84}	LD_{16}	LD_{50}	LD_{84}	LD_{16}	LD_{50}	LD_{84}
noSAL/noMOV	118	8.30	9.00	9.80	7.40	8.10	8.85	7.05	7.70	8.50
noSAL/MOV	118	8.20	8.90	9.70	7.60	8.15	8.80	7.10	7.65	8.40
8SAL/noMOV	45	8.00	8.65	9.35	7.60	8.00	8.35	6.90	7.40	8.00
8SAL/MOV	45	8.15	8.80	9.50	7.75	8.10	8.40	7.60	8.00	8.50
16SAL/noMOV	80	8.15	8.90	9.70	7.45	8.05	8.70	7.45	7.95	8.50
noMOV	243	8.15	8.90	9.80	7.50	8.10	8.70	7.10	7.75	8.30
MOV	163	8.20	8.95	9.80	7.60	8.10	8.60	7.30	7.80	8.40
noSAL	236	8.25	8.95	9.75	7.50	8.15	8.80	7.05	7.70	8.45
SAL	170	8.10	8.80	9.55	7.55	8.05	8.55	7.30	7.85	8.40
Total noETOH	406	8.15	8.90	9.70	7.50	8.10	8.70	7.15	7.75	8.45
4ETOH/noMOV	88	8.30	9.00	9.80	7.80	8.50*	9.20	7.70	8.30*	8.85
8ETOH/noMOV	74	8.80	9.40	10.00	8.45	8.90	9.40	8.05	8.70	9.40
8ETOH+8SAL/noMOV	91	8.60	9.60	10.70	8.40	9.10	9.85	8.25	8.90	9.55
Total 8ETOH	165	8.70	9.50†	10.30	8.40	9.00†	9.65	8.15	8.80†	9.50

Lethal doses are expressed in g/kg; confidence limits were between + 0.25–0.40 g/kg.
*Significantly different ($p < 0.05$) from total noETOH and total 8ETOH groups.
†Significantly different ($p < 0.05$) from SAL group.

novelty of environment. It does appear that only amplitude of the waves of deaths had a strong dose-response relationship. Thus, the time points of 2 h, 24 h, and 10 days after injections are convenient points to determine dose-effect functions. In a number of studies done in mice (4,6,7,11,13), the LD_{50} has been assessed; none, however, of them employed Swiss Webster mice and 20% (w/v) solution of ethanol. Further, the LD_{50} obtained in this study could not be verified by data obtained from the literature and the present study is thus the first to have assessed the relationship between mortality and ethanol dose in Swiss Webster mice in the wide ranges of doses and times after injections.

EXPERIMENT 3: ETHANOL PREEXPOSURE

The purpose of this experiment was to determine the effects of preexposure to ethanol on its lethal effects.

METHOD

Two-hundred and 98 mice were divided into four groups. All mice were moved from the colony room into Rm-1, where twice daily they were injected with ethanol over 2 (4EtOH/noMOV group, $n = 91$) or 4 days (8EtOH/noMOV group, $n = 82$). The third group of mice received ethanol injections in the morning and saline injections in the afternoon for 8 days (8EtOH + 8SAL/noMOV group, $n = 92$). The fourth group received no saline or 16 prior saline injections followed by the 20-day period of alternating ethanol and saline injections (0-16SAL/20EtOH + 20SAL/noMOV group, $n = 34$). On the final day, mice were given ethanol overdoses (from 6.5–10.5 g/kg). At the end of the subchronic ethanol period in the 8EtOH/noMOV group, five mice died and three additional seemed in poor condition. In the 4EtOH/noMOV group, three more mice were in poor condition. Finally, in 8SAL/8EtOH/noMOV and 0-16SAL/20EtOH + 20SAL/noMOV groups one and two mice died, respectively. All these mice were excluded from statistical analysis.

RESULTS AND DISCUSSION

Distributions of survival time in Swiss Webster mice with 4, 8, or 20 prior injections of ethanol also had three waves of deaths and did not differ significantly from each other (for all three pairs of groups, F-test yielded $p > 0.1$). Therefore, the data were collapsed. A comparison between ethanol-naive (combined naive and saline groups) and ethanol-preexposed mice also did not reveal a difference between distributions of survival time, $F(344, 504) = 1.14, p > 0.08$.

ANOVA showed a significant effect of prior injections of 3.5 g/kg ethanol but not saline injections on latency to LORR, $F(2, 452) = 19.11$, $p < 0.0001$, and RORR, $F(2, 174) = 18.56$, $p < 0.00001$. Ethanol preexposure resulted in an increase in latency to LORR and decrease in latency to RORR.

Dose-response curves fitted straight lines ($p < 0.01$) after ethanol preexposure (Fig. 2). Analysis by the method of Litchfield and Wilcoxon revealed a significant difference between LD_{50} for SAL, 4EtOH, and 8EtOH groups (Table 1). Ethanol preexposure resulted in a shift to the right of dose-response curves. However, the lethal effects of 9.0 and 9.5 g/kg in mice exposed to ethanol for 20 days did not differ from the lethal effects in ethanol-naive animals in terms of mortality (Fig. 3) as well as survival time.

The finding that intermittent ethanol exposure resulted in increased tolerance to the lethal effects of ethanol in Swiss Webster mice is in agreement with the data obtained by Melchior and Tabakoff (13) in BALB/c mice. In the present study, the LD_{50} at 24 h increased by 5.6 and 13.7% following administration of a total of 14.0 or 28.0 g/kg ethanol, respectively. However, 20 injections totaling of 70 g/kg ethanol did not result in significant changes in lethality as compared to ethanol-naive mice. It seems unlikely that the changes observed in the 4EtOH and 8EtOH groups' increase in tolerance was compensated by an increase in the sensitivity to the lethal effects due to aging. Nevertheless, this assumption remains to be studied in an experiment with a parallel 20SAL control group.

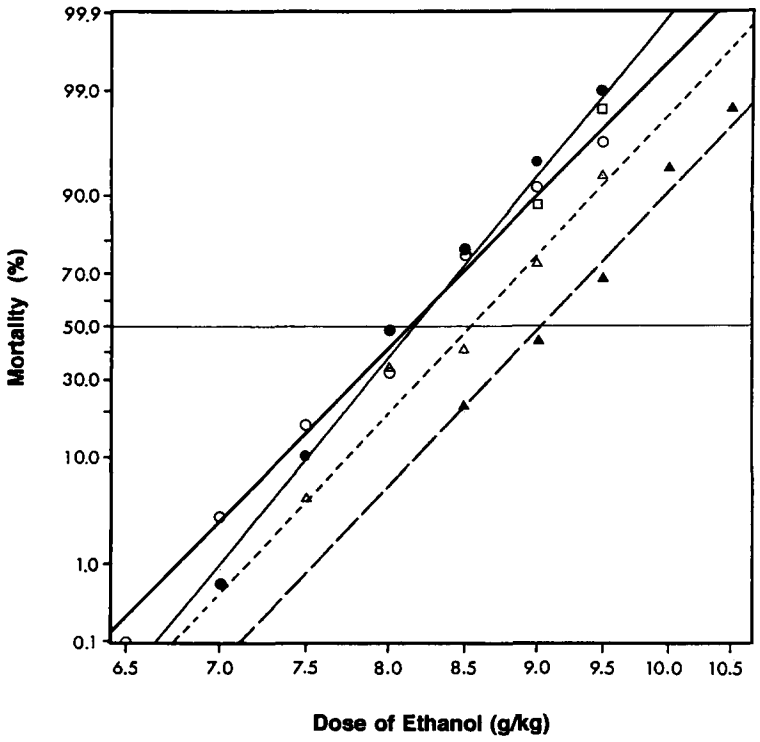

FIG. 2. Dose-response curves at 24 h following ethanol injections in naive Swiss Webster mice (○) and in mice preexposed to 8 injections of saline (●) or 4 (△), 8 (▲), and 20 (□) injections of 3.5 g/kg ethanol. The Litchfield and Wilcoxon method revealed significant differences ($p < 0.05$) between LD_{50} for 8SAL and 4EtOH and 8EtOH groups (see Table 1).

EXPERIMENT 4: TOLERANCE TO LETHAL EFFECTS OF ETHANOL IN BALB/C MICE

There are great strain differences in the sensitivity to ethanol-induced lethal effects (11,12). The purpose of this experiment was to determine and compare to Swiss Webster survival time distribution, dose-response relationship, and tolerance to the lethal effects of ethanol in BALB/c mice.

METHOD

Twice daily (at 0900 and 1500 h) cages with BALB/c mice were moved from the animal colony to Rm-1 and there mice received injections of saline or ethanol for 4 consecutive days. On the morning of day 5, mice were given overdoses of ethanol ranging from 4.5–7.5 g/kg in the same Rm-1 (8SAL/noMOV and 8EtOH/noMOV groups) or in a different adjacent room (large, brightly lit room, Rm-0, 8SAL/MOV group). Because of health problems induced by subchronic ethanol injections, six mice of the 8EtOH/noMOV group were excluded from further analysis.

Seven days after the overdose injections, 16 control mice that survived injections of 6.0 g/kg EtOH were given twice daily 12 injections of 3.5 g/kg EtOH (total of 48 g/kg; one mouse died during this period) and these same mice then received again 6.0 ($n = 7$), 6.5 ($n = 6$), or 7.5 g/kg ($n = 2$) of EtOH (12EtOH/noMOV group). Fourteen experimental mice that survived injections of 6.0 g/kg ethanol were given an additional 23 injections of 3.5 g/kg twice daily for 8 days and daily for 7 days (total of 114.5 g/kg). One mouse died and was excluded from analysis. The rest of the mice received again 7.0 g/kg ($n = 5$) or 7.5 g/kg ($n = 8$) of ethanol in Rm-1 (31EtOH/noMOV group).

RESULTS AND DISCUSSION

Cumulative mortality curves for all groups and doses showed that 99.3% of deaths occurred within 5–200 min after ethanol injections. Only one death in ethanol-naive BALB/c mice occurred at 40 h following injection of 7.5 g/kg (Fig. 4). Survival time distribution fitted a lognormal distribution (κ^2 test, $p = 0.055$). Therefore, ANOVA was used to determine the influence of novelty, ethanol dose, and ethanol preexposure as independent factors on latencies to LORR and RORR as well as survival time.

In ethanol-naive mice, latency to RORR increased significantly, $F(3, 42) = 113.28$, $p < 0.00001$, with increases in ethanol doses from 4.5 g/kg (89.0 ± 2.9 min) to 6.5 g/kg (583.9 ± 76.6 min). However, dose did not affect latency to LORR, $F(4, 31) = 2.08$, $p > 0.1$. Novelty did not affect latency to RORR, $F(1, 42) = 0.72$, $p > 0.4$, or latency to LORR, $F(1, 31) = 1.29$, $p > 0.05$. Also, dose of ethanol, $F(1, 12) = 0.73$, $p > 0.4$, and novelty, $F(1, 12) = 0.50$, $p > 0.5$, did not change survival time in saline-preexposed BALB/c mice.

Ethanol preexposure decreased latency to RORR, $F(1, 58) = 22.24$, $p < 0.0001$, but did not affect survival time, $F(1, 36) = 1.65$, $p > 0.2$, or latency to LORR, $F(1, 54) = 0.30$, $p > 0.5$. In ethanol-experienced mice, doses of ethanol also did not affect latency to LORR, $F(2, 54) = 2.38$, $p > 0.1$, but increased latency to RORR, $F(2, 39) = 23.80$, $p < 0.00001$. Regression analysis did not reveal any significant

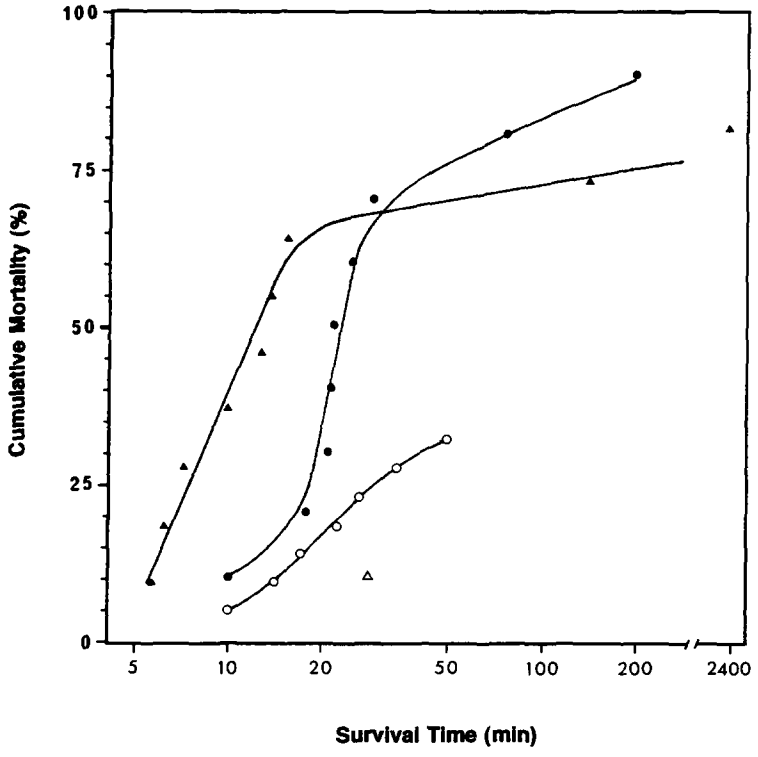

FIG. 3. Cumulative mortality following injections of 6.5 g/kg (○) or 7.0 g/kg (●) of ethanol in saline-preexposed and 6.0 g/kg (△) or 7.5 g/kg (▲) of ethanol in ethanol-preexposed BALB/c mice.

correlations between any pairs of independent variables in any groups of mice. Thus, latency to LORR could not be a predictor for latency to RORR or survival time.

Intermittent administration of 28 g/kg ethanol during 4 days resulted in a tolerance to the lethal effects of ethanol as well as to the hypnotic effects. Mortality increased by 8.3% ($p = 0.05$) as compared to ethanol-naive mice (Table 2). Again, as in Experiment 2 with Swiss Webster mice, we obtained the surprising result that administration of 48 or 114.5 g/kg ethanol had no effect on tolerance to the lethal effects (data were therefore collapsed; see the 12-31EtOH/noMOV group, Table 2). It must be noted that the number of animals in this group was insufficient to determine LD_{50}.

TABLE 2

EFFECTS OF NOVELTY AND ETHANOL INJECTIONS ON MORTALITY IN BALB/c MICE

		200 min		
	n	LD_{16}	LD_{50}	LD_{84}
8SAL/noMOV	32	6.40	6.70	7.00
8SAL/MOV	36	6.10	6.55	7.00
Total SAL	68	6.20	6.60	7.00
8ETOH/noMOV	41	6.80	7.15*	7.65
12-31ETOH/noMOV	28	6.10	6.70	7.20

Lethal doses are expressed in g/kg.

*Significantly different ($p < 0.05$) from both SAL groups and 12-31ETOH group.

In this experiment, the LD_{50} in control BALB/c male mice was 6.60 g/kg (95% confident interval 6.40–6.80 g/kg). This dose is higher than that reported by Melchior and Tabakoff (13) (about 4.8–5.2 g/kg). One of the possible explanations for this difference is the marked difference in the weight and, presumably, age of animals in the two studies (19–23 and 22–28 g, respectively). It has been shown that sensitivity to the lethal effects of ethanol increases with aging in rats (2,14,18). Unfortunately, no other comparable studies on mice are available at present. Further, the identical procedure of subchronic ethanol preexposure to BALB/c mice resulted in tolerance increases by 30% in Melchior and Tabakoff's study (13) but only less than 10% in the present experiment. It is possible that this difference might also be due to age difference.

GENERAL DISCUSSION

The results of Experiments 1 and 2 showed that the probability density function of ethanol-induced lethality in Swiss Webster mice did not describe a monotonous function and had several extremes. The localization in time of the two longest intervals with low density of lethality did not appear to depend significantly upon factors such as novelty, saline injections, ethanol preexposure, and even ethanol dose. In other words, the temporal pattern of lethality was strictly constant. It would seem reasonable to assume that different processes were triggered by overdoses of ethanol that were responsible for deaths in different time periods following injections. We

speculated that the first wave of deaths (3–20 min) could have been caused by respiratory depression, the second wave (2–22 h) by cardiovascular malfunctioning, and the third wave (26–46 h) by gastrointestinal damage. Moreover, the data suggested that 7.0 g/kg ethanol, a dose slightly over the lethal threshold, caused deaths with latency mostly in the third wave. Most deaths that occurred following injections of the highest doses (9.5–10.0 g/kg) of ethanol happened during the 3- to 20-min period. Doses of 8.0–8.5 g/kg, which are around the LD_{50}, produced a whole spectrum of survival times, predominantly, however, within the second wave of deaths. It seems reasonable to assume that three putative triggers for different causes of death require different strengths for being switched "on." That is why with the increase in ethanol dose survival time skipped from one wave with presumably an easier-to-switch trigger to another wave with a high threshold trigger. This view, while a speculation, is nevertheless consistent with the surprising finding that survival time in BALB/c mice did not depend upon the ethanol dose they received and yielded only one wave of deaths with a peak between 5–50 min and with only an occasional death in 40 h. Respiratory depression was assumed to be the main cause of death in BALB/c mice. It is also believed that BALB/c mice are less sensitive than Swiss Webster mice to the adhesive and irritating effects of IP-injected ethanol (C. Melchior, personal communication).

The straight lines of dose–response relationships had steep slopes in Swiss Webster and also in BALB/c mice. Despite the small difference between the LD_{16} and LD_{84}, Swiss Webster but not BALB/c mice showed significantly different survival times at lower and higher lethal doses of ethanol. Regardless of dose, BALB/c mice were more sensitive to the lethal effects of ethanol during the first 2 h (LD_{50} was 6.60 and 9.00 g/kg in the two strains, respectively). The fact that 7.0 g/kg ethanol caused death in 90% of BALB/c mice within 10–200 min but only in 20% of Swiss Webster mice, who died within 14–100 h, suggested that the trigger threshold of a long-latency cause (gastrointestinal damages?) of death in Swiss Webster mice will be lower than thresholds of other triggers in these mice but nevertheless higher than the trigger threshold of a short-latency cause (respiratory depression?) in BALB/c mice. In other words, the difference between survival time patterns in Swiss Webster and BALB/c mice might be explained by the higher susceptibility of BALB/c mice to respiratory depression. Apparently, we can observe a three-wave pattern in Swiss Webster mice because the lower-threshold triggers control more long-latent causes of death.

There was no significant influence of saline injections and novelty on LD_{50} and slope functions and only subchronic ethanol administration changed mortality. Intermittent preexposure to ethanol resulted in increased tolerance to ethanol-induced lethality. This effect increased from 5.6 to 13.8% when the total preexposed dose increased from 14–28 g/kg but nearly disappeared after preexposure to injections totaling 70 g/kg ethanol in Swiss Webster mice. In BALB/c mice, tolerance increased by 8.3% after preexposure to injections of 28 g/kg but disappeared after administration of injections totaling 48–114.5 g/kg. Tolerance to the hypnotic effects of ethanol also developed in the course of chronic and subchronic administration of 3.5 g/kg ethanol (in preparation). Latency to RORR decreased following eight injections of ethanol by 6.2% (2.4–9.0% at different doses) in BALB/c mice and by only 2.5% (at any doses used) in Swiss Webster mice. Extraordinary small changes in tolerance to the lethal and hypnotic effects of ethanol seem to be rare among drugs and cannot be explained by low preexposure doses (around 10% of animals died within 3–4 days of the preexposure period) or by the short span of the preexposure period (up to 3 weeks).

The lethal effects of ethanol are highly sensitive to and increase with the enhancement in body temperature (6,7,11). This fact suggests that the development of tolerance to the hypothermic effect of ethanol should counteract the development of tolerance to the lethal effects of ethanol and thus might account for the small degree of tolerance development. Among the pharmacokinetic factors exerting the greatest influence on the development of tolerance to ethanol-induced lethality, one might include the rate of ethanol absorption (Tsibulsky and Amit, in preparation), the volume of ethanol distribution (13,17), and the rate of its elimination (17). Contribution of these and other factors to the rate of tolerance development remains to be investigated. The purpose of our next study is to determine a role of Pavlovian conditioning of environmental stimuli in tolerance increases following ethanol exposure in mice and rats (14).

In conclusion, the obtained data suggested that in Swiss Webster mice ethanol-induced lethality has a steady temporal pattern that included periods with a low probability of deaths. This pattern is resistant to the influence of variables such as lethal dose of ethanol, novelty, or preexposure to saline or ethanol. Also, ethanol-induced lethality is not a unitary phenomenon and deaths occurring within distinct waves may probably be ascribed to different causes. Mice strains differ in terms of the susceptibility to different causes of death. Finally, preexposure to 3.5 g/kg ethanol resulted in small but significant increases in tolerance to ethanol-induced lethality.

ACKNOWLEDGEMENT

The authors thank Wayne Bowers for assistance in the preparation of this article.

REFERENCES

1. Aragon, C. M. G.; Spivak, K.; Amit, Z. Effect of 3-amino-1,2,4-triazole on ethanol-induced narcosis, lethality and hypothermia in rats. Pharmacol. Biochem. Behav. 39:55–59; 1991.
2. Chesler, A.; LaBelle, G. C.; Himwich, H. E. The relative effects of toxic dose of alcohol on fetal, newborn and adult rats. Q. J. Stud. Alcohol 3:1–4; 1942.
3. Chin, J. H.; Goldstein, D. B. Electron paramagnetic resonance studies of ethanol on membrane fluidity. In: Gross, M. M., ed. Alcohol intoxication and withdrawal—IIIa. Biological aspects of alcohol. New York: Plenum Press; 1977:111–122.
4. Deimling, M. J.; Schnell, R. C. Circadian rhythms in the biological response and disposition of ethanol in the mouse. J. Pharmacol. Exp. Ther. 213:1–8; 1980.
5. Deitrich, R. A.; Dunwiddie, T. V.; Harris, R. A.; Ervin, V. G. Mechanism of action of ethanol: Initial central nervous system actions. Pharmacol. Rev. 41:489–537; 1989.
6. Dinh, T. K. H.; Gailis, L. Effect of body temperature on acute ethanol toxicity. Life Sci. 25:547–552; 1979.
7. Finn, D. A.; Bejanian, M.; Jones, B. L.; Syapin, P. J.; Alkana, R. L. Temperature affects ethanol lethality in C57BL/6, 129, LS and SS mice. Pharmacol. Biochem. Behav. 34:375–380; 1989.
8. Klingman, G. I.; Haag, H. B. Studies on severe alcohol intoxication in dogs. I. Blood and urinary changes in lethal intoxication. Q. J. Stud. Alcohol 19:203–225; 1958.
9. Lee, E. T. Statistical methods for survival data analysis. Belmont, CA: Wadsworth; 1980.

10. Litchfield, J. T.; Wilcoxon, F. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96:99–113; 1949.
11. Malcolm, R. D.; Alkana, R. L. Temperature dependence of ethanol lethality in mice. J. Pharm. Pharmacol. 35:306–311; 1982.
12. Melchior, C. L. Conditioned tolerance provides protection against ethanol lethality. Pharmacol. Biochem. Behav. 37:205–206; 1990.
13. Melchior, C. L.; Tabakoff, B. Environment-dependent tolerance to the lethal effects of ethanol. Alcohol. Clin. Exp. Res. 6:306; 1982.
14. Tsibulsky, V. L.; Amit, Z. The role of environmental cues as Pavlovian conditioned stimuli in enhancement of tolerance to ethanol effects. I. Lethal effects in mice and rats. Pharmacol. Biochem. Behav. (submitted).
15. Wiberg, G. S.; Coldwell, B. B.; Trenholm, H. L. Toxicity of ethanol-barbiturate mixtures. J. Pharm. Pharmacol. 21:232–236; 1969.
16. Wiberg, G. S.; Samson, J. M.; Maxwell, W. B.; Coldwell, B. B. Further studies on the acute toxicity of ethanol in young and old rats: Relative importance of pulmonary excretion and total body water. Toxicol. Appl. Pharmacol. 20:22–29; 1971.
17. Wiberg, G. S.; Trenholm, H. L.; Coldwell, B. B. Increased ethanol toxicity in old rats: Changes in LD_{50} in vivo and in vitro metabolism, and liver alcohol dehydrogenase activity. Toxicol. Appl. Pharmacol. 16:718–727; 1970.
18. Wood, W. G.; Armbrecht, H. J. Behavioral effects of ethanol in animal: Age differences and age changes. Alcohol. Clin. Exp. Res. 6:3–12; 1982.