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Effects of Exercise on Ethanol-Induced Hypothermia and Loss of Righting Response in C57BL/6J Mice

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MOLLENAUER, S., R. BRYSON, C. SPECK AND J. R. CHAMBERLIN. *Effects of exercise on ethanol-induced hypothermia and loss of righting response in C57BL/6J mice.* PHARMACOL BIOCHEM BEHAV **43**(1) 285–290, 1992.—C57BL/6J mice were given 5 weeks of voluntary wheel running and then studied for ethanol (EtOH) sensitivity as indicated by EtOH-induced hypothermia and loss of righting response (LORR) after 3.8 g/kg EtOH (20% w/v). Mice were assigned to wheel (free access to a running wheel in the home cage) or no wheel conditions, and wheel counts were monitored by a computer at 5-min intervals around the clock. In Experiment 1, duration of EtOH-induced LORR was assessed as amount of time required for the animal to right itself three times in a 30-s period, and body temperature was assessed by rectal probe. Wheel animals showed significantly shorter LORR and significantly less hypothermia at regaining the righting response than no wheel controls. In Experiment 2, temperature was assessed at 45 and 90 min after EtOH challenge. Baseline temperatures for wheel and no wheel animals did not differ, but wheel animals showed dramatic resistance to EtOH-induced hypothermia at both time points. Together with our earlier work, these results provide evidence that prior exercise can offset the effects of EtOH intoxication in several domains of EtOH sensitivity.

Exercise Voluntary wheel running Ethanol Temperature Loss of righting response C57BL/6J mice

THE beneficial effects of exercise are now the subject of intense interest, but relatively little research has studied the effects of exercise in conjunction with ethanol (EtOH) use or intoxication. A few investigations have looked at the combined effects of exercise and EtOH on physiological mechanisms of cardiac and skeletal muscle function (7,8) and, more recently, hepatic function (3). The latter work by Ardies et al. (3) focused primarily on EtOH disposition; rats subjected to chronic exercise showed increased activity in the microsomal EtOH oxidizing system. This finding suggests that exercise causes accelerated clearance of EtOH and, by inference, suggests that exercise might be beneficial in offsetting EtOH intoxication. In earlier work and in the research reported here, we studied the effectiveness of voluntary exercise in offsetting EtOH intoxication in C57BL/6J mice.

We recently reported preliminary evidence that 5 weeks of voluntary wheel running appeared to cause resistance to loss of the righting response (LORR) after 3.16 g/kg EtOH in C57BL/6J mice (14). Likewise, 5 weeks of voluntary wheel running attenuated the behavioral impairment of activity and avoidance caused by an intoxicating dose of EtOH (2.4 g/kg) in C57BL/6J mice (15). The present experiments were designed to determine the effectiveness of voluntary wheel running in another domain of EtOH sensitivity, namely, EtOH-induced hypothermia. The phenomenon of EtOH-induced hypothermia is well established (6,12,13) and is assumed to represent a different and possibly independent domain of EtOH sensitivity than that represented by LORR or behavior (12,16). Because these differing domains of EtOH response may model different aspects of human response to EtOH, it is of interest to assess protective effects of exercise in each area.

Experiment 1 assessed the effects of prior wheel running on duration of LORR and EtOH-induced hypothermia at return of the righting response (RORR) after a 3.8-g/kg dose of EtOH. This dose was selected because it has been used extensively in studies of blood ethanol concentration in C57BL/6J mice and is in line with doses used in studies of EtOH-induced hypothermia in these animals (1,5,17). Experiment 2 assessed the effects of prior wheel running on EtOH-induced hypothermia at fixed time points following a 3.8-g/kg dose of EtOH. Temperature was assessed just prior to injection and at 45 and 90 min after injection.

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METHOD

Animals

Animals for Experiment 1 were 26 C57BL/6J mice, 16 males and 10 females, 2 months old at the beginning of the experiment; of these, 1 animal died and the data for 1 animal were lost due to apparatus failure during the course of experiment. Animals for Experiment 2 were 30 C57BL/6J mice, 16 males and 14 females. Eight additional mice, four males and four females, were used for videotape assessment of computer wheel counts. All animals were first-generation offspring bred from stock obtained from Jackson Laboratories (Bar Harbor, ME). Animals were separated by sex at approximately 1 month of age and reared in same-sex litter groups with no more than four animals per cage. Animals were maintained on a 12 L:12 D cycle with light off at 1:00 a.m. All injections were administered between 8:00–10:00 a.m., with exercise conditions evenly represented across hours. Animals had ad lib access to water and mouse lab chow throughout the exercise phase.

Exercise Conditions

At 2 months of age, male and female mice were randomly assigned in equal proportions to wheel or no wheel conditions, with body weight balanced across conditions. Animals were individually housed in rectangular acrylic cages, 25 × 45 cm and 25 cm deep, with a wire cloth floor and wire cloth lid. Each cage was housed in a sound-attenuated chamber equipped with a fan and low-watt lighting; male and female mice were housed in separate rooms. Half the cages were equipped with small rodent running wheels (Ward Co.), measuring 18 cm in diameter. Wheel revolutions (counts) were registered by photobeam and recorded by computer at 5-min intervals, except during the hour when animals were interrupted for servicing. Chambers were opened for servicing at 12:00 noon each day, and animals were otherwise left undisturbed.

Validation of Computer Counts

To validate the computer assessment of running, four male and four female mice were allowed 5 weeks of voluntary wheel running and then videotaped for later correlation with computer counts recorded during the same period. Animals were taped for 1-h periods at 2:00 a.m., the hour of peak running, and at 9:00 a.m., when running had declined.

Experiment 1 Procedure

After 5 weeks in wheel or no wheel conditions, animals were tested for EtOH sensitivity, assessed by LORR, following an injection of 3.8 g/kg EtOH (20% w/v in normal saline). Each mouse was removed from its chamber 30 min before injection; it was weighed at this time and placed in an individual holding cage. Body temperature was measured just prior to injection and again when the animal regained the righting response (RORR). Temperature was measured with a Thermalert temperature monitor (Sensortech., Inc., Saugus, CA), equipped with a Number 8 Type T thermocouple temperature probe (Mallinckrodt, Inc., St. Louis, MO). For this procedure, the mouse was gently restrained with a gloved hand and the probe, lubricated with surgical lubricant, was inserted 1.5 cm into the rectal cavity and left until equilibrium was reached, approximately 30 s. After injection, the mouse was placed on its back in a V-shaped trough and duration of LORR was recorded. LORR was defined as the time during which the animal could not right itself three times within a 30-s period.

Experiment 2 Procedure

In Experiment 2, exercise procedures were the same as for Experiment 1 but animals were removed from the exercise chambers 60 min prior to injection to lessen any acute effects of exercise on baseline temperature. Body temperature was assessed at three fixed time points: just prior to injection and at 45 and 90 min postinjection. The EtOH dose, 3.8 g/kg, and injection procedures were the same as in Experiment 1. The procedures for assessing temperature were also the same, with one important exception: The probe employed was a mouse rectal probe, RET-3 (Physitemp Instruments Co., Columbus, OH); with this probe, temperatures stabilized in approximately 15 s, meaning the animal was restrained half as long as in Experiment 1.

RESULTS

Wheel Running

Figure 1 summarizes 24-h wheel running, averaged across the last 3 days of training, with data from the two experiments pooled. These data, mean number of wheel counts per 5-min period for each hour, were analyzed by analysis of variance (ANOVA) with gender as a between factor and hour as a within factor. As expected, there was a strong circadian effect in which wheel running was confined largely to the dark period, $F(22, 528) = 92.4$, $p < 0.01$.

Inspection of the figure also shows an interaction between time and gender, in which levels of wheel running during the dark phase were higher for females than males, $F(22, 528) = 3.63$, $p < 0.01$. In the simple effects comparisons, mean wheel counts were significantly higher for females during the first 9 h of the dark period. Circadian effects and gender differences appeared to be stable across days. An analysis of wheel counts on the preceding 3-day block produced a virtually identical pattern of results.

Validation of Computer Wheel Counts

Complete wheel revolutions were counted from the videotapes for 30-min segments and these counts correlated with the computer counts for the same times. At 2:00 a.m., the mean computer count per 5-min period for male mice was 526 and the mean observation count was 433 ($r = 0.996$); the mean computer count for female mice was 884 and the mean observation count was 747 ($r = 0.969$). At 9:00 a.m., the mean computer count for male mice was 329 and the mean observation count was 339 ($r = 0.997$); the mean computer count for female mice was 735 and the mean observation count was 639 ($r = 0.978$). These correlations indicate that the computer counts are an extremely accurate estimate, if not a literal measure of running behavior.

LORR

The effects of exercise on duration of LORR, are summarized in Fig. 2. As the figure shows, wheel animals regained the righting response significantly faster than no wheel animals or, in other words, showed significantly shorter duration of EtOH-induced LORR, $F(1, 18) = 4.56$, $p < 0.05$. There was no significant effect of gender ($F < 1$) and no interaction between wheel and gender ($F < 1$). For male mice, mean

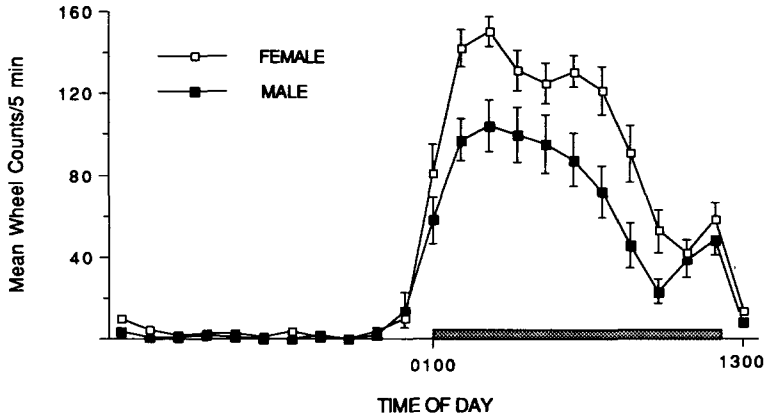

FIG. 1. Mean number of wheel counts per 5-min period by hour (±SEM), averaged over the last 3 days of exercise, for 14 male and 12 female C57BL/6J mice that had free access to a running wheel throughout the 5-week period. Bar indicates 12-h dark period.

LORR times for no wheel and wheel animals were 95.6 and 67.0 min, respectively; for female mice, mean LORR times for no wheel and wheel animals were 80.5 and 60.4 min, respectively.

Body Weight

In the analysis of body weight, from Experiment 1 wheel and no wheel animals did not differ significantly [$F(1, 18) = 1.73$]; nor did wheel interact with gender ($F < 1$). For male mice, mean body weight was 26.6 and 26.9 g for no wheel and wheel animals, respectively; for female mice, mean body weight was 20.5 and 21.9 g for no wheel and wheel animals, respectively. As expected, the gender differences were highly reliable, $F(1, 18) = 78.7, p < 0.01$.

Temperature at RORR

Temperatures taken prior to injection and at RORR are presented in Table 1. As expected, EtOH induced significant hypothermia, $F(1, 18) = 667.5$, $p < 0.01$. Although the interaction between time and wheel condition was not significant, planned comparisons of simple effects indicated that wheel animals showed significantly higher temperatures than no wheel animals at RORR, $F(1, 33) = 6.27, p < 0.05$, suggesting that prior running had attenuated the EtOH-induced hypothermia. The wheel and no wheel animals did not differ at preinjection [$F(1, 33) = 2.49$]. The ANOVA also resulted in a significant main effect of wheel, $F(1, 18) = 6.42$, $p < 0.05$, presumably due to differences at RORR. Gender had no significant effect [$F(1, 18) = 2.14$]; nor did gender interact with exercise ($F < 1$).

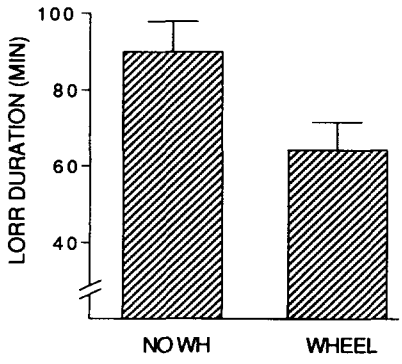

FIG. 2. Mean duration of LORR in minutes (±SEM), after 3.8 g/kg EtOH in C57BL/6J mice. LORR was recorded as the time that the mouse could not right itself three times within 30 s. Mice had free access to a running wheel (Wheel) or no access (No WH) for 5 weeks prior to EtOH treatment.

Temperature at Fixed Times

The data from Experiment 2 are summarized in Fig. 3. As the figure shows, EtOH did induce marked hypothermia, $F(2, 52) = 128.9, p < 0.05$, and wheel animals showed significant resistance to hypothermia, as reflected in an interaction between time and wheel, $F(2, 52) = 5.57, p < 0.05$. While there was no significant difference in wheel and no wheel preinjection temperatures, follow-up comparisons of simple effects confirmed that wheel animals showed significantly higher temperatures than no wheel animals at 45 and 90 min, $F(2, 52) = 6.57$ and 7.65, $p < 0.05$, respectively. This pattern of results also resulted in a significant main effect of wheel condition, $F(1, 52) = 4.32$, $p < 0.05$. There were no significant differences attributable to gender either as a main effect or an interaction.

TABLE 1

BODY TEMPERATURES FOR WHEEL AND NO WHEEL MICE

		Mean Temperature (°C) (±SEM)	
Exercise	*n*	Injection	RORR
No wheel	11	37.14 ± 0.10	33.83 ± 0.20
Wheel	11	37.44 ± 0.09	34.36* ± 0.14

*Differs significantly from no wheel at RORR, $p < 0.05$.

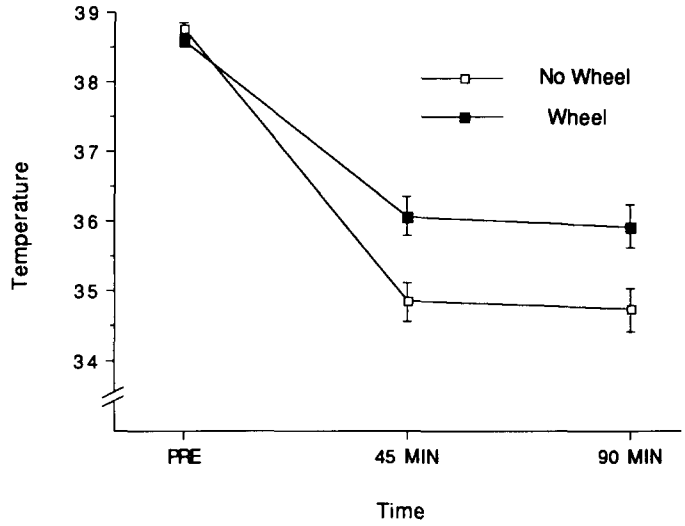

FIG. 3. Mean body temperature (±SEM) assessed by rectal probe in C57BL/6J mice prior to injection (PRE) and at 45 and 90 min after injection of 3.8 g/kg EtOH. Mice had free access to a running wheel (Wheel) or no access (No Wheel) for 5 weeks prior to EtOH treatment.

DISCUSSION

The present results confirm and extend our previous findings that voluntary wheel running provides protection against acute EtOH intoxication in C57BL/6J mice. The results of Experiment 1 confirmed that 5 weeks of wheel running caused C57BL/6J mice to resist EtOH-induced LORR. Wheel animals showed a 30% reduction in the duration of LORR as compared to no wheel controls after 3.8-g/kg EtOH challenge (Fig. 2). The results of Experiment 1 also suggested that voluntary wheel running altered EtOH sensitivity in another domain, namely, EtOH-induced hypothermia. As compared to no wheel animals, wheel animals showed a small, but significant, reduction in EtOH-induced hypothermia at regaining the righting response. This phenomenon was confirmed in Experiment 2, in which wheel animals showed dramatic resistance to EtOH-induced hypothermia (Fig. 3). Thus, the present results on LORR and hypothermia, together with earlier work on behavior (15), provide evidence that prior exercise confers protection in several different domains of EtOH response.

An important feature of the present research is the fact animals were tested at rest. In Experiment 2, animals were removed from the exercise chambers 60 min before EtOH challenge and all injections were administered during the later part of the dark period, when activity levels were normally low (Fig. 1). Any acute effects of wheel running on body temperature would presumably have returned to baseline prior to challenge. This assumption is supported by a recent review of exercise and drug pharmacokinetics that reported that hemodynamic variables generally return to preexercise levels very rapidly, particularly in animals that have received chronic exercise (4), and also by the fact that the baseline temperatures of wheel and no wheel animals were virtually identical (Fig. 3). Thus, the present results are consistent with the idea that voluntary wheel running protects against future response to EtOH.

Although the pattern of results for temperature was the same in Experiments 1 and 2, a comment may be in order regarding the difference in absolute temperature levels in these two experiments. Recorded temperatures were on the order of 1.5° lower in Experiment 1. Animals were restrained for a longer period of time in Experiment 1, but this would be expected to cause an elevation in baseline temperature. Thus, we assume that the lower temperatures in Experiment 1 were attributable to the probe employed, a silicone-coated surgical probe; in contrast, Experiment 2 employed an uncoated mouse rectal probe. Presumably, the difference in absolute temperatures was attributable to this difference in the measurement instruments and represents a constant in the data. The variance was extremely low in both experiments, as might be expected for temperature readings in an inbred strain, and, more important for interpretation of the present results, there was no reason to expect an interaction between sensitivity of the probe and wheel condition.

The analysis of wheel count data in the present research showed that female mice exhibited higher levels of running than male mice during most of the active period (Fig. 1); analyses of several blocks of days indicated that this pattern of activity for male and female mice was stable across days. Previous studies of wheel running in C57BL/6J mice have generally used only male animals (10,18,19), but the rat literature suggests that females show higher levels of running than males (20) and that running patterns vary with estrous cycle (2). Gender differences in running patterns will become important for the present line of research in the event that we see gender differences in the effects of exercise on EtOH response. Thus far, we have not seen gender differences in effects of exercise on EtOH-induced LORR, hypothermia, or impairment of activity and avoidance.

The overall levels of wheel running seen in the present research were consistent with those previously reported for C57BL/6J mice given 24-h free access to running wheels (10,18). Goodrick (10) studied the effect of voluntary wheel

running on food and water intake and body weight in C57BL/6J mice and several mutant strains. In that research, the C57 mice increased food and water intake to compensate for energy expended in running and maintained normal body weight over the course of 20 days. In accord with this earlier work, exercised animals of the present research maintained normal body weight even over the longer period of 5 weeks.

Despite the absence of any differences in body weight, it can be assumed that the wheel animals did develop differences in body composition as a consequence of exercise. It has recently been reported that after 24 weeks of voluntary wheel running rats showed no change in body weight but did show a change in body composition, notably, a reduction in the proportion of body fat (2). Also in unpublished work recently completed in our laboratory C57BL/6J and BALB/cByJ mice were killed after 5 weeks of voluntary running and carcasses were desiccated (9) to assess body fat, which provides an accurate estimate of % body water (% BW). Wheel mice had significantly higher % BW than no wheel animals ($p < 0.05$). The actual differences were quite small, on the order of 1-2%, but were reliable given the extremely low variance in body weight in the inbred strains. These findings provide evidence, in addition to the EtOH response, that voluntary running did result in alteration of the animals' physiology.

The differences in % BW also bear on the question of whether the present effects of exercise are attributable to differences in EtOH disposition, specifically, volume of distribution. To the extent that wheel animals have a higher % BW it can be assumed that they have a higher volume of distribution or an effectively lower dose of EtOH (9). Thus, differences in volume of distribution may have contributed in part to the effect of wheel running on the various indices of EtOH response. However, the magnitude of the difference in % BW was so low as to argue against the possibility that this effect accounts entirely for the protective effects of wheel running on EtOH response.

Another factor that may have affected EtOH disposition in the present experiment is accelerated clearance of EtOH. There have been two reports that exercise imposed after EtOH challenge results in more rapid clearance of blood EtOH (3,21). Ardies et al. (3) also reported that prior exercise (treadmill) training accelerated the action of the microsomal EtOH oxidizing system and resulted in more rapid clearance of EtOH from the blood of rats tested at rest. However, closer examination of the results of that research suggests that the major effect on clearance was shown by animals given additional treadmill exercise after EtOH challenge. In other words, previous training amplified effects of postchallenge exercise but produced little effect on clearance for animals tested without postchallenge exercise. Because the animals in our research were not given additional postchallenge exercise, it seems unlikely that the protective effects of prior exercise can be attributed primarily to differences in rate of clearance.

In summary, it seems unlikely that dispositional factors, such as volume of distribution or accelerated clearance, will account in full for the protective effects of voluntary exercise. The literature now suggests that chronic exercise alters neurotransmitter function in several brain areas (11,19), and it is conceivable that voluntary exercise exerts its protective effects in part by actions on one or more of these systems. In any case, it remains for future research to determine the exact combination of mechanisms that account for the protective effects of voluntary exercise. Whatever mechanisms prove to be implicated in these effects, we have now accumulated considerable evidence to suggest that prior exercise confers protection against EtOH intoxication. These effects are seen in several different domains of EtOH response, namely, EtOH-induced LORR, hypothermia, and behavioral impairment.

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