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Effect of Ethyl Alcohol on Thermoregulation in Mice Following the Induction of Hypothermia or Hyperthermia[1]

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GORDON, C. J. AND A. G. STEAD. *Effect of ethyl alcohol on thermoregulation in mice following the induction of hypothermia or hyperthermia.* PHARMACOL BIOCHEM BEHAV **29**(4) 693–698, 1988.—This study was designed to assess the effects of ethyl alcohol (ethanol) administration on behavioral and autonomic thermoregulation in mice subjected to severe hypothermia or hyperthermia. Male mice of the BALB/c strain were injected intraperitoneally with ethanol at dosages of 0, 0.3, 1.0, or 3.0 g/kg and then placed within a hot environmental chamber to raise their body temperature to 41°C or, alternatively, within a cold chamber to lower it to 28°C. Once the desired hypothermic or hyperthermic state was achieved, the mice were removed from the chamber and placed in either a temperature gradient to monitor behavioral thermoregulatory responses or in an environmental chamber thermostabilized at an ambient temperature (T_a) of 28°C to monitor metabolic rate. The 3.0 g/kg dosage significantly affected behavioral thermoregulatory responses of the hyperthermic mice when initially placed in the temperature gradient. The ability to increase metabolic rate following hypothermia was significantly suppressed at 3.0 g/kg. Dosages of 1.0 and 3.0 g/kg inhibited metabolic rate of hyperthermic mice. Both hypothermic and hyperthermic mice given 3.0 g/kg of ethanol had colonic temperatures significantly below normal after placement in the temperature gradient and metabolic chamber. In conclusion, relatively large dosages of ethanol impair behavioral and autonomic thermoregulation and may lower the set-point for the control of body temperature in mice.

Ethyl alcohol Metabolic rate Preferred ambient temperature Metabolic rate Colonic temperature Set-point

NUMEROUS studies have demonstrated a profound effect of ethyl alcohol (ethanol) on the control of body temperature in rodents and other mammals [4,9]. At normal room temperatures (e.g., 22°C), relatively large doses of ethanol result in hypothermia. On the other hand, administering ethanol at warm ambient temperatures (T_a) generally attenuates the hypothermia and may even lead to hyperthermia [3,9].

In view of the numerous clinical reports of accidental hypothermia and hyperthermia associated with ethanol intoxication, there has been substantial interest in understanding the effects of ethanol on autonomic and behavioral thermoregulatory responses [4]. There is considerable debate over the effects of ethanol on behavioral thermoregulation. Acute administration of ethanol in rats had no effect on the preferred T_a in spite of a significant reduction in colonic temperature [13]. In contrast, a recent study in our laboratory showed that mice given an acute dose of ethanol (3 g/kg) and placed in a temperature gradient underwent a significant reduction in their preferred T_a along with concomitant hypothermia [3]. This appears to be paradoxical because the intoxicated animals were free to select a warmer T_a and thereby block the ethanol-induced hypothermia.

Most studies on the effects of ethanol on thermoregulation have utilized animals which are normothermic. Considering the potential danger of hypothermia and hyperthermia in combination with ethanol intoxication in man, it would be beneficial to develop an experimental model to study the thermoregulatory effects of ethanol in an animal which is not normothermic and has a compromised thermoregulatory control. For example, would a hypothermic animal subjected to ethanol intoxication be able to select a warm environment and/or raise metabolic heat production in order to return body temperature to normothermia? The purpose of the present study was to assess the effects of acute ethanol ad-

[1]This paper has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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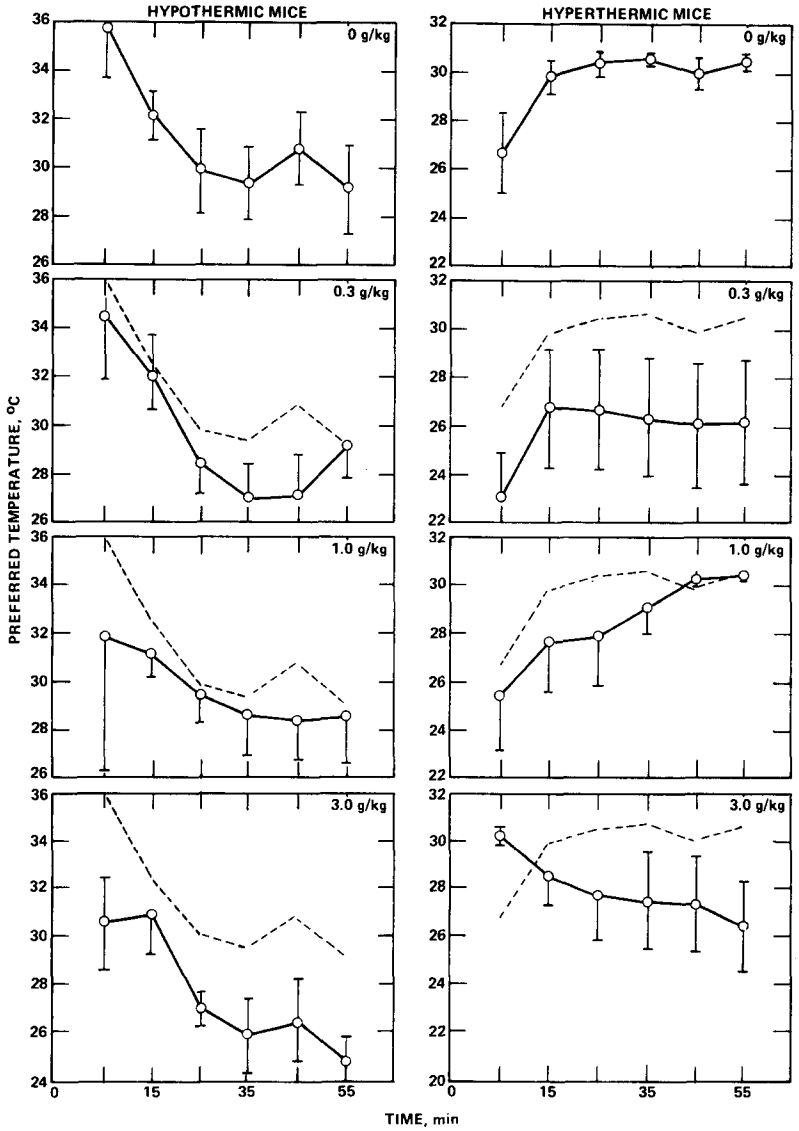

FIG. 1. Time course of preferred ambient temperature (T_a) of mice injected with ethanol, forced into hypothermia (T_{col}=28°C) or hyperthermia (T_{col}=41°C), and then placed in the temperature gradient for 60 min. For ease of comparison, the response of the control mice is graphed as a dashed line along side each of the treated groups. N=7 for each ethanol dosage.

ministration on behavioral and autonomic thermoregulation in mice when previously subjected to severe hypothermia or hyperthermia.

METHOD

Subjects

Animals used in this study were young-adult male mice of the BALB/c strain (Charles River Labs.). The mice were housed in groups of 10 in cages lined with wood shavings. The mice were maintained at a T_a of 22°C with a relative humidity of 50% and a 12:12 L:D photoperiod. Food (Lab Blox) and water were provided ad lib.

Behavioral Experiments

Preferred T_a of individual mice was measured using a temperature gradient as described previously [1,3]. Position of the mice in the gradient was automatically recorded at 1 min intervals, converted to preferred T_a, and plotted on a digital-analog recorder (Dianachart, DG-5).

The experimental protocol consisted of five principal components; (I) a naive mouse was placed in the temperature gradient and allowed to accomodate for 60 min; (II) the mouse was removed from the gradient and injected intraperitoneally (IP) with one of four dosages of ethyl alcohol dissolved in 0.9% saline (USP grade; 0, 0.3, 1.0, or 3.0 g/kg in a volume of 0.5 ml/100 g body mass); (III) a type-K thermocouple was inserted 2.5 cm past the anal sphincter and taped to the tail. The restrained mouse was then placed in a small environmental chamber set at a cold temperature of 0°C or a warm termperature of 55°C; body temperature of the mouse was continually monitored until the designated

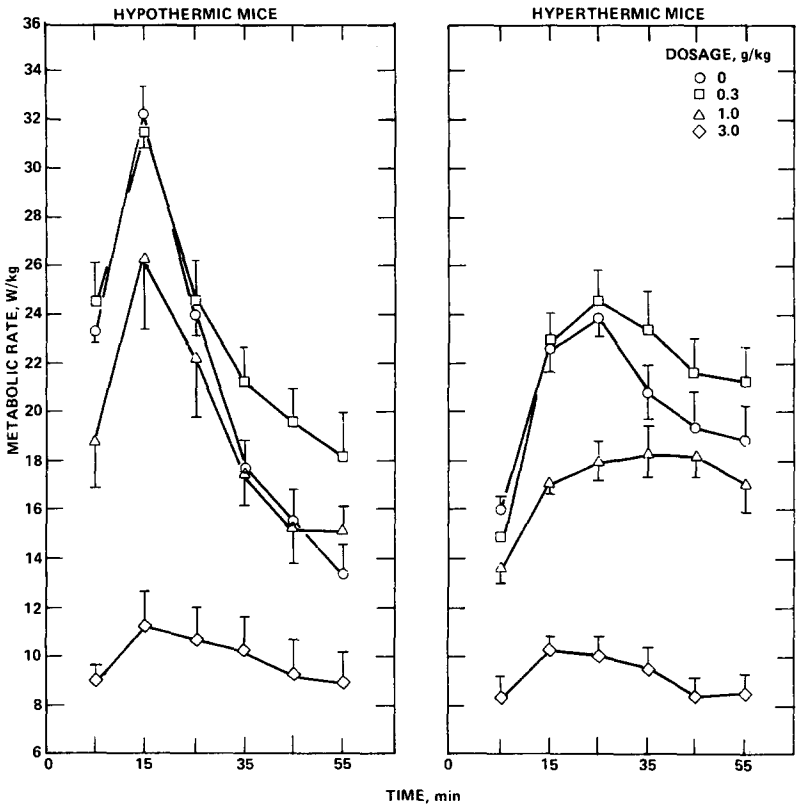

FIG. 2. Time course of metabolic rate (MR) of mice injected with ethanol, forced into hypothermia or hyperthermia and then placed in an environmental chamber set at a T_a of 28°C. Each data point represents the mean±S.E. N=5 for each ethanol dosage.

hypothermic (colonic temperature=28°C) or hyperthermic state (colonic temperature=41°C) was achieved; the rate of heating and cooling was related to the dose of alcohol (cf., Fig. 4); (IV) the mouse was quickly removed from the chamber, the thermocouple was removed from the colon and the mouse was placed in the temperature gradient for 60 min while preferred T_a was continuously recorded; (V) after 60 min in the gradient the mouse was quickly removed from the gradient and its colonic temperature was determined.

In the hypothermia experiments the gradient was adjusted such that the low temperature was 22°C and the high temperature was 42°C. After the hypothermic treatment the mouse was always placed at the coolest end of the gradient. In the hyperthermic experiments the gradient was adjusted such that the low temperature was 16°C and the high temperature was 31°C. After the hyperthermic treatment the mouse was always placed at the warmest end of the gradient. Thus, in both experiments the animals were placed in the gradient in a way that they were forced to use behavioral mechanisms to warm or cool themselves depending on the previous thermal treatment. The mean (±S.E.) body mass was 21.5 (±0.3) and 24.6 (±0.2) g for the hypothermic and hyperthermic groups, respectively.

Autonomic Experiments

In these experiments naive mice were forced into a condition of hypothermia or hyperthermia as described above (steps I–III) and then placed in an environmental chamber set at a T_a of 28°C while metabolic rate (MR) was recorded. The characteristics of the chamber and technique for measuring MR have been described in detail [2]. Briefly, dry air was pulled through a stainless steel temperature-controlled chamber at a constant flow rate. Percent oxygen of the effluent chamber air was continually monitored using an oxygen analyzer (Applied Electrochemistry, S-3A). The fractional change in oxygen of the air passing through the chamber was used to calculate MR.

The protocol was similar to that of the behavioral experiments except that there was no accomodation period in the metabolic chamber prior to the ethanol treatment (i.e., no step I). Following the hypothermic or hyperthermic treatment, the mouse was placed in the chamber while MR was automatically recorded at 2.0 min intervals. Sixty min after placement in the chamber the colonic temperature was quickly measured. The mean (±S.E.) body mass was 25.0 (±0.3) and 24.1 (±0.3) g for the hypothermic and hyperthermic groups, respectively.

Statistical Analysis

The behavioral and metabolic responses for each mouse were averaged to produce 6 time points. Four separate analyses were conducted: One for each endpoint and pre-exposure condition. A general linear models procedure was used to perform multivariate analysis of variance (AOV) within a repeated measures framework [6]. For those situations where no time-by-ethanol interactive effects were detectable, profile analysis was employed [12]. Individual one-way AOVs were also used to test for possible dosage-

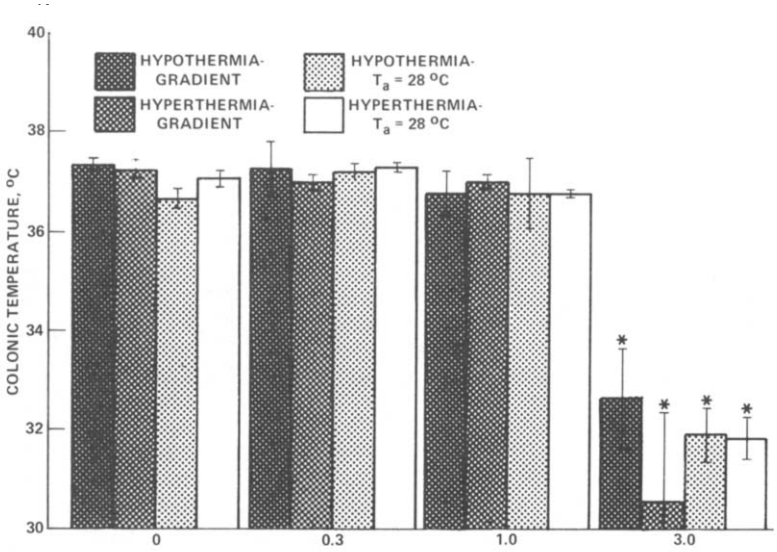

FIG. 3. Mean±S.E. of colonic temperature of mice from hypothermic and hyperthermic groups measured 60 min after placement in either the temperature gradient or metabolic chamber. N=7 for the behavioral experiment and N=5 for the metabolic experiments.

related effects of ethanol at each time point. If the response during a particular interval was significant, a multiple comparison technique due to Ryan [11], among others, was invoked to examine pairwise differences between ethanol dosage group means. For these comparisons, Type I experiment-wise error was controlled at 5%. Significant differences in colonic temperature were assessed using Dunnett's multiple comparison *t*-test.

RESULTS

Behavioral Experiments

Hypothermic mice. Control mice placed in the gradient quickly moved to the warm end with a mean preferred T_a of 36°C during the first 10 min of testing (Fig. 1). These mice gradually moved to cooler temperatures, eventually settling to a preferred T_a of ~30°C. Individual AOVs could not detect any ethanol-related effects at any of the 6 time points. It is interesting to note that the 3 g/kg group was significantly hypothermic at the end of one hour in the temperature gradient; however, this treatment failed to elicit a significant change in preferred T_a.

Hyperthermic mice. Control mice placed in the gradient initially moved to the cool end with a preferred T_a of 27°C during the first 10 min of testing (Fig. 1). These mice were similar to the hypothermic animals by exhibiting a gradual movement to a T_a of 30°C during the latter stages of the testing period. AOVs of preferred T_a indicated possible dosage-related effects during the first 10 min in the gradient. Specifically, the 3 g/kg dose group had a preferred T_a of 30° C which was significantly different from the preferred T_a of the 0.3 g/kg group ($p=0.039$). Colonic temperature of the 3.0 g/kg group was significantly below that of the controls (Fig. 3).

Metabolic Experiments

Hypothermic mice. Mice given 0, 0.3, or 1.0 g/kg alcohol exhibited a profound overshoot in MR during recovery from hypothermia (Fig. 2). Individual analysis of variance of MR at each time point showed ethanol effects ($p<0.01$) at each of the 6 time points (Fig. 2). MR of the 3 g/kg group was significantly below that of the other groups for the first 50 min. Otherwise, there were no significant effects of MR between the controls and the lower dosage groups. Colonic temperature of the 3 g/kg group was significantly depressed after one hour in the metabolic chamber (Fig. 3).

Hyperthermic mice. As with the hypothermic groups, mice recovering from hyperthermia exhibited an overshoot in MR during recovery; however, the response was attenuated when compared to the hypothermic mice (Fig. 2). AOVs of MR showed that a significant ($p<0.01$) ethanol dosage-related effect occurred at each time point (Fig. 2). Multiple comparison techniques showed that MR at 3 g/kg was significantly lower than MR of the other dosages. At the 2nd and 3rd time points, MR for the 1 g/kg group was significantly lower than for controls or the 0.3 g/kg group. As with the other treatments, colonic temperature of the 3 g/kg dosage was significantly below that of the controls (Fig. 3).

Ethanol treatment had a significant effect on the rate of cooling but not heating when the animals were restrained and subjected to cold or heat exposure (Fig. 4). There was a dose-related decrease in cooling of colonic temperature in the cold-exposed mice. The rate of heating was not significantly influenced by ethanol treatment. Mice of the hypothermic and hyperthermic groups given 3.0 g/kg initially survived the treatment but died 24 to 48 hr after injection. No deaths were noted at the lower ethanol dosages.

DISCUSSION

The data show that an IP administration of ethanol in mice impairs some aspects of behavioral and autonomic thermoregulation when challenged with a severe hypothermic or hyperthermic stress. The effects on metabolic heat production were much more evident than the effects on behavioral thermoregulation. Both the 1.0 and 3.0 g/kg dosages had depressant effects on metabolic heat production. It was

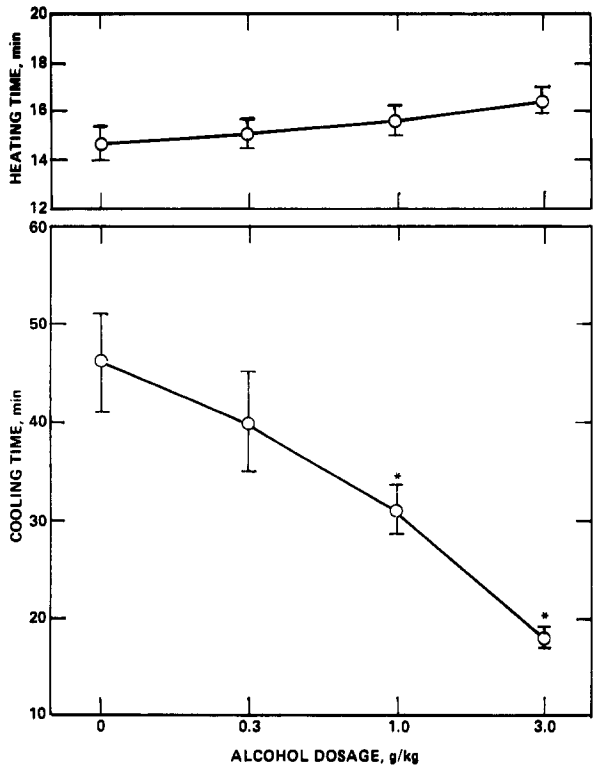

FIG. 4. Effect of ethanol dosage on the rate of cooling and warming of colonic temperature of mice placed in the cold and hot environmental chambers, respectively. *Indicates significant difference from controls ($p<0.02$).

recently shown that a dosage of 3.0 but not 1.0 g/kg of ethanol significantly inhibited MR in normothermic mice [3]. The ability of a hypothermic mammal to increase heat production would be crucial to rewarming. The 3 g/kg group forced into hypothermia had a severely attenuated MR response which was probably responsible for the hypothermic body temperature 60 min after placement in the 28°C environmental chamber. It is interesting to note that the hyperthermic mice administered 0 to 1.0 g/kg underwent an overshoot in MR which would impede the ability of the mice to lower body temperature. On the other hand, the MR of the 3 g/kg mice after the hyperthermic treatment was severely depressed which probably accounted for the hypothermia 60 min after placement in the metabolic chamber. It is not clear whether the inhibitory action of acute ethanol treatment on metabolic rate is attributable to an effect on the central nervous system or is a direct inhibition of tissue metabolism. Clearly, ethanol affects several aspects of lipid and carbohydrate metabolism in vivo and in vitro (e.g., [15]). Further work is needed to differentiate between the central and peripheral effects of ethanol on whole-body metabolism.

Only the 3 g/kg dosage of ethanol in hyperthermic mice had a significant effect on behavioral thermoregulation. In those animals the preferred T_a did not decrease as occurred in the other groups when placed in the warm end of the temperature gradient. A dosage of 3 g/kg is very toxic to mice and generally resulted in death within 48 hr. However, it is interesting to note the interaction between body temperature and the behavioral thermoregulatory response of these animals. That is, at the end of one hour in the temperature gradient, the hypothermic and hyperthermic mice given 3 g/kg of ethanol had a preferred T_a of 24 to 28°C, albeit their colonic temperature was only 30 to 32°C. This would suggest a severe impairment of thermoregulatory control in these animals since they had the option of moving to the warmer part of the temperature gradient and thereby raise their colonic temperature. A recent study from this laboratory showed that normothermic mice given 3 g/kg of ethanol underwent a slight but significant decrease in the preferred T_a (Gordon and Stead [3]). Spencer *et al.* [13] found that rats injected IP with ethanol had no change in preferred T_a when placed in a temperature gradient in spite of a significant decrease in colonic temperature. On the other hand, such a behavioral effect was only apparent at extremely high doses. It is not clear how the effects of ethanol on motor activity impact on behavioral thermoregulatory responses. For example, the failure to rapidly select a cooler T_a in the hyperthermic mice might be a result of an ethanol-induced change in locomotor activity rather than a direct effect on behavioral thermoregulation. In a related study, Le *et al.* [5] reported a more rapid development of tolerance to hypothermia in rats to repeated alcohol administration when treatment was carried out at a T_a of 4°C, where hypothermia was observed, compared to a T_a of 36°C where no hypothermia was observed.

It is possible that ethanol may cause a decrease in the set-point for body temperature which would explain the low preferred T_a of hypothermic mice in the temperature gradient. Several studies have shown an effect of ethanol on the concentration, turnover, and/or release of various neuromodulating substances in the central nervous system [4, 7, 14]. Rezvani *et al.* [10] found that the hypothermic action of alcohol in the rat was closely tied to the turnover of calcium in the central nervous system. Since the control of the set-point for body temperature has been shown to be closely dependent on the turnover of calcium as well as other neuromodulating substances in the hypothalamus and preoptic area [8], it is possible that ethanol administration could modulate the set-point.

This study has demonstrated a useful model for examining the interaction between forced hyperthermia and hypothermia and the impact of acute ethanol administration. Ethanol and other agents may exert different effects in animals which are temperature-stressed (i.e., hypothermic or hyperthermic) compared to unstressed individuals. In the case of this study, ethanol affected the initial behavioral response of the hyperthermic mouse to select a cool T_a and also attenuated the metabolic response during rewarming from hypothermia. However, these effects were only observed at very toxic dosages. It will be of interest to apply these methods using other species and other routes of administration.

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