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Genetic Selection Alters Thermoregulatory Response to Ethanol

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O'CONNOR, C. S., L. I. CRAWSHAW AND J. C. CRABBE. *Genetic selection alters thermoregulatory response to ethanol.* PHARMACOL BIOCHEM BEHAV 44(3) 501-508, 1993.—The present study examined the effect of ethanol on the regulated temperature of two lines of mice selected in replicate for a smaller (HOT1 and HOT2) or greater (COLD1 and COLD2) decline in rectal temperature after IP ethanol. Mice were implanted with indwelling telemetry devices for remote monitoring of internal temperature and trained in a temperature gradient (8-40°C). Both internal and selected temperature were tracked and recorded with a computer after injections of NaCl or various doses of ethanol. All animals responded similarly to control injections, with a transient rise in body temperature. After an effective dose of ethanol, mice showed clear evidence of a regulated decline in body temperature, as evidenced by selection of low temperatures in the gradient at the same time internal temperatures were falling. COLD mice were more sensitive than HOT mice; this was apparent in both replicates of the selected lines, indicating that a difference in the CNS regulator of body temperature has been selected for in these animals.

COLD mice Ethanol HOT mice Selected lines Temperature gradient Thermoregulation

THERE is considerable individual variability in the response of body temperature to an acute dose of ethanol (EtOH), although the response in general involves a lowering of body temperature. However, if the output of a thermoregulatory effector mechanism is not measured an observation that internal temperature is lowered by EtOH or any other drug at a given ambient temperature says little about whether the regulated temperature has been altered. A variety of thermoregulatory effector mechanisms are available to animals, including vascular responses, evaporative water loss, and metabolic adjustments; also available are thermoregulatory behaviors, which include postural modifications and selection of an appropriate environmental temperature. Thermoregulatory behavior is a powerful and favored effector mechanism (10). If a sufficiently broad range of ambient temperatures is available to an animal, measurement of preferred environmental temperature (when considered along with changes in internal temperature) provides a sensitive means with which to assess alterations in the regulated temperature (19,38).

High doses of EtOH disrupt all physiological processes (11), including thermoregulation (30). However, moderate doses of EtOH elicit a decrease in body temperature regulated by the CNS in both the rat (25,26) and the mouse (18,31). This effect has been most clearly demonstrated in a behavioral situation for both rats and mice (26,31). If EtOH acted only on peripheral effector mechanisms, for example, causing dilation of tail vasculature, without affecting the regulated temperature, an animal whose internal temperature began to fall would, if able to exercise a behavioral option, oppose that fall by selecting a warmer environment. In fact, after moderate doses of EtOH mice select cooler ambient temperatures at the same time internal temperature is falling, indicating a downward adjustment of the regulated temperature by EtOH (31). Such a regulated change is sometimes referred to as a change in "set point."

Much of the individual variability in sensitivity to the hypothermic effect of EtOH reflects genetic differences (6). Bidirectional genetic selection for a lesser or greater decrease in internal temperature after IP-injected EtOH has produced lines of mice that differ substantially and reliably in this characteristic (9). To confirm the reliability of similarities or differences detected between lines, this selection experiment was performed in replicate. For a given acute dose of EtOH, insensitive HOT1 and HOT2 mice experience a much smaller decline in rectal temperature than do the sensitive COLD1 and COLD2 mice.

Although the magnitude of the differences between HOT and COLD mice has been clearly established (5,9), the mecha-

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nisms underlying the genetic difference have not been elucidated. At least for the first 3 h postinjection, their rate of EtOH clearance is similar (5). During genetic selection, mice were characterized at 24°C, an ambient temperature well below thermoneutrality for the mouse (36). Because rectal temperature was the only response to EtOH assessed, it was not possible to distinguish between a regulated fall in body temperature and a decline in internal temperature due to the direct activation of heat loss effectors. To understand how selective breeding has altered body temperature responses in these lines of mice, it is essential to know the extent to which the observed divergence in body temperature after EtOH is due to a direct effect on the CNS regulator. A selection-induced difference in the regulated responses of HOT and COLD mice would open the exciting possibility of using these selected lines to elucidate the specific mechanisms by which EtOH affects CNS control of body temperature.

METHOD

Animals

Thirty-six male mice 8–10 weeks old were used in this study. Mice were from generation 20 of four selected lines. These lines have been selected in replicate for an attenuated (HOT1 and HOT2) or augmented (COLD1 and COLD2) hypothermic response to an initial IP injection of 3 g EtOH/kg (9). The within-family selection protocol involved injecting naive mice at 24°C and measuring internal temperature with a rectal probe before and 30 and 60 min after injection. By the 18th selected generation, the average changes from baseline temperature 30 min after a 3-g EtOH/kg injection were: HOT1, −2.0°C; HOT2, −1.0°C; COLD1, −5.3°C; COLD2, −5.5°C (33).

Housing

Mice were housed, four or five to a cage, on hardwood shavings in standard mouse cages. The animal room was maintained at 26 ± 2°C on a 12 L : 12 D artificial photoperiod, with lights on at 6:30 a.m. Food (Purina Rodent Lab Chow) and water were available ad lib. All mice were naive to both the gradient test device and EtOH at the start of testing.

Surgical procedures

All mice were implanted in the intraabdominal space with radio transmitters (Model XM-FH, Mini-mitter Co., Inc., Sunriver, OR) that broadcast an AM pulse rate inversely proportional to temperature (600–300 Hz in the range of 32–42°C). Mice were anesthetized with a ketamine-xylazine cocktail, and a calibrated transmitter, coated with paraffin, was inserted into the peritoneal cavity of each mouse as previously described in detail (31). The abdominal muscle was sutured and the skin incision closed with 7.5-mm wound clips. During surgery, corneal surfaces were coated with ophthalmic ointment. After surgery, mice were given an IM injection of 3 mg chloramphenicol (Chloromycetin, Parke-Davis Co., Detroit, MI), and body temperature was kept at 37°C until waking. Mice were allowed 6 days of recuperation before participating in an experiment.

Apparatus for quantifying internal and preferred temperature

Temperature selection of mice was quantified in a temperature gradient (31). Briefly, Plexiglas tubes (2.2 m long × 4.5 cm in diameter) were submerged, one per lane, in the nine lanes of an aquatic temperature gradient device. The ends of each tube made a 90° bend upward out of the water to enable mice to enter and exit; removable baffles prevented premature departure. The water in each lane was heated at one end and cooled at the other. A nearly linear temperature gradient (about 8–40°C) was thereby established in the water, generating a similar gradient in the air of each submerged tube. During experiments, lights were kept dim. A low-light videocamera mounted above the gradient was connected to an image analyzer installed in a Zenith 386 computer. This allowed the position of each animal to be recorded every 6 s, while output to two video monitors permitted continuous observation of mice. After every experiment, the water temperature was measured at 10 locations in each lane. These temperatures were used to translate recorded animal positions into selected temperatures with the use of a correction factor to take into account a small, regular difference between air and water temperature along the length of the gradient tubes. To monitor internal temperatures, each tube was wound with wire, which acted as an antenna to carry the transmitter signal to its receiver (Model RA-1000-TH, Mini-mitter), which in turn relayed the signal to the computer for recording.

Procedure for quantifying internal and preferred temperature

Two days before mice were implanted, they were trained in gradient tubes until they entered the tubes promptly and exited willingly. Training consisted of an initial bout in the gradient lasting about 1.5 h, a rest period of 2 h, and a second bout in the gradient of approximately 45 min. All mice received the same amount of training time. Beginning with the first training run and continuing through the several experimental runs, insertion and removal of mice was randomized between the hot and cold ends of the tubes. In addition, no mouse was run in the same lane on more than 1 day. Tubes were thoroughly washed with running water and dried between days of use. We previously demonstrated that mice position themselves in the gradient tubes in response to the temperature gradient, not odor or lighting cues (31). All training and testing occurred between 10:00 a.m. and 2:00 p.m.

Because only nine mice could be tested in 1 day, animals were divided into four groups of nine mice each. Each group consisted of a mixture of HOT1, HOT2, COLD1, and COLD2 mice (i.e., Group 1 contained 3 HOT1, 2 HOT2, 2 COLD1, and 2 COLD2 animals; Group 2 contained 2 HOT1, 3 HOT2, 2 COLD1, and 2 COLD2 animals, etc.) so that each genotype of mouse was represented on each day of testing (grand total of nine mice of each genotype). A few mice died over the course of these experiments, so results from nine animals of each genotype are not available for every dose.

On the day of an experiment, each mouse was weighed and released into one of the gradient tubes. Both behaviorally selected temperature and internal temperature were monitored for 1 h; then, mice were removed and returned to their home cages for about 1 h to rest. Next, mice were injected IP with 10% w/v EtOH (2.0, 2.25, 2.65, 2.85, or 3.0 g EtOH/kg body wt) or with a volume of 0.9% NaCl equivalent to the volume of a 2.65-g EtOH/kg injection. Because HOT and COLD animals differed substantially in their sensitivity to EtOH, it was not appropriate to test all mice at all doses. However, each animal was tested at multiple doses; the specific doses and order of administration are given in Table 1. After injection, each mouse was immediately returned to the same tube occupied before injection and internal and selected temperature were monitored for 1 h. For each mouse, gradient runs were always 4 days or more apart.

During the course of these experiments, qualitative obser-

TABLE 1
INJECTIONS GIVEN TO HOT AND COLD MICE

	Injection Order			
Genotype	1st	2nd	3rd	4th
HOT1	2.65 g/kg	NaCl	2.85 g/kg	–
HOT2	2.65 g/kg	NaCl	2.85 g/kg	3.0 g/kg
COLD1	2.65 g/kg	NaCl	2.25 g/kg	2.0 g/kg
COLD2	2.65 g/kg	NaCl	2.25 g/kg	2.0 g/kg

vations were also made about differences in reactivity and apparent emotionality in the four groups of mice.

Data Analysis

Temperature-response data were quantified in two ways. First, internal temperatures (monitored with the implanted transmitters) and selected temperatures (deduced from position in the gradient), collected every 6 s during the course of the experiments, were analyzed. The mean of the last 10 min of the preinjection period was utilized as baseline. Postinjection temperatures were calculated as 10-min means for the 60-min postinjection period.

After an injection of either NaCl or EtOH, changes are observed in both the internal and selected temperatures of a mouse. If the effect of injection is to alter the set point for body temperature, effector response and internal temperature will exhibit coordinated postinjection changes. We established the thermoregulatory index (TI) (31) to allow a quantitative evaluation of postinjection changes from preinjection baseline data, and temperature responses were also compared using this measure. The calculation to obtain the TI compares the postinjection temperature status of each individual against its own preinjection internal and selected temperature, allowing each animal to be its own frame of reference to determine whether an injection has had an effect. We employed an additive model (21) to represent the interaction between internal and selected (ambient) temperatures in the following equation:

$$\mathrm{TI} = \alpha(T_{c_e} - T_{c_i}) + (T_{\mathrm{sel}_e} - T_{\mathrm{sel}_i}),$$

where

TI = thermoregulatory index;
α = weighting for internal temperature;
T_{c_e} = core temperature after injection;
$T_{c_i}^{e}$ = initial (baseline) core temperature;
T_{sel_e} = selected temperature after injection;
$T_{\mathrm{sel}_i}^{e}$ = initial (baseline) selected temperature.

We assume a value of 10 for α, which represents the weighting of internal temperature relative to ambient temperature by the regulator with respect to relative importance as stimuli in the activation of thermoregulatory effector responses. This value reflects the results of appropriate comparisons on medium and small mammals by several investigators (20,22,29). Ten-minute preinjection values were utilized as initial (baseline) internal or selected temperature in this calculation.

Activity was quantified as the distance (cm) moved in the gradient tube per 6-s sample interval.

Factorial analysis of variance (ANOVA) with repeated measures was used to evaluate the effect of EtOH. Selected lines such as HOT and COLD mice, whose progenitors were randomly selected from a pool of genetically heterogeneous mice, differ in the frequency of genes that control the trait upon which selection pressure has been applied. Replication of the selection experiment, that is, independent development of the HOT1-COLD1 and HOT2-COLD2 replicate pairs, controls for trait-irrelevant inbreeding and genetic drift. The detection of similar response differences in both replicate pairs is evidence that the difference has developed as a result of selection pressure and not by chance. Therefore, between-subjects factors of both selected line and genetic replicate, as well as drug or dose, and within-subjects factor time were utilized in the analyses, although not all factors were used in every analysis (see below). A posthoc Fisher's least significant differences (LSD) test was employed where appropriate to detect significant differences between levels of drug, dose, or time.

To compare the effect of the 2.65-g EtOH/kg injection initially given to all mice, the internal and selected temperatures of each animal, recorded every 6 s, were summed to individual 10-min means. Responses were evaluated over the −10-min (preinjection) to +60-min (postinjection) period and analyzed using the factors selected line, genetic replicate, and time. Analysis of the TI values after NaCl and 2.65-g EtOH/kg injections utilized the same factors in the ANOVA protocol as were used to compare the raw temperature data, with the addition of the factor drug to compare the effects of control and ethanol injections.

For analysis of dose-response curves, a 60-min average of the six 10-min postinjection TI values was calculated for each animal after NaCl and the various doses of EtOH and utilized in the repeated-measures ANOVA protocol. HOT and COLD mice received different doses of EtOH, so responses were only compared between genetic replicate (within-factor dose). Because HOT2 mice appeared to have a slightly higher threshold for EtOH hypothermia than did HOT1 mice, they received an additional dose of 3.0 g EtOH/kg. A separate one-way ANOVA was performed to examine the effects of control injection and all levels of EtOH on HOT2 mice.

Analysis of locomotor activity after control and EtOH injections followed the same procedure as that explained above for the dose-response analyses, using 60-min average activity calculated for each animal at each condition.

An α level of $p < 0.05$ was accepted as significant. Significant F and p values are reported within the text. All measures of variability refer to SEM.

RESULTS

The responses of all HOT and COLD mice to the common dose of EtOH (2.65 g/kg) are illustrated in Fig. 1. Internal temperatures are shown in the upper panel and selected tem-

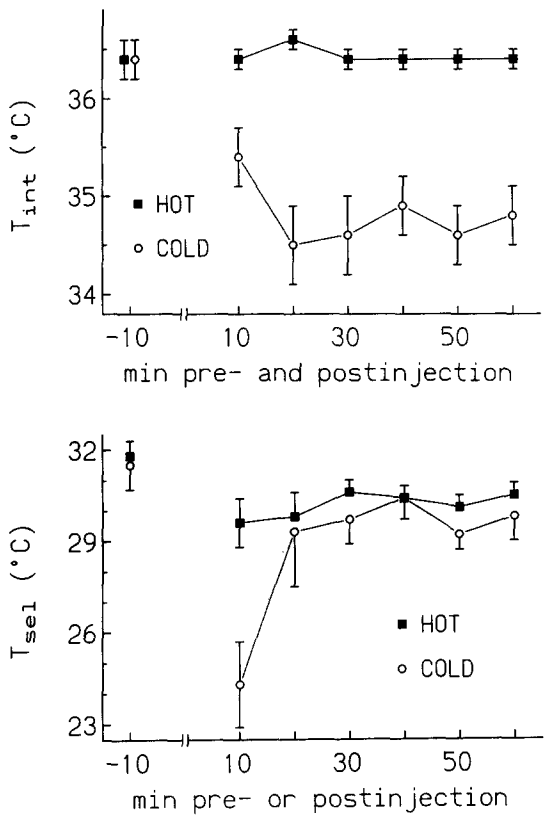

FIG. 1. Internal (T_{int}, upper plot) and selected (T_{sel}, lower plot) temperature responses to 2.65 g ethanol (EtOH)/kg IP of HOT (n = 17) and COLD (n = 16) mice. Shown are 10-min mean ± SEM temperatures for 10 min before and 60 min after injection. (Preinjection internal temperatures were identical, so symbols are slightly offset.)

peratures in the lower panel for 10 min before and 60 min after injection. Because ANOVA reported no significant effect of genetic replicate on either internal or selected temperatures, HOT and COLD mice were grouped for this figure.

Analysis of internal temperatures revealed a significant effect of line, $F(1, 31) = 43.98$, $p < 0.0001$, and time, $F(6, 186) = 6.19$, $p < 0.0001$, and a significant line × time interaction, $F(6, 186) = 7.10$, $p < 0.0001$. The postinjection internal temperatures of COLD mice were significantly lower than preinjection temperatures at all postinjection time intervals ($p < 0.05$), while the postinjection and preinjection internal temperatures of the HOT mice did not differ.

Analysis of selected temperatures showed no effect of line, but a significant effect of time, $F(6, 174) = 9.31$, $p < 0.0001$, and a significant line × time interaction, $F(6, 174) = 3.79$, $p < 0.01$. HOT and COLD mice were therefore compared at each level of time. The selected temperatures did not differ before injection; after injection, HOT and COLD mice differed only for the first 10-min postinjection time interval, $F(1, 31) = 11.64$, $p < 0.01$. All postinjection selected temperatures were significantly lower than those selected before injection.

As illustrated in Fig. 1, during the first 10 min after this dose of EtOH the internal temperatures of COLD mice declined substantially below their preinjection values. At the same time, these mice selected very cool temperatures. Thus, they utilized a powerful effector mechanism (thermoregulatory behavior) to lower internal temperatures yet further, as evidenced by the additional decrease in internal temperature during the second 10-min postinjection period. For HOT mice, because this dose had a marginal effect the situation was more complex. The internal temperatures of HOT mice were similar before and after injection, while selected temperatures after injection remained depressed below preinjection levels. Because internal and selected temperatures are not independent measures for freely behaving animals in a temperature gradient, it is misleading to consider one without the other. To clarify the relationship between the measured variables (internal and selected temperature) and the calculated TI, the data from Fig. 1 have been replotted in Fig. 2 as changes in the TI over time after the common dose of 2.65 g EtOH/kg. To compare the effects of control and EtOH injections on the regulated temperature, Fig. 2 also shows the TI values after NaCl control injections.

Analysis of the TI of all genotypes after NaCl and 2.65 g EtOH/kg revealed no significant effect of genetic replicate. ANOVA reported significant main effects of line, $F(1, 31) = 63.13$, $p < 0.0001$, time, $F(5, 155) = 8.93$, $p < 0.0001$, and drug, $F(1, 31) = 44.03$, $p < 0.0001$, and significant line × drug, $F(1, 31) = 16.55$, $p < 0.001$, and time × drug, $F(5, 154) = 7.92$, $p < 0.0001$, interactions. The three-way interaction was not significant. To further clarify the analysis, a two-way ANOVA (factors line and time) was run separately on the responses to EtOH or NaCl. Only the factor line was significant for the response to EtOH, $F(1, 31) = 49.52$, $p < 0.0001$. In contrast, time (but not line) was significant for the response to NaCl, $F(5, 155) = 75.05$, $p < 0.0001$. Posthoc Fisher's LSD reported that the first 20 min differed significantly from the last 40 min, and the third and fourth 10 min periods differed from all others, during the response to NaCl injection ($p < 0.05$ for all comparisons). For neither two-way ANOVA (on NaCl or EtOH) was there a significant line × time interaction.

These statistical results make it clear that, in the original multifactorial analysis, the significance of the factor time was due to the response to control injection. After an NaCl injection, the TI is significantly elevated for about 20 min, then gradually declines to preinjection levels. HOT and COLD animals do not differ in this response. In contrast, HOT and

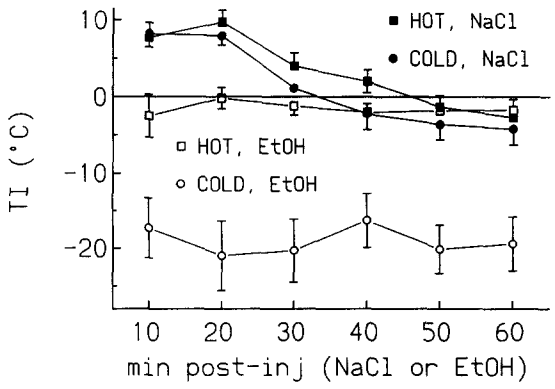

FIG. 2. Thermoregulatory index (TI) of responses to NaCl and 2.65 g ethanol (EtOH)/kg (HOT mice, n = 17; COLD mice, n = 16). Shown are sequential 10-min mean ± SEM TI values for 60 min after injection. For explanation of TI, see the text.

COLD animals differ significantly in their response to 2.65 g EtOH/kg, but time has no significant effect on the drug response for the 60-min postinjection period.

Due to the markedly greater sensitivity of COLD mice to acute EtOH, which necessitated utilizing different dose ranges in the genotypes, 2.65 g EtOH/kg was the only dose level common to all mice. To examine the response of the mice to varying doses of EtOH, COLD mice were further tested at doses of 2.0 and 2.25 g EtOH/kg, while HOT mice were given an additional dose of 2.85 g EtOH/kg. Because HOT2 mice appeared to have a higher threshold for EtOH hypothermia than did HOT1 mice, an additional dose of 3.0 g EtOH/kg was used for HOT2 mice only. The 60-min mean postinjection TI values for control and all EtOH injections are shown in Fig. 3. The degree of negativity of the TI closely estimates the extent to which the regulated temperature was decreased. Because the dose ranges differed, the responses of HOT and COLD mice were analyzed separately.

When ANOVA was applied to the responses of COLD mice, a significant effect of dose on the TI was reported, $F(3, 39) = 16.69, p < 0.0001$. No significant effect of genetic replicate or significant replicate × dose interaction was detected. Posthoc analysis reported that the effect of NaCl injection (0.0 g EtOH/kg) was significantly different from the effects of all doses of EtOH and that the effects of the 2.0- and 2.65-g EtOH/kg doses differed from each other ($p < .05$); the effect of the 2.25-g EtOH/kg dose was intermediate to both and did not differ from either.

Analysis of the responses of HOT mice revealed a different pattern. ANOVA of responses to the 0.0-, 2.65-, and 2.85-g EtOH/kg doses detected a significant effect of both replicate, $F(1, 15) = 7.79, p < 0.05$, and dose, $F(2, 30) = 7.12, p < 0.01$, on the TI of HOT mice, although the replicate × dose interaction did not reach significance. Posthoc analysis indicated that the effects of control injection and the 2.85-g EtOH/kg dose were significantly different ($p < 0.05$). A separate one-way ANOVA was performed on HOT2 mice only, including data from the 3.0-g EtOH/kg dose given only to those animals. The main effect of dose did not achieve significance.

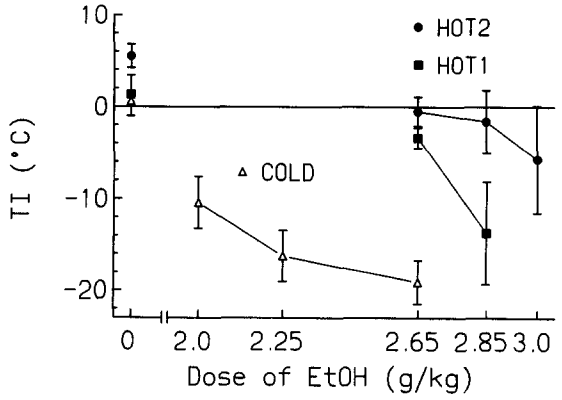

FIG. 3. Dose-response curves; thermoregulatory index (TI) of response for 60 min after IP injection of NaCl (0) or indicated doses of ethanol (EtOH). COLD1 and COLD2 mice did not differ and are combined (NaCl and 2.65 g/kg, $n = 16$; 2.25 g/kg, $n = 15$; 2 g/kg, $n = 14$). Differences were detected between HOT1 ($n = 9$/dose) and HOT2 ($n = 8$/dose) mice and they are shown separately. For each individual mouse, a single 60-min postinjection TI was calculated by taking a mean of six consecutive postinjection 10-min TI values. These values for individuals were averaged to yield mean ± SEM TI. For explanation of TI, see the text.

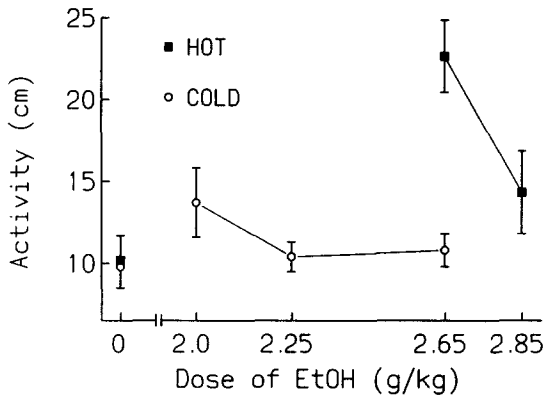

FIG. 4. Activity of mice after IP injection of NaCl (0) or indicated doses of ethanol (EtOH), expressed as mean ± SEM cm traveled in the gradient tubes per 6-s sample interval, averaged over the 60-min test. Shown are combined HOT1 and HOT2 mice ($n = 17$/dose) and COLD1 and COLD2 mice (NaCl and 2.85 g/kg, $n = 16$; 2.25 g/kg, $n = 15$; 2 g/kg, $n = 14$).

Figure 4 shows the activity of animals in the thermal gradient tubes after injections of NaCl or EtOH. Once again, because the dose ranges employed in the lines were different the activity responses of HOT and COLD mice were analyzed separately. Because ANOVA detected no significant effect of genetic replicate, the responses of COLD and HOT mice were grouped for this figure. Analysis of the activity of COLD mice revealed a significant dose effect, $F(3, 39) = 4.75, p < 0.01$). Posthoc Fisher's LSD reported that activity of COLD mice was significantly elevated by the 2.0-g EtOH/kg dose, above activities measured after control or the other EtOH injections ($p < .05$). Similarly, analysis of responses of HOT mice detected a significant dose effect, $F(2, 45) = 9.82, p < 0.001$). Posthoc analysis revealed that activity of HOT mice after 2.65 g/kg was significantly elevated ($p < 0.05$) compared to activities after NaCl or the 2.85-g/kg EtOH dose.

DISCUSSION

Both HOT and COLD mice responded to EtOH with a regulated decrease in body temperature. Because the dose of EtOH required to elicit an effect was greater in HOT than in COLD mice, we believe a major difference in the response of the CNS regulator of body temperature to EtOH has been selected for in these mice. These results were apparent in both replicates of the selected lines, which suggests that they truly represent pleiotropic effects of genes determining the acute EtOH sensitivity on which selection was performed.

Experiments involving measurement of rectal temperature before and after various doses of EtOH (5) likewise revealed a substantial difference between the lines. A dose-response curve showing change in rectal temperature after 1, 2, 3, or 4 g EtOH/kg in HOT and COLD mice from the seventh selected generation (5) revealed that COLD mice experienced the same decline in rectal temperature after a 2-g EtOH/kg injection, as did HOT mice after a 4-g EtOH/kg injection (about 2°C). More recent studies show that with increasing generations of selection equivalent responsiveness is now seen at doses of about 5 g EtOH/kg (HOT) and 2 g EtOH/kg (COLD) (Feller and Crabbe, in preparation). Given the constraints of our

behavioral experiments, where animals must retain motor coordination to exercise thermoregulatory choice, it was indeed a challenge to find a common dose of EtOH at once large enough to affect the regulated body temperature of HOT mice but not so large as to produce ataxia and loss of motor function in COLD mice.

A typical response to injection of a dose of EtOH sufficient to exert a thermoregulatory effect is illustrated by the response of COLD mice to 2.65 g EtOH/kg presented in Fig. 1. During the initial 10-min postinjection period, when internal temperatures of COLD mice were below preinjection levels, they also chose very cool temperatures in the gradient. By thus utilizing the behavioral effector mechanism available to them in the gradient tubes, COLD mice lowered their internal temperatures even further. Internal temperatures remained low, although in subsequent time intervals COLD mice selected warmer temperatures. The time course of effect on internal temperature and selected temperature was quite different.

By combining the two temperature measures, the TI yielded a more comprehensive measure of thermoregulatory drive than either parameter (internal or selected temperature) expressed alone. The factors interacting in this measurement have been previously discussed in more detail (31). The TI of both HOT and COLD mice was significantly lower after an effective dose of EtOH than after control injections of NaCl. Although sensitivity varied among the genotypes, the data clearly indicated a decrease in the CNS-regulated temperature of both COLD and HOT mice in response to EtOH. The genotypes did not seem to differ in their fundamental response to EtOH but only in the magnitude of response to a given dose; COLD mice were significantly more sensitive than HOT mice. In addition, although the difference was not significant HOT1 mice tended to exhibit slightly greater sensitivity to EtOH hypothermia than did HOT2 mice (Fig. 3). However, this observation is in contrast to previously published findings that, for the last several generations, HOT1 mice have exhibited smaller hypothermic responses to 3 g EtOH/kg than HOT2 mice when tested by measuring rectal temperature response at room temperature (33). Further testing of a larger number of HOT mice in the gradient would be required to reveal or disprove the existence of a significant difference between the replicates in their thermoregulatory response to EtOH. Meanwhile, the substantial differences between the two experimental paradigms make any direct comparison between them difficult.

The results of this study confirm our previous conclusions about the thermoregulatory effects of moderate doses of EtOH, derived from studies in genetically heterogeneous mice (31), and agree with the work of others on the regulatory effects of moderate doses of EtOH in rodents (18,25,26). The mechanisms by which EtOH induces a decline in regulated body temperature are currently not understood. There is evidence for the involvement of a wide variety of neurotransmitter systems, including dopamine, norepinephrine, serotonin, acetylcholine, and several amino acids (24,34), although the precise role of each neurotransmitter has yet to be elucidated. Selection for insensitivity and sensitivity to EtOH, respectively, in HOT and COLD mice may have been achieved by a modification of one or more of these neurotransmitter systems, perhaps by altering neurotransmitter release or affecting receptor numbers or affinity. We demonstrated, for example, that COLD mice exhibit an increased hypothermic response to ICV-injected serotonin, as compared with the response of HOT mice (32). The divergent sensitivity to serotonin appears to be a regulated effect, similar to that which occurs after EtOH administration. It has also been postulated that an effect of EtOH on calcium channels in the brain is responsible for decreased body temperature after EtOH (37). In light of the importance of calcium for synaptic transmission, an effect on brain calcium channels might well influence a variety of physiological processes, including thermoregulation.

COLD mice achieved a lower rectal temperature than HOT mice after IP administration of a variety of alcohols and sedative hypnotics (propanol, *n*-butanol, *t*-butanol, pentanol, diazepam, phenobarbital, pentobarbital, methyprylon, and ethchlorvynol) (13). COLD mice also exhibited significantly lower rectal temperatures than did HOT mice after IP injections of the opiate agonists morphine and levorphanol, although their sensitivity to dopaminergic agonists and antagonists, an α-1 adrenergic agonist, a nicotinic agonist, and amphetamine did not differ (14). The principal difference between selected lines is in the frequencies of genes that control the trait upon which selection pressure was applied. Therefore, detection of differences between HOT and COLD mice in body temperature response to a neurotransmitter-selective drug suggests a possible role for that neurotransmitter in the mediation of EtOH hypothermia. However, because both these studies measured rectal temperature change at discrete time intervals after drug administration in mice held at 21°C no conclusions can be drawn about differential effects on the regulated temperatures of HOT and COLD mice.

COLD mice experienced a greater decrease in rectal temperature than did HOT mice after IP hydralazine, which principally acts as a peripheral vasodilator (13). It was concluded by the authors that selection has produced some alteration in vascular mechanisms between HOT and COLD mice, accounting for some of their difference in sensitivity to EtOH. Although a difference in vasodilatory response to EtOH may exist, it is unlikely to have made an important contribution to the difference in response to EtOH that we observed in the gradient situation because a behaviorally thermoregulating mouse could easily compensate for heat loss due to peripheral vasodilation by adjusting its position in the gradient.

The regulated decrease in body temperature observed after EtOH appears to serve an important function by increasing the probability of survival after potentially toxic doses of EtOH in mice (12,15,28). Decreased temperature also increases the threshold for depression and impaired motor coordination caused by the drug in both mice and rats (27,40,35). EtOH, like high temperature, causes a decrease in cell membrane viscosity (16,17); this effect is intensified at higher temperatures (4). Lower temperatures would be expected to oppose the membrane disordering effects of EtOH. A regulated lowering of body temperature after EtOH may have developed as an adaptive strategy in an animal whose wild ancestors consumed fermenting fruits; consumption of fruit during certain parts of the year (especially late summer) has been documented for wild wood mice (39).

There was no effect of genetic replicate on activity responses to injections in this study. Both HOT and COLD mice experienced a significant increase in activity at lower doses of EtOH (2.0 g EtOH/kg for COLD mice, 2.65 g EtOH/kg for HOT mice) and a depression of activity at higher doses. This response is consistent with previous studies that have shown locomotor stimulation by low to moderate doses of EtOH and depression of activity by high doses of the drug (1-3,7,23). In one study, HOT and COLD mice from selected generation 10 were injected with 2 g EtOH/kg and tested in an open-field apparatus (8). Under those conditions, where body temperature freely varied, COLD1 and HOT1 mice showed a similar

stimulation of activity by EtOH compared to activity after an injection of NaCl. COLD2 mice, however, showed less activation and HOT2 mice more activation by EtOH than did the COLD1-HOT1 pair. Unlike Crabbe et al., we observed no difference between the replicates in the effect of EtOH injection on activity. However, two-dimensional movement in a thermally homogeneous, brightly lit open field is quite different from one-dimensional movement in a dimly lit, thermally heterogeneous tube.

We observed indications of increased emotionality in HOT2 mice as compared to HOT1 or COLD mice. HOT2 mice became noticeably more reactive to handling during the course of these experiments than did other animals. They were more difficult to catch in the home cage and much more likely to squeak in response to restraint and injection. HOT2 mice may also have exhibited a tendency toward a higher threshold than HOT1 mice for the hypothermic effect of EtOH, as discussed above, although the effect was not statistically significant.

We conclude that a major aspect of the way in which the HOT and COLD mice differ in body temperature response to EtOH involves a selection-induced difference in the sensitivity to EtOH of the CNS regulator of body temperature. The mechanism by which EtOH affects the regulated body temperature is not currently understood. Any EtOH-mediated effect that resulted in increased firing rates of warm-sensitive hypothalamic neurons, or decreased firing rates of cold-sensitive neurons, could explain the decrease in set point we observe (11) and could have been the target of the selection experiments that produced these lines of mice. Elucidation of the neurophysiological and neuropharmacological mechanisms underlying the difference between HOT and COLD mice is clearly of great interest and holds promise for substantially advancing our understanding of the mechanism by which EtOH exerts its thermoregulatory effects.

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REFERENCES

1. Ahlenius, S.; Brown, R.; Engel, J.; Svensson, T. H.; Waldeck, B. Antagonism by nialamide of the ethanol-induced locomotor stimulation in mice. J. Neural Trans. 35:175–178; 1974.
2. Buckalew, L.; Cartwright, G. General and differential behavioral effects of five ethanol dosages on the albino rat. Psychol. Rep. 23:1151–1154; 1968.
3. Carlsson, A.; Engel, J.; Svensson, T. H. Inhibition of ethanol-induced excitation in mice and rats by α-methyl-*p*-tyrosine. Psychopharmacologia 26:307–312; 1972.
4. Chin, J. H.; Goldstein, D. B. Membrane-disordering action of ethanol: Variation with membrane cholesterol content and depth of the spin label probe. Mol. Pharmacol. 19:425–431; 1981.
5. Crabbe, J. C.; Feller, D. L.; Dorow, J. S. Sensitivity and tolerance to ethanol-induced hypothermia in genetically selected mice. J. Pharmacol. Exp. Ther. 249:456–461; 1989.
6. Crabbe, J. C.; Janowsky, J. S.; Young, E. R.; Kosobud, A.; Stack, J.; Rigter, H. Tolerance to ethanol hypothermia in inbred mice: Genotypic correlations with behavioral responses. Alcohol. Clin. Exp. Res. 6:446–458; 1982.
7. Crabbe, J. C.; Johnson, N.; Gray, D.; Kosobud, A.; Young, E. R. Biphasic effects of ethanol on open-field activity: Sensitivity and tolerance in C57BL/6N and DBA/2N mice. J. Comp. Physiol. Psychol. 96:440–451; 1982.
8. Crabbe, J. C.; Kosobud, A.; Feller, D. J.; Phillips, T. J. Use of selectively bred mouse lines to study genetically correlated traits related to alcohol. In: Kuriyama, K.; Takada, A.; Ishii, H., eds. Biomedical and social aspects of alcohol and alcoholism. Amsterdam: Elsevier Science Publishers; 1988:427–430.
9. Crabbe, J. C.; Kosobud, A.; Tam, B. R.; Young, E. R.; Deutsch, C. M. Genetic selection of mouse lines sensitive (COLD) and resistant (HOT) to acute ethanol hypothermia. Alcohol Drug Res. 7:163–174; 1987.
10. Crawshaw, L. I. Temperature regulation in vertebrates. Annu. Rev. Physiol. 42:473–491; 1980.
11. Crawshaw, L. I.; O'Connor, C. S.; Wollmuth, L. P. Ethanol and the neurobiology of temperature regulation. In: Watson, R. R., ed. Alcohol: Neurobiology and neurophysiology. Boca Raton, FL: CRC Press; 1992:341–360.
12. Dinh, T. K. H.; Gailis, L. Effect of body temperature on acute ethanol toxicity. Life Sci. 25:547–552; 1979.
13. Feller, D. L.; Crabbe, J. C. Effect of alcohols and other hypnotics in mice selected for differential sensitivity to hypothermic actions of ethanol. J. Pharmacol. Exp. Ther. 256:947–953; 1991.
14. Feller, D. L.; Crabbe, J. C. Effect of neurotransmitter-selective drugs in mice selected for differential sensitivity to the hypothermic actions of ethanol. J. Pharmacol. Exp. Ther. 256:954–958; 1991.
15. 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.
16. Goldstein, D. B. Pharmacology of alcohol. New York: Oxford University Press; 1983.
17. Goldstein, D. B. The effects of drugs on membrane fluidity. Annu. Rev. Pharmacol. Toxicol. 24:43–64; 1984.
18. Gordon, C. J.; Stead, A. G. Effect of alcohol on behavioral and autonomic thermoregulation in mice. Alcohol 3:339–343; 1986.
19. Hammel, H. T. Regulation of internal body temperature. Annu. Rev. Physiol. 30:641–710; 1968.
20. Hammel, H. T. Concept of the adjustable set temperature. In: Hardy, J.D.; Gagge, A.P.; Stolwijk, J.A.J., eds. Physiological and behavioral temperature regulation. Springfield, IL: Charles C. Thomas; 1970:676–683.
21. Hammel, H. T.; Jackson, D. C.; Stolwijk, J. A. J.; Hardy, J. D.; Strømme, S. B. Temperature regulation by hypothalamic proportional control with adjustable set temperature. J. Appl. Physiol. 18:1146–1154; 1963.
22. Heller, H. C. Hypothalamic thermosensitivity in mammals. Experientia (suppl.) 32:267–276; 1978.
23. Jarbe, T. U. C.; Ohlin, G. C. Interactions between alcohol and other drugs on open-field and temperature measurements in gerbils. Arch. Int. Pharmacodyn. Ther. 227:106–117; 1977.
24. Kalant, H.; Lê, A. D. Effects of ethanol on thermoregulation. Int. J. Pharmacol. Ther. 23:313–364; 1984.
25. Lomax, P.; Bajorek, T.-A.; Chaffee, R. R. J. Thermoregulatory mechanisms and ethanol hypothermia. Eur. J. Pharmacol. 71: 483–487; 1981.
26. Lomax, P.; Bajorek, J. G.; Chesarek, W. A.; Chaffee, R. R. J. Ethanol-induced hypothermia in the rat. Pharmacology 23:288–294; 1980.
27. Malcolm, R. D.; Alkana, R. L. Temperature dependence of ethanol depression in mice. J. Pharmacol. Exp. Ther. 217:770–775; 1981.
28. Malcolm, R. D.; Alkana, R. L. Temperature dependence of ethanol lethality in mice. J. Pharm. Pharmacol. 35:306–311; 1983.
29. Mercer, J. B.; Simon, E. A comparison between total body thermosensitivity and local thermosensitivity in mammals and birds. Pflugers Arch. 400:228–234; 1984.
30. Meyers, R. D. Alcohol's effect on body temperature: Hypother-

mia, hyperthermia or poikilothermia? Brain Res. Bull. 7:209–220; 1981.
31. O'Connor, C. S.; Crawshaw, L. I.; Kosobud, A.; Bedichek, R. C.; Crabbe, J. C. The effect of ethanol on behavioral temperature regulation in mice. Pharmacol. Biochem. Behav. 33:315–319; 1989.
32. O'Connor, C. S.; Hayteas, D. L.; Crawshaw, L. I.; Crabbe, J. C. Mice selected for a difference in hypothermic response to ethanol also show a difference in body temperature response to i.c.v. 5-HT. Fed. Proc. Fed. Am. Soc. Exp. Biol. 4:A989; 1990.
33. Phillips, T. J.; Crabbe, J. C. Behavioral studies of genetic differences in alcohol action. In: Crabbe, J.C.; Harris, R.A., eds. The genetic basis of alcohol and drug actions. New York: Plenum Press; 1991:25–104.
34. Pohorecky, L. A.; Brick, J. Pharmacology of ethanol. Pharmacol. Ther. 36:335–427; 1988.
35. Pohorecky, L. A.; Rizek, A. Biochemical and behavioral effects of acute ethanol in rats at different environmental temperatures. Psychopharmacology (Berl.) 72:205–209; 1981.
36. Prosser, C. L.; Brown, F.A., Jr. Comparative animal physiology. Philadelphia, PA: W.B. Saunders; 1961:266.
37. Rezvani, A. H.; Mack, C. M.; Crovi, S. I.; Myers, R. D. Central Ca^{++}-channel blockade reverses ethanol-induced poikilothermia in the rat. Alcohol 3:273–279; 1986.
38. Satinoff, E. Drugs and thermoregulatory behavior. In: Lomax, P.; Schönbaum, E., eds. Body temperature: Regulation, drug effects, and therapeutic implications. New York: Marcel Dekker; 1979:151–181.
39. Watts, C. H. S. The foods eaten by wood mice (*Apodemus sylvaticus*) and bank voles (*Clethrionomys glareolus*) in Wytham Woods, Berkshire. J. Anim. Ecol. 37:25–41; 1968.
40. Wenger, J. R.; Alkana, R. L. Temperature dependence of ethanol depression in C57BL/6 and BALB/c mice. Alcohol 1:297–303; 1984.