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The Effect of Ethanol on Behavioral Temperature Regulation in Mice

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O'CONNOR, C. S., L. I. CRAWSHAW, A. KOSOBUD, R. C. BEDICHEK AND J. C. CRABBE. *The effect of ethanol on behavioral temperature regulation in mice.* PHARMACOL BIOCHEM BEHAV **33**(2) 315–319, 1989.—Mice were injected with 20% ethanol in 0.9% NaCl, or with 0.9% NaCl without ethanol during sessions of behavioral thermoregulation in a tubular temperature gradient (ambient temperature range approximately 9–38°C). Internal temperature was monitored with an implanted telemetry device. An imaging system recorded the position (selected temperature) of the mouse within the gradient every 5 sec. A dose of either 2.25 or 2.60 g ethanol/kg body wt. produced significantly lower body temperatures than control (NaCl) injections. The 2.60 g/kg dose produced significantly lower selected temperatures than either the NaCl or 2.25 g/kg injections. Doses of 2.75 g ethanol/kg and above incapacitated the mice, precluding accurate behavioral thermoregulation. Utilizing a thermoregulatory index to compare the responses following experimental and control injections indicated that 2.25 or 2.60 g ethanol/kg leads to a decrease in the regulated temperature of mice.

Behavioral thermoregulation Ethanol Mice Thermoregulation Temperature gradient Hypothermia

ETHANOL can produce a broad range of physiological effects which include changes in vascular state (20), a range of primary and secondary alterations in nervous system function (5), and modification of cell membrane properties throughout the body (18). Such effects could impinge at various points upon the thermoregulatory network, which involves sensing, integrating, and responding to thermal challenges. Indeed, ethanol has been reported to decrease panting, shivering, and preoptic-anterior hypothalamic neuron firing rates, and to alter respiratory depth and frequency, sweat secretion and behavior (10). At the systems level, ethanol could affect the central nervous thermoregulatory mechanism. The set point of the regulated body temperature could be adjusted upwards or downwards, or abolished completely; or the range within which body temperature is regulated could be broadened. If ethanol acted to lower the set point, an endotherm would activate behavioral and physiological responses to lower its body temperature. Such an animal would seek out cooler environmental temperatures, while peripheral vasodilation augmented the effect of the behavioral response. If ethanol acted to raise the set point, opposite behavioral and vascular changes would occur as the animal sought to raise its body temperature.

If ethanol were mainly disruptive, low doses would be expected to broaden the set point, and an animal would allow its body temperature to rise or fall several degrees before acting to oppose the change. Such a disruption could be detected, not only by measuring an increased variation in internal temperature, but also by observing a decreased precision of thermoregulatory behavior. Higher doses of ethanol would be expected to abolish the set point, and the animal's body temperature would drift with ambient temperature.

If ethanol left the set point unchanged, but acted on an effector system, an animal would utilize remaining, uncompromised systems to oppose induced thermal loads. If, for instance, ethanol caused peripheral vasodilation, an animal in a cold environment would be subjected to excessive heat loss. The animal would subsequently defend its (unchanged) regulated temperature by shivering, piloerecting and approaching heat sources.

Experiments designed to evaluate the mechanism whereby ethanol affects body temperature have produced divergent results. Intragastric gavage with ethanol (2.0 or 4.0 g/kg) disrupted thermoregulatory mechanisms in rats (14). On the other hand, injecting rats intraperitoneally with ethanol (1.5 g/kg) at a thermoneutral temperature (26°C) produced a significant fall in body temperature (11). Although the change occurred without observed alterations in either metabolic rate or tail temperature, it was concluded that the fall was caused by a decrease in the thermoregulatory set point.

Investigations of the effect of ethanol on behavioral thermoreg-

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ulatory responses have also indicated an effect on the set point. In one study (12), rats avoided a heat source during the ethanol-induced fall in core temperature. Likewise, after intraperitoneal injections of ethanol (3.0 g/kg) mice were observed to select cooler ambient temperatures (7).

While useful, the foregoing behavioral investigations do not allow a clear conclusion to be drawn about the effects of ethanol on thermoregulatory mechanisms, since core and ambient temperatures were not continuously monitored. However, behavior is the most powerful mechanism for controlling body temperature. Indeed, the first response of an endotherm to a thermal stimulus is behavioral; autonomic responses are activated to augment the behavioral response (4). Therefore, the effector we have chosen to monitor is behavior. We here report the results of experiments designed to clarify the effect of ethanol on the thermoregulatory system of the mouse by utilizing nonstressful remote monitoring techniques for measuring both internal and selected temperature in unrestrained animals.

METHOD

Animals and Environment

Male mice of an outbred stock (WSC), bred by J. C. Crabbe, were housed, four to a cage, on hardwood shavings in small animal cages. The animal room was maintained at about 22°C and exposed to the ambient photoperiod. Food (Purina Rodent Laboratory Chow) and water were available ad lib.

Temperature Selection Measurements

Temperature selection of eight mice was simultaneously quantified in Plexiglas tubes about 2.2 m long and 4.5 cm in diameter. The tubes were weighted with iron rods and submerged, one to a lane, in the lanes of an aquatic temperature gradient which has been previously described (16). The ends of each tube made a 90° bend upwards out of the water to allow insertion and removal of mice. Removable barriers at each end prevented escape but were loosely fitted to allow air circulation through the tubes. One end of each lane of the gradient was equipped with a heat exchanger coupled to a heat source, the other end similarly equipped and coupled to a cold source, yielding an ambient temperature range of about 9–38°C. Measurements revealed that the air temperature inside the tubes was within 0.75°C of the water temperature outside the tubes, except at temperatures above 30°, where the discrepancy could reach 1.5°C. The data were corrected for these systematic discrepancies where appropriate. During experiments, the light level was kept dim. A low light video camera mounted above the gradient was connected to an Oculus image analyzer (Coreco Co., Quebec, Canada) installed in an IBM PC XT computer. This arrangement allowed recording and storage of each animal's position within its tube at 5-sec intervals (16). Output from the image analyzer to a video monitor permitted observation of the animals' behavior. After each experiment, the apparatus was calibrated by measuring the water temperature at 10 locations in each lane. The temperature information obtained during calibration was used to translate animals' position into selected temperature for data analysis.

Internal Temperature Measurements

In rats, stress produced by procedures such as restraint (15) and measuring body temperature with rectal probes (6,17) causes a rise in internal temperature. To avoid perturbing one of the measured parameters, eight male mice were implanted with Model X mini-mitters (Mini-Mitter Co., Inc., Sunriver, OR) which broadcast an AM (550–1600 KHz) radio signal inversely proportional to temperature. Mice were anesthetized with a cocktail of ketamine hydrochloride (Ketaset, Bristol-Meyers Co.) and xylazine hydrochloride (Rompun, Haver-Lockhart Co.) (400 μl 50 mg/ml Ketaset: 200 μl 20 mg/ml Rompun: 400 μl 0.9% NaCl, 3.3 μl/g mouse wt.). A left parasagittal incision approximately 1.25 cm long was made in the hypogastric region. A previously calibrated mini-mitter was then inserted into the peritoneal cavity, and secured with nonabsorbable suture to the abdominal wall. Each animal received 0.5 ml gentamycin antibiotic solution in the peritoneal cavity. Mice were allowed to rest and recuperate for four days before experimentation was begun. To pick up the radio emissions of the mini-mitters, each submersible tube was wound with wire which acted as an antenna to carry the signal out of the gradient. A radio receiver was used to monitor the signal at 5-min intervals (preinjection) or 2-min intervals (postinjection). The preimplant calibration data for each mini-mitter were used to translate pulse rate into internal temperature for data analysis.

Temperature Selection Procedure

Before the mice were implanted with mini-mitters they were trained in the gradient for several hours. Training included several insertions into and removals from the tubes, until all mice entered the tubes willingly and exited promptly. 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 about an hour to record baseline information. After 1 hr, mice were removed from the tubes and placed in their home cages for about 45 min. Next, each mouse was injected IP with ethanol (20% v/v in 0.9% NaCl) or with an equivalent amount of 0.9% NaCl, and immediately inserted into the same tube it had occupied before injection. Behavioral temperature selection was monitored beginning 5 sec or less after each mouse entered his tube. Internal temperature was measured at 2-min intervals starting less than 30 sec after each mouse entered his tube. Data were recorded from the animals for a period of 70 min following injection. The mice were then removed from the tubes and returned to their home cages. All tubes were thoroughly washed with running water after every run.

On a given day, four mice were injected with the desired dose of ethanol, the others with saline. During the next run the mice who had received the saline injection were given ethanol, and vice versa. Although mice develop short-term tolerance to the hypothermic effect of injected ethanol (3), tolerance disappears by 48 hr after the last ethanol injection. In our study ethanol injections were always at least four days apart.

Ethanol Dose Determination

In preliminary experiments, groups of two or three mice were administered ethanol at doses of 1.5, 2.25, 2.60, 2.75, 2.90 and 3.10 g/kg body wt. Following ethanol administration, the mice were placed in the temperature selection apparatus. Selected temperature and core temperature were measured, and the behavior of the mice was carefully observed.

Validation of Behavioral Temperature Selection Protocol

To ascertain whether cues other than ambient temperature could be causing the behavior observed in the gradient tubes, an experiment was performed in the absence of a thermal gradient. Eight male mice were trained in the gradient tubes as described above. On the day of the experiment, the gradient heater was used to raise the temperature of the warm end of the gradient to about

32°C. The heater was turned off, and submersible pumps were used to circulate the water in each lane until measurements showed the temperature throughout the lanes had stabilized at about 29°C. The tubes were placed in the gradient and allowed 45 min to come into equilibrium with the water temperature. Water temperature was measured at 27 locations throughout the gradient to verify that thermal equilibrium had been achieved; temperature was about 27°C at all locations. Six mice were injected with 2.60 g ethanol/kg and placed individually in gradient tubes, and activity and preferred location were monitored for 60 min.

Evaluation of Ambient Temperature

In a separate experiment to determine the effect of low ambient temperature on internal temperature, four implanted mice were individually placed at 26.0±0.3°C in plastic containers with thermal characteristics similar to the Plexiglas tubes. After a 40-min baseline period, the mice were injected with NaCl (volume equivalent to that of a dose of 2.60 g ethanol/kg) and returned to the plastic containers. Internal temperature and ambient temperature were monitored for 45 min after injection.

Data Analysis

ANOVA (analysis of variance) with repeated measures was used to evaluate the effect of ethanol over time. The means of the last 20 min of internal and selected temperature values before injection were used as a baseline, and at 2-min intervals the postinjection change from that baseline was calculated for each animal. The values obtained were used to calculate the data points utilized in the ANOVA protocol. All measures of variability refer to the standard error of the mean.

The effects of ethanol on temperature regulation are best understood during the period immediately following the ethanol injection, when all responses are having their maximal effect and body temperature is exhibiting the greatest rate of change. If the main effect of ethanol were to alter the central nervous system set point for body temperature, effector responses and body temperature should exhibit coordinated postinjection changes. To allow a quantitative evaluation of this hypothesis, we established the thermoregulatory index. This index combines postinjection changes in both internal and selected (ambient) temperature into a single value. An increased deviation of the thermoregulatory index from zero indicates an increased likelihood that observed alterations in body temperature are due to coordinated regulatory changes rather than disruptions of effector or regulatory mechanisms. Positive values indicate increases in the regulated temperature, while negative values indicate decreases in the regulated temperature.

We chose to utilize an additive model (2, 8, 19) to represent the interaction between core and selected (ambient) temperature in the determination of thermoregulatory responses. Thus,

$$TI = \alpha\,(T_{c_e} - T_{c_i}) + (T_{sel_e} - T_{sel_i})$$

where TI = thermoregulatory index
α = weighting for internal temperature
T_{c_e} = core temperature after ethanol injection
T_{c_i} = initial (baseline) core temperature
T_{sel_e} = selected temperature after ethanol injection
T_{sel_i} = initial (baseline) selected temperature

We assumed a value of 10 for α, the weighting of core temperature relative to ambient temperature by the regulator in the elicitation of thermoregulatory effector responses. This value reflects the results of appropriate comparisons on medium and small mammals by several investigators (8, 9, 13).

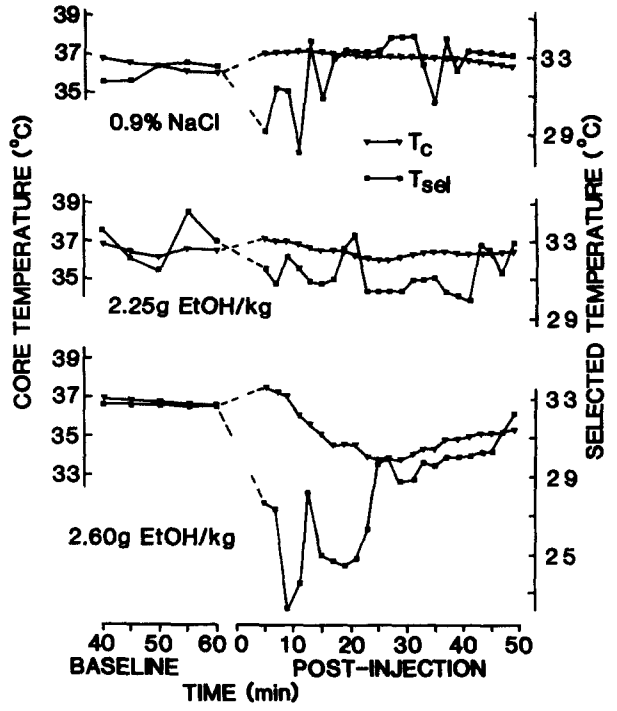

FIG. 1. Individual records of internal temperature and selected temperature before and following a control injection and the injection of two doses of ethanol (EtOH).

RESULTS

In the preliminary dose-response evaluation, doses of 1.50, 2.25, and 2.60 g ethanol/kg did not produce observable decrements in locomotor behavior; 1.50 g ethanol/kg also produced little or no change in core temperature. Doses of 2.75 g ethanol/kg and above produced increasing behavioral derangement, and the mice would often become insensible in thermally untenable locations within the gradient. The doses we chose to analyze further were 2.25 and 2.60 g ethanol/kg.

Activity of mice injected with 2.60 g ethanol/kg and placed in the tubes in the absence of a thermal gradient was normal, but all six mice preferred the end of the tube opposite to the end into which they had been inserted, and repeatedly attempted to escape by pushing up the barriers and crawling out. Animals in tubes with the thermal gradient in place spend virtually no time at the ends of the tubes, and escape attempts are very rare. Temperatures at the hot and cold extremes of the tubes are intended to be sufficiently aversive to discourage the mice from spending appreciable time at those locations, and to insure that a large enough range of ambient temperature is available that the animals can express their thermal preference without choice limitations. The behavior of these animals in the absence of temperature cues demonstrated that the mice normally position themselves in the tubes in response to the temperature gradient, rather than in response to some other cue such as lighting variation or odor.

Figure 1 depicts continuous records of internal and selected temperature for individual mice before and after the injection of 0.9% NaCl (top graph), 2.25 g ethanol/kg (middle graph) or 2.60 g ethanol/kg (bottom graph). Points are plotted every 5 min for the

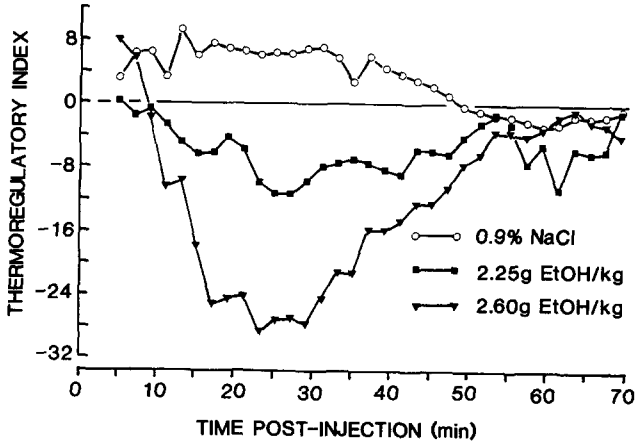

FIG. 2. The postinjection data from Fig. 1 replotted as changes from baseline of the thermoregulatory index (TI).

preinjection period, and every 2 min postinjection. The changes in internal and selected temperature illustrated in these graphs are typical of the responses to NaCl and ethanol of all mice used in this study. After NaCl injection, core temperature is elevated above baseline for approximately 40 min. The transient decrease in selected temperature that occurs after the NaCl injection is likely a response to the increase in core temperature produced by the injection procedure. After injection of 2.25 g ethanol/kg, both core and selected temperature decline below preinjection levels. This effect is pronounced after an injection of 2.60 g ethanol/kg; mice select markedly cooler temperatures for a long period after injection, and core temperature declines also.

To clarify the relationship between the measured variables and the thermoregulatory index (TI), the data shown in Fig. 1 have been replotted in Fig. 2 as changes in the TI as a function of time. Figure 3 shows the change in TI for all animals. In Figs. 2 and 3, the top line represents the change in TI after an injection of NaCl; the middle line shows the change after an injection of 2.25 g ethanol/kg; and the bottom line represents the change after 2.60 g ethanol/kg. In both these figures, the negative values for TI following ethanol administration indicate a decrease in the regulated temperature. To analyze the experiments statistically, mean TI was calculated for the 0 to 40 min postinjection period and used as 1 datum to represent a single animal in a particular trial. ANOVA with repeated measures revealed a significant difference between the response to NaCl and either the lower, $F(1,18) = 78.95$, $p<0.0001$, or the higher, $F(1,18) = 218.05$, $p<0.0001$, dose of ethanol, and also between the two doses of ethanol, $F(1,18) = 44.13$, $p<0.0001$.

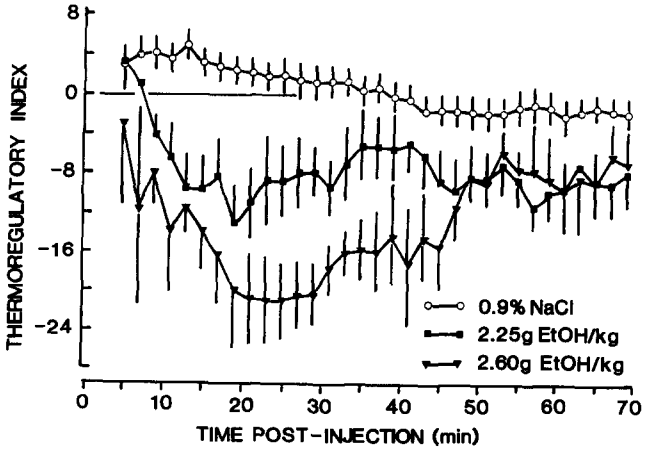

FIG. 3. Group data (mean ± S.E.M.) for postinjection changes in the thermoregulatory index (TI) following control injections (n = 11) and the injection of two doses of ethanol (EtOH); 2.25 g EtOH/kg mouse wt. (n = 7) or 2.60 g EtOH/kg mouse wt. (n = 4).

A similar analysis of core and selected temperatures for the 40-min postinjection period showed that core temperature was significantly lower after injection of 2.25 g ethanol/kg, $F(1,18) = 123.47$, $p<0.0001$, or 2.60 g ethanol/kg, $F(1,18) = 202.75$, $p<0.0001$, than after NaCl injection; core temperature was also significantly lower after 2.60 g ethanol/kg than after 2.25 g ethanol/kg, $F(1,18) = 17.73$, $p<0.0001$. Temperatures selected after 2.25 g ethanol/kg or after NaCl were not significantly different, $F(1,18) = 1.6$, $p<0.20$, but the animals chose significantly cooler temperature after 2.60 g ethanol/kg than after NaCl, $F(1,18) = 19.69$, $p<0.0001$; in addition, the animals preferred significantly lower temperatures after 2.60 g ethanol/kg than after 2.25 g ethanol/kg, $F(1,18) = 10.79$, $p<0.0012$. During the 20 min following the 2.60 g ethanol/kg injections, when core temperature was rapidly falling, the mice selected about 26°C. The four mice that were injected with 0.9% NaCl and placed in plastic containers at 26°C showed a slight decrease in core temperature of 0.4 ± 0.3°C.

DISCUSSION

The highly significant decrease in the thermoregulatory index indicates that, at the doses tested, ethanol administration leads to a decrease in the regulated temperature of mice. The fall in core temperature that followed the ethanol injections involved both physiological and behavioral responses, since the decreased ambient temperature selected by the mice was not, by itself, sufficient to cause a major fall in body temperature. Previous studies utilizing behavioral measures have suggested that ethanol produces a decrease in the thermoregulatory set point, but changes in core temperature were not monitored. In one study, 10 min after a 1.5 g/kg IP injection of ethanol, rats escaped a radiant heat source faster than controls (12). Other data collected by that laboratory indicated that core temperature was probably falling during the period when the behavioral measurements were being made. Likewise, IP ethanol injections of 3.0 g/kg caused BALB/c strain male mice to select cooler temperatures (7).

The above findings appear to contradict a study (14) which reports a poikilothermic (thermolytic) effect of ethanol on thermoregulation. The most likely explanation for this discrepancy lies in the dose levels utilized. At lower doses, such as those employed in the present study, ethanol lowers the regulated body temperature. At slightly higher doses, physiological impairment affects all aspects of physiological function, including temperature regulation. For example, at doses of ethanol above 1.5 g/kg, rats became ataxic and were unable to respond behaviorally (12). We noted the same effect in our mice at doses of 2.75 g ethanol/kg and above. Since the mass specific metabolic rate of the mouse is about twice that of the rat (1), high doses (2.0 or 4.0 g/kg ethanol) (14) were probably more disruptive to the rats than our injections were to the mice.

Control injections, like ethanol injections, caused our mice to choose a cooler environment. However, to consider changes in selected temperature apart from simultaneous changes in core temperature is misleading. After NaCl injection, mice select cooler temperatures while their internal temperature is elevated. Because internal and selected temperatures move in opposite directions, we can infer that the animals are using their ability to select a cooler environment as a means to oppose the rise in

internal temperature induced by the stress of handling and injection. In contrast, after ethanol injection the animals select cooler temperatures during a time when their internal temperature is depressed. The consistent choice of a cooler environment in concert with a lowered body temperature reflects a concerted drive towards reducing body temperature, suggesting a decrease in the thermoregulatory set point. The difference between a decrease in selected temperature (coupled with a rise in internal temperature) after NaCl and a decrease in selected temperature (coupled with a decrease in internal temperature) after ethanol is clearly illustrated by the differences in the thermoregulatory index.

The physiological adjustments that aid in the ethanol-induced fall in body temperature are currently unclear. When tail skin and rectal temperature were measured in rats at a constant ambient temperature of 26°C (11), no increase in tail temperature (which would indicate peripheral vasodilation) was observed during an ethanol-induced decrease in core temperature. After the fall in core temperature reached its maximum, however, tail temperature declined and remained about 1°C below preinjection levels during recovery.

Because moderate doses of ethanol cause both lowered core temperatures and a concomitant selection of cooler ambient temperatures in behaviorally-thermoregulating mice, we conclude that moderate doses of ethanol cause a decrease in the set point of the regulated body temperature in the mouse. We believe that the poikilothermia that has been observed by other investigators after ethanol administration is an effect of using much higher doses of the drug. High doses disrupt behavior and all aspects of physiological function, including temperature regulation.

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