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Ethanol Anapyrexia in Rats

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BRIESE, E. AND L. HERNANDEZ. *Ethanol anapyrexia in rats.* PHARMACOL BIOCHEM BEHAV **54**(2) 399-402, 1996.—In the present series of experiments we tested whether ethanol decreases body temperature by impairing thermal regulation (poikilothermia) or by shifting the set point downwards. The central temperature of rats kept in a thermocline and the selected ambient temperature were recorded by telemetry. After an IP injection of 2 g/kg of ethanol the rats selected an ambient temperature 7°C lower than the one they selected before the ethanol injection and 8°C lower than the one selected by the same rats after saline injection. At the same time the central temperature decreased by 2.5°C. After about 40 min the rats preferred warmer ambient temperatures and 10 min later the central temperature began to rise. When, after ethanol, the rats were kept at 30°C the central temperature remained at the normal level. At 35-36°C the central temperature of normal rats without ethanol rose, in 1 h, from 37°C to 39.75°C. The results suggest that ethanol hypothermia is due to a downward shift of the set point and, in fact, is an anapyrexia, a condition inverse to fever.

Ethanol Hypothermia Anapyrexia Set point Temperature regulation Behavioral temperature regulation

AT room temperature ethanol produces a fall of central temperature (T_c). However, it is still not clear whether this is due to the action of ethanol on the set point or simply to an impairment of temperature regulation caused by a general anesthetising action (7). Myers (10) found that, after ethanol administration, the T_c falls if the animal is left at 22°C but rises if it is placed at 36°C and concluded that ethanol suppresses thermoregulation. However, other works imply that during the fall of T_c due to ethanol the animals regulate their T_c to a lower level. In other words, ethanol would produce a downward shift of the set point. For example, ethanol lowered the T_c in rats that, at the same time, avoided a heat source (9) and reduced the latency to escape from radiant heat (8). Mice selected a lower ambient temperature (T_a) in a thermocline after receiving 3 g/kg of ethanol (5). Goldfishes in 1% ethanol in water selected a cooler T_a (11). With doses of 2.25 or 2.60 g/kg ethanol decreased the T_c in mice while the animals selected a cooler T_a. The response to an equivalent volume of saline injection was the opposite: the mice "selected a cooler T_a while their internal temperature was elevated" (12). The authors concluded that alcohol hypothermia was a regulated change with a downward set point shift whereas the rise of T_c after the saline injection was a nonregulated hyperthermia with a stable set point because the animals opposed the rise of T_c by selecting a cooler T_a. The nonregulated hyperthermia probably was an emotional hyperthermia (3).

The results reported in the present work suggest that ethanol hypothermia is due to a downward shift of the set point and, thus, is an anapyrexia (6).

METHOD

The effect of ethanol was investigated simultaneously on two responses: 1) the IP temperature considered here as the T_c and 2) the preferred T_a selected by the animal in a thermocline. In addition, the effect of ethanol on T_c was investigated when the animal, after the ethanol injection, was confined within the warm compartment of the thermocline at about 30°C. Finally, the T_c was recorded in six normal rats that did not receive ethanol before, during, and after they were placed for 1 h at 35-36°C.

The rats used in the present experiments were adult males of Wistar origin with a body weight between 305 and 390 g. The experiments were carried out in a chamber with the temperature regulated at 24.5°C. The rats had food and water ad lib. Lights were on at 0600 h and off at 1800 h. Under ketamine anesthesia, a crystal-controlled radio transmitter (model TM from Minimitter Co.) was implanted into the peritoneal cavity of each rat. To record the T_a selected by the animal in the thermocline a second radiotransmitter was fixed on a support made from a cut-off disposable syringe fixed on the animal's skull with stainless steel screws and acrylic cement. Each

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crystal-controlled radiotransmitter worked on a different frequency, allowing the simultaneous recording of multiple temperatures. The signals from the transmitters were picked up by an antenna from a 27-MHz radioreceptor and transmitted through a special EMI suppression cable to a Mini-Mitter Dataquest III system and an IBM PC.

The thermocline was a 61 × 26 × 40 cm plywood box with a transparent Plexiglas cover. The box had three intercommunicating compartments where the air was maintained at about 19, 25, and 30°C by forced convection from three heat exchangers through which water circulated from constant temperature baths. A baffle made the air flow upward from the floor. Due to the openings between the three compartments of the thermocline the rats could choose intermediate temperatures between the 19 and 30°C extremes.

Recovery After Surgery

Because the anesthesia and surgery disturbed the circadian rhythms, before starting the ethanol or control injections, a sufficient recovery period was allowed until the T_c and selected T_a normal rhythms were restablished, as shown in Fig. 1.

Procedure

After surgery three experiments were conducted. In the first experiment the rats were placed in the thermocline and the T_c and selected T_a were recorded by telemetry every 2, 3, or 4 min. After 3–5 days the circadian temperature rhythms became normal, as shown in Fig. 1, and the animal received an IP injection of 2 g/kg of ethanol in a 40% solution in 0.9% saline or the same volume of saline. Half of the animals received the saline injection first and half of them the ethanol first. At least 3 days were allowed between the injections. All injections were done at 1030 h. Immediately after the injection the rat was placed in the middle compartment of the thermocline at about 24.5°C. There were six ethanol sessions and six saline sessions.

The same procedure was followed for the second experiment, in which the rat could not choose a preferred T_a. After the ethanol injection, the rat was placed in the warm compartment of the thermocline at about 30°C with the opening blocked.

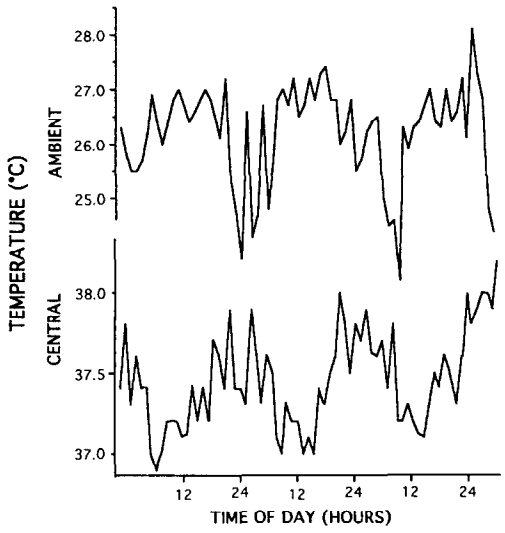

FIG. 1. Normal circadian rhythm of central temperature of a rat and ambient temperature selected in a thermocline.

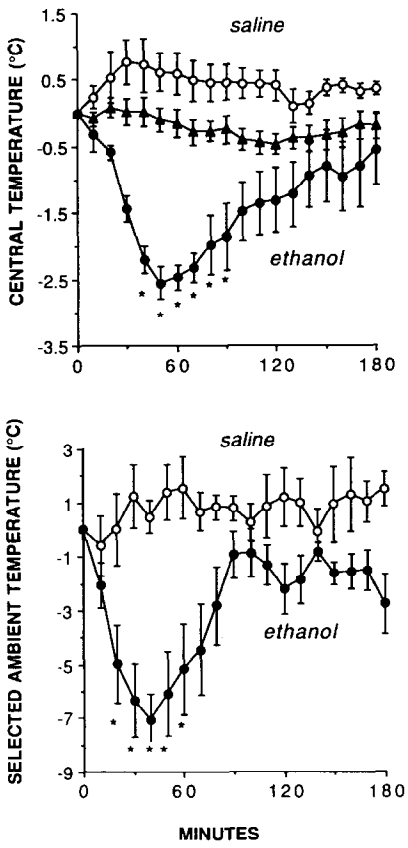

FIG. 2. Mean changes of central temperature and ambient temperature selected by six rats in a thermocline after a 2 g/kg IP injection of ethanol (●) and after an injection of the same volume of saline (○). Changes (▲) in central temperature in seven rats when the animals, after the ethanol injection (2 g/kg), were kept at about 30°C. Vertical bars represent the SEM. *Significantly different means, at 0.05 level, from the first point.

In the third experiment six rats with implanted radiotransmitters and usually kept at 24.5°C were placed for 1 h in a plastic pail at 35–36°C without any previous treatment. The T_c was recorded while the rats were in the hot ambient and for 1 h before and after.

Analysis of the Results

The T_c and the preferred T_a recordings were analysed over 4 h : 1 h before the injection, from 0930 to 1030 h, which was the baseline, and 3 h after the injection. The results were expressed as changes with respect to the baseline mean. The averages at 10-min intervals were calculated. There were six averages each hour (i.e., 24 for one session). The mean of the six averages of the hour before the injection was taken as the baseline. Next, the differences between the baseline mean and the 18 averages of the following 3 h gave the temperature changes after the injections. Finally, the means and SEMs of the temperature changes for the six ethanol and six saline sessions were calculated (Fig. 2). The same procedure was followed for the analysis of the data from the rats confined at 30°C and for the rats confined at 35–36°C.

The statistical significance of T_c and selected T_a changes was assessed by one-way and two-way ANOVA followed by Newman-Keuls related t-test. The correlation between the T_c and selected T_a changes was assessed by regression analysis.

RESULTS

After the ethanol injection the rats preferred a cool T_a while their T_c decreased (Fig. 2). A two-way ANOVA showed that the three curves in the upper part of Fig. 2 are significantly different from each other, $F(2, 15) = 84.34$, $p < 0.001$. The selected T_a after ethanol was also significantly different from the selected T_a after saline (bottom of Fig. 2), $F(1, 34) = 56.58$, $p < 0.001$. The decrease of the T_c and of the selected T_a after ethanol was significant; for the selected T_a, $F(18, 95) = 4.66$, $p < 0.001$, and for the T_c, $F(18, 95) = 4.17$, $p < 0.001$. The T_c reached the lowest mean point, 2.55°C lower than the baseline, 50 min after the injection. The lowest point of the selected T_a was reached 10 min earlier, at 40 min after the ethanol injection. Apparently, the longer delay for the maximum T_c descent would indicate that the behavioral response preceded the autonomic response. This view was corroborated by the regression analysis of ethanol-induced T_c vs. T_a changes. With the same abscissa for both curves (Fig. 2), the regression was $R = 0.519$, $F(1,16) = 5.911$, $p < 0.002$. However, displacing the T_a curve so that the lowest points of both curves coincided, the correlation was better: $R = 0.841$, $F(1, 5) = 36.13$, $p < 0.001$ (Fig. 3).

The subsequent rise of T_c and preferred T_a also suggests that behavioral responses preceded the autonomic ones, because the recorded T_a selected after ethanol reached a higher stable level 90 min after the injection whereas T_c was still going up 180 min after the injection (Fig. 2).

When, after the ethanol injection, the animals were confined at about 30°C, the ethanol did not produce a lowering of the T_c (Fig. 2, triangles). At 30°C the variations of T_c after ethanol were not significant, $F(18, 114) = 1.07$, NS.

The results of the experiment in which the rats were placed in a 35-36°C environment without any previous treatment are given in Fig. 4. The ANOVA showed that the rise of T_c was significant, $F(17, 90) = 12.85$, $p < 0.001$. The means indicated by asterisks in Fig. 4 are significantly higher than the means recorded before the animals were placed at 36°C.

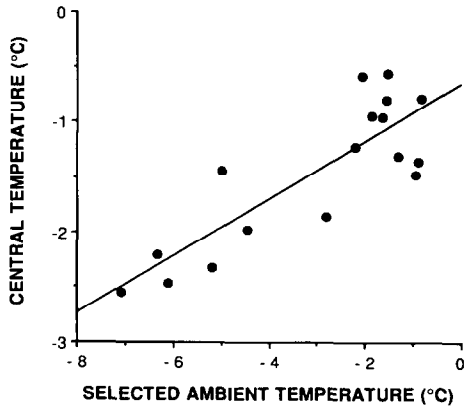

FIG. 3. Regression analysis of change of central temperature vs. change of selected ambient temperature after an ethanol IP injection (2 g/kg). The equation of the best fitting line was $Y = -0.7 + 0.26X$.

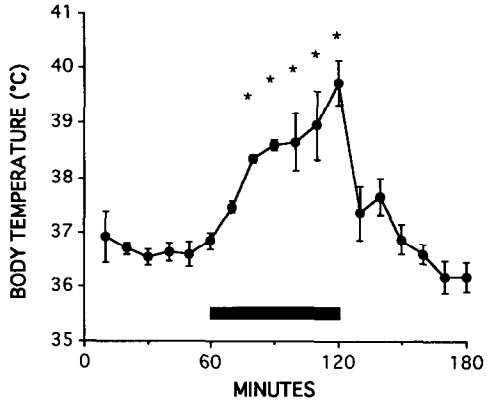

FIG. 4. Mean IP temperature of six rats placed for 1 h at 35-36°C. The horizontal bar indicates the period for which the rats were in the warm environment. Vertical bars represent SEM. *The mean temperatures significantly different from the first five means.

DISCUSSION

Regulation of T_c in homeotherms is usually attributed to a mechanism similar to a thermostat with a set point. Changes in T_c might be due to passive gain or loss of heat with a stable set point or to shifts of the set point. When the level of the set point is stable the organism responds to a gain or loss of heat—and the consequent change in Tc—by activating thermoeffectors to counteract the changes. That means that the thermoregulatory responses oppose the passive change of T_c. On the other hand, when the origin of the T_c change is a shift of the set point, the organism activates thermal responses to bring the T_c toward the new level prescribed by the set point. This means that the thermal responses are in the same direction as the set point shift.

Fever is the classic example of an upward shift of the set point. In fever, thermoeffector responses (skin vasoconstriction, shivering, cold sensation, and preference of a warm environment) are aimed to generate and conserve heat, which results in a T_c rise.

In small laboratory animals the simplest way to tell a fever from a passive hyperthermia is to investigate the T_a preferred by the animals in a thermal gradient. The rise in T_c is a fever if the animal selects a warm environment and it is a hyperthermia if the animal selects a cooler environment.

At room temperature ethanol produces a fall of T_c. Similar to the method used to differentiate a fever from a hyperthermia, we can tell a passive hypothermia from a regulated fall in T_c by behavioral methods. If the fall in T_c is a passive hypothermia the animal should select a warm T_a to reestablish the previous level of T_c because the set point, in this case, has not changed and the T_c is below the set point. If, on the contrary, the fall in T_c is due to a downward shift of the set point, the animal should respond by loosing heat and will select a low T_a. This last condition is inverse to fever; it is a regulated change of T_c but in the opposite direction, and it has been designated by the Thermal Commission of IUPS (6) as anapyrexia. The experiments reported here suggest that ethanol produces an anapyrexia rather than a hypothermia or poikilothermia because the T_c fall is concomitant with a low T_a preference.

This was recognized a long time ago. In the fifth edition of Gaddum's Pharmacology (4) we have: "The effect of alcohol on circulation is to cause an increased blood-flow through the skin at the expense of other tissues. This effect is produced by an action on the central nervous system. It leads to a feeling of warmth but a falling internal temperature."

Some previous works also imply that ethanol produces a regulated fall in T_c. Ethanol decreased the T_c in mice, which concomitantly selected a lower T_a in a thermal gradient (12). But in that study the results are expressed as a "thermoregulatory index," which combines postethanol injection changes of both T_c and the selected T_a in a single value. Such a mode of presentation rises some doubt. The "thermoregulatory index" was calculated as the change of T_c multiplied by a coefficient of 10 plus the concomitant change in the selected T_a. "An increased deviation of the thermoregulatory index from zero indicates an increased likelihood that the observed alterations in body temperature are due to coordinated regulatory changes rather than disruption of effector or regulatory mechanisms" (12). However, with that equation even opposite variations of T_c and selected T_a (which are not coordinated regulatory changes) could give an index different from zero and could erroneously be taken as coordinated regulatory changes. For instance, a 0.2°C increase in T_c and an 8°C decrease of selected T_a would give a negative index thermoregulatory (index $= 0.2 \times 10 - 8 = -6$), which would indicate a coordinated decrease of T_c when, in fact, such T_c and selected T_a variations ought to indicate a nonregulated increase of T_c.

By contrast, in the present work, the changes of T_c and T_a are presented separately. It is the first time that a significant correlation has been established between T_c after ethanol and the selected T_a and, consequently, that ethanol induces an anapyrexia. In addition, the present study shows that ethanol does not decrease the T_c when the animals are kept at 30°C (Fig. 2), which stresses the importance of the thermal regulatory behavior in producing the T_c decrease.

The separate analysis of T_c and selected T_a changes have made it possible to observe that the behavioral response (selection of T_a) significantly preceded by about 10 min the autonomic recorded response (T_c fall). This was not reported previously. It seems unlikely that this might be due to two set points, one for the behavioral and another one for the autonomic response. It is more likely that a downward shift of the set point would produce an immediate feeling of warmth that triggers the behavioral response, whereas it takes some time for the organism to lose heat through autonomic responses to lower the T_c.

Because at 22°C the T_c fell and at 36°C the T_c rose, Myers (10) concluded that under ethanol the animal becomes poikilothermic. However, at 30°C, for physical reasons, the animals can hardly lose heat and at 36°C, on the contrary, they gain heat from the environment. This was demonstrated by the fact that at 36°C the rats became hyperthermic, even without ethanol as shown in the present and an previous work (1). In spite of this experiment, the poikilothermic effect on ethanol-intoxicated animals cannot be completely ruled out. No ethanol-intoxicated rats were placed at 36°C to find out whether or not normal and ethanol-intoxicated rats behave the same at a 36°C ambient temperature. Moreover, a single dose of ethanol was used in the present study. Larger doses of ethanol might make temperature regulation impairment more conspicuous.

In any event, the reciprocal circadian rhythm of T_c and selected T_a (Fig. 1) confirms the previous findings (1,2) that the T_c circadian rhythm is opposed by an inverse rhythm of T_a preference. This indicates that the methods used in the present work were reliable because they could distinguish a nonregulated hyperthermia or hypothermia, as in the case of the circadian rhythm, from a regulated hypothermia, as in the case of the ethanol effect.

We can conclude that ethanol in rats at the dose of 2 g per kg produces an anapyrexia, that is, a regulated fall of T_c, a condition inverse to fever.

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