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Temperature dependence of ethanol lethality in mice†

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The present study provides systematic evidence indicating a direct relationship between environmental temperature, rectal temperature and ethanol lethality. Male, C57 BL/6J mice, previously housed at room temperature (23 ± 1 °C), were injected intraperitoneally with 4·8 to 9·2 g kg^{-1} ethanol and then exposed for 24 h to ambient temperatures that did not appreciably exceed the thermally neutral range for sober mice (20 to 35 °C). There was a direct relationship between temperature and ethanol lethality at 8 and 24 h after injection. The 8 h LD50 increased by 64%, from 5·3 to 8·7 g kg^{-1}, as environmental temperature decreased from 35 to 20 °C. The 24 h LD50 increased by 51%, from 5·3 to 8·0 g kg^{-1}, across this temperature range. Each 5 °C reduction in ambient temperature induced a significant decrease in the rectal temperature of ethanol-injected mice. Mean rectal temperature ranged from 2·2 °C above baseline at an ambient temperature of 35 to 15 °C below baseline in the 20 °C environment. Ethanol induced a significant dose-related hypothermia in mice exposed to the 20, 25 and 30 °C environments but did not produce hypothermia in animals kept in the 35 °C environment. These findings indicate that the potency of potentially lethal ethanol doses varies with body temperature in accordance with partition and membrane expansion-fluidization theories of anaesthesia.

Recently, attention has been focused on investigating the effect of ethanol on the body temperature of homeothermic animals. At this time, it is clear that rodents administered sub-hypnotic and hypnotic doses of ethanol display a dose-dependent degree of hypothermia when housed at normal room temperatures (Freund 1973; Ritzmann & Tabakoff 1976a; Lomax et al 1980). This hypothermia appears to result from a decrease in the set point of the central thermostat and a diminished ability to regulate body temperature around the new set point (Lomax et al 1980; Hirvonen & Huttunen 1977). These findings have been extended to show that the intoxicated animal behaves somewhat like a poikilotherm. The degree and direction of the body temperature change following ethanol are determined largely by the gradient between ambient and normal body temperature (Lomax et al 1981; Malcolm & Alkana 1981; Myers 1981).

Despite the frequent use of hypothermia as an indicator of ethanol's effects (Ritzmann & Tabakoff 1976b; Crabbe et al 1979; Deimling & Schnell 1980; Muñoz & Guivernau 1980; Cappel et al 1981) and recent data demonstrating the importance of ambient temperature in determining the body temperature of intoxicated animals (Malcolm & Alkana 1981; Myers 1981), there is a paucity of information regarding the influence of temperature on the behavioural and toxic effects of ethanol. A link between temperature and brain sensitivity to ethanol is suggested by theories of anaesthetic drug action. These postulate that a decrease in body temperature, and hence the temperature of brain membranes, would reduce the fluidizing-expanding effect of ethanol that is thought to cause depression (Lever et al 1971; Seeman 1972; Hill & Bangham 1975; Halsey et al 1978), or would decrease the partition of ethanol into hydrophobic regions of membranes (Meyer 1899, 1901). Either of these changes would decrease the potency of ethanol. In contrast, an increase in body temperature would have the converse effect and increase ethanol potency.

In-vivo studies using sleep-time, swim performance and motor activity (Malcolm & Alkana 1981; Pohorecky & Rizek 1981) support the contention that changes in temperature can influence the potency of ethanol. The present study extends previous work by systematically exploring the relationship between ambient temperature, body temperature and the lethal effect of ethanol.

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METHODS

Subjects and procedures

Male, C57 BL/6J mice, 38 to 70 days old, 16 to 30 g, at testing, were housed five per cage on a 12-h light-dark cycle (0700 on) in a room thermostatically maintained at 23 ± 1 °C for a minimum of one week

before testing. Animals were injected intraperitoneally (i.p.) with 4·8 to 9·2 g kg^{-1} ethanol (20% w/v made by diluting 95% v/v ethanol, U.S.P., with freshly made 0.9% NaCl (saline) between 0830 and 1000 h. Immediately after injection, each mouse was placed into a 9 × 6 cm compartment constructed by partitioning a standard cage into four sections. The compartmentalized cages, containing the singly housed mice, were maintained in ambient temperatures of 20, 25, 30 or 35 °C for the 24 h test period. To insure an adequate supply of oxygen and to prevent the build-up of carbon dioxide or other metabolic wastes, breathing air supplied from compressed air cylinders flowed through the chambers at a rate of 0.5 litre min^{-1}. Separate groups of animals were injected with a volume of saline equivalent to that for the 7·6 g kg^{-1} ethanol dose and exposed to ambient temperatures of 20 or 35 °C. Mice were randomly assigned to conditions in order to avoid bias from day to day or age differences.

Lethality

Death was defined as the absence of respiration for a period of one min (Dinh & Gailis 1979). The number of surviving animals was recorded at 8 and 24 h after injection.

Rectal temperature

Rectal temperatures were monitored in all mice immediately before injection and at 1, 2, 4, 8 and 24 h post-injection in surviving animals using a telemetric technique previously described (Freund 1973; Malcolm & Alkana 1981). If blood was detected on the rectal probe, the animal was eliminated from the study.

Environmental temperature

Environmental temperatures of 25, 30 and 35 °C were maintained within ±1 °C with a Thelco incubator (Model 6, GCA/Precision Scientific, Chicago, IL.). The cool environmental temperature was achieved with an upright cold box (Vering, Los Angeles, CA) which enabled cage temperature to be held at 20 ± 1 °C. Environmental temperatures were monitored with probes (Yellow Springs Instrument Co., Model 403) mounted slightly above the cage floors.

Data analysis

The LD50 and 95% fiducial limits were calculated for each environmental temperature at 8 and 24 h after ethanol using the SAS 'Probit' computer program. The mean rectal temperature ±s.e. was calculated at each time point for all saline and ethanol dose-environmental temperature combinations. Initial comparisons between groups at each time point were made using one-way analysis of variance. If warranted, further comparisons were made using Duncan's new multiple range test. The relationship between mean rectal temperature and ethanol dose was evaluated at every time point for each environmental temperature using least squares regression analysis. A P value of $< 0{\cdot}05$ was taken as significant in all statistical analysis.

RESULTS

Ethanol mortality was directly related to environmental temperature at 8 and 24 h after injection. The 8 h log dose-% mortality curve was shifted to the right (Fig. 1A) and the LD50 was significantly increased (Table 1) by each 5 °C reduction in the ambient temperature. The 8 h LD50 increased by 64% from 5·3 g kg^{-1} in the 35 °C environment to 8·7 g kg^{-1} when the ambient temperature was 20 °C. The statistical significance of the difference in survival rates at each temperature is indicated by the lack of overlap in the 95% fiducial limits for the corresponding LD50s (Table 1). Similar temperature effects were seen 24 h after injection (Fig. 1B, Table 1). The 24 h LD50s at 25, 30 and 35 °C did not differ significantly from the 8 h values. However, the 24 h mortality curve for mice kept in the 20 °C environment shifted to the left of the 8 h curve and the LD50 decreased to 8·0 g kg^{-1}.

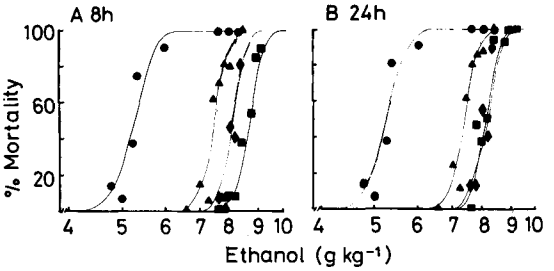

FIG. 1. The effect of environmental temperature on ethanol lethality (A) 8 h and (B) 24 h after injection. The % mortality is shown for groups of mice that were injected with 4·8 to 9·2 g kg^{-1} ethanol and exposed to environmental temperatures of 20 (■), 25 (◆), 30 (▲), or 35 °C (●). The mortality curves were plotted from probit analysis of the raw data and illustrate the response at each ambient temperature. See Table 1 and Results for LD50s and statistical analysis.

Fig. 2 illustrates the relationship between environmental and rectal temperature following injection of 7·6 g kg^{-1} ethanol or an equivalent volume of saline. Analysis of variance demonstrated a significant

Table 1. The effect of environmental temperature on rectal temperature and ethanol LD50.

Environmental temp. °C	Approx. mean rectal temp.[a] °C	LD50 (95% Fiducial limits) g kg^{-1} 8 h	LD50 (95% Fiducial limits) g kg^{-1} 24 h	n
35	37·6–37·9	5·3 (5·2–5·5)	5·3 (5·2–5·5)	121
30	34·0–34·2	7·5 (7·1–7·7)	7·4 (7·2–7·5)	159
25	29·1–30·0	8·1 (8·0–8·2)	8·0 (7·7–8·5)	167
20	22·6–23·6	8·7 (8·5–8·8)	8·0 (7·7–8·2)	151

[a] The rectal temperature ranges shown are the mean 8 h values in surviving animals given the nearest ethanol dose above and below the calculated LD50 since we did not actually test this dose.

effect of ambient temperature on rectal temperature at all time points following injection [$F(5,53–78) = 24·9–209·1$, $P < 0·001$]. Further comparisons by Duncan's procedure indicated that the rectal temperature of intoxicated mice exposed to 35 °C did not differ from the rectal temperature of saline controls that were kept in the 35 °C environment. Similar comparisons indicated that the rectal temperatures of intoxicated mice exposed to 30, 25 and 20 °C were significantly lower than both saline groups (35 and 20 °C). Furthermore, the degree of hypothermia in the intoxicated mice increased significantly with each 5 °C reduction in ambient temperature at 1, 2, 4 and 8 h post-injection. Similar relationships were observed between ambient temperature and the rectal temperatures of mice given other doses of ethanol (data not shown). Exposure of saline-injected mice to environmental temperatures of 20 and 35 °C did not produce significant differences in rectal temperature between the two groups.

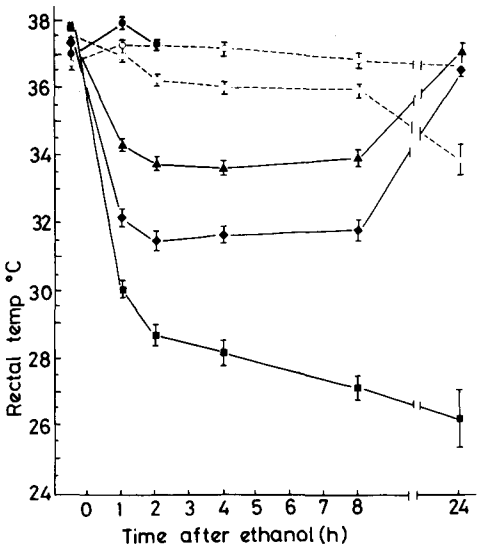

FIG. 2. The effect of environmental temperatures from 20 to 35 °C on the rectal temperatures of intoxicated mice. Animals were injected with 7·6 g kg^{-1} ethanol or an equivalent volume of saline and exposed to ambient temperatures of 20 (■), 25 (◆), 30 (▲) or 35 °C (●). Shown are the mean ± s.e. Due to ethanol mortality, the n per treatment varied with time from 20 to 5 animals. The 2 h value for the 35 °C environment represents that of the only surviving mouse. See Results for statistical analysis. Saline 35 °C (○); 20 °C (□).

Fig. 3 shows the dose-response relationship for ethanol's effects on rectal temperature in animals kept in different ambient temperatures. Ethanol induced a dose-dependent hypothermia in mice exposed to the 20 and 25 °C environments (Fig. 3A and B). Least squares regression analysis demonstrated a significant [$F(1,3) = 7·0–56·1$, $P < 0·05 > 0·001$] inverse linear relationship between dose and mean rectal temperature at 1 ($r^2 = 0·70$), 2 ($r^2 = 0·84$), 4 ($r^2 = 0·98$) and 8 ($r^2 = 0·95$) h after ethanol for animals kept in the 20 °C ambient temperature. Similar significant [$F(1,1) = 75·0–300·1$, $P < 0·05$] inverse linear relationships were found at 4 ($r^2 = 0·99$) and 8 ($r^2 = 0·99$) h post-injection for mice exposed to 25 °C. Although not as pronounced, the ethanol-induced hypothermia in mice exposed to 30 °C was also dose-related (Fig. 3C). Regression analysis demonstrated a significant [$F(1,2) = 10·6$, $P < 0·05$] inverse relationship between ethanol dose and mean rectal temperature at 2 h ($r^2 = 0·84$) post-injection. Intoxicated animals kept in the 35 °C environment became mildly hyperthermic (Fig. 3D). The degree of hyperthermia was not dose-related [$F(1,3) = 0·001–2·9$, $P > 0·19$].

DISCUSSION

The present study provides systematic evidence indicating a direct relationship between environmental temperature, rectal temperature, and ethanol lethality. Although the span of ambient temperatures tested did not appreciably exceed the thermally neutral range for sober mice, each 5 °C reduction induced a significant decrease in rectal temperature and lethality in ethanol-injected mice. This finding is consistent with work at sub-hypnotic and hypnotic doses indicating that temperature represents an important variable that can alter brain sensitivity to

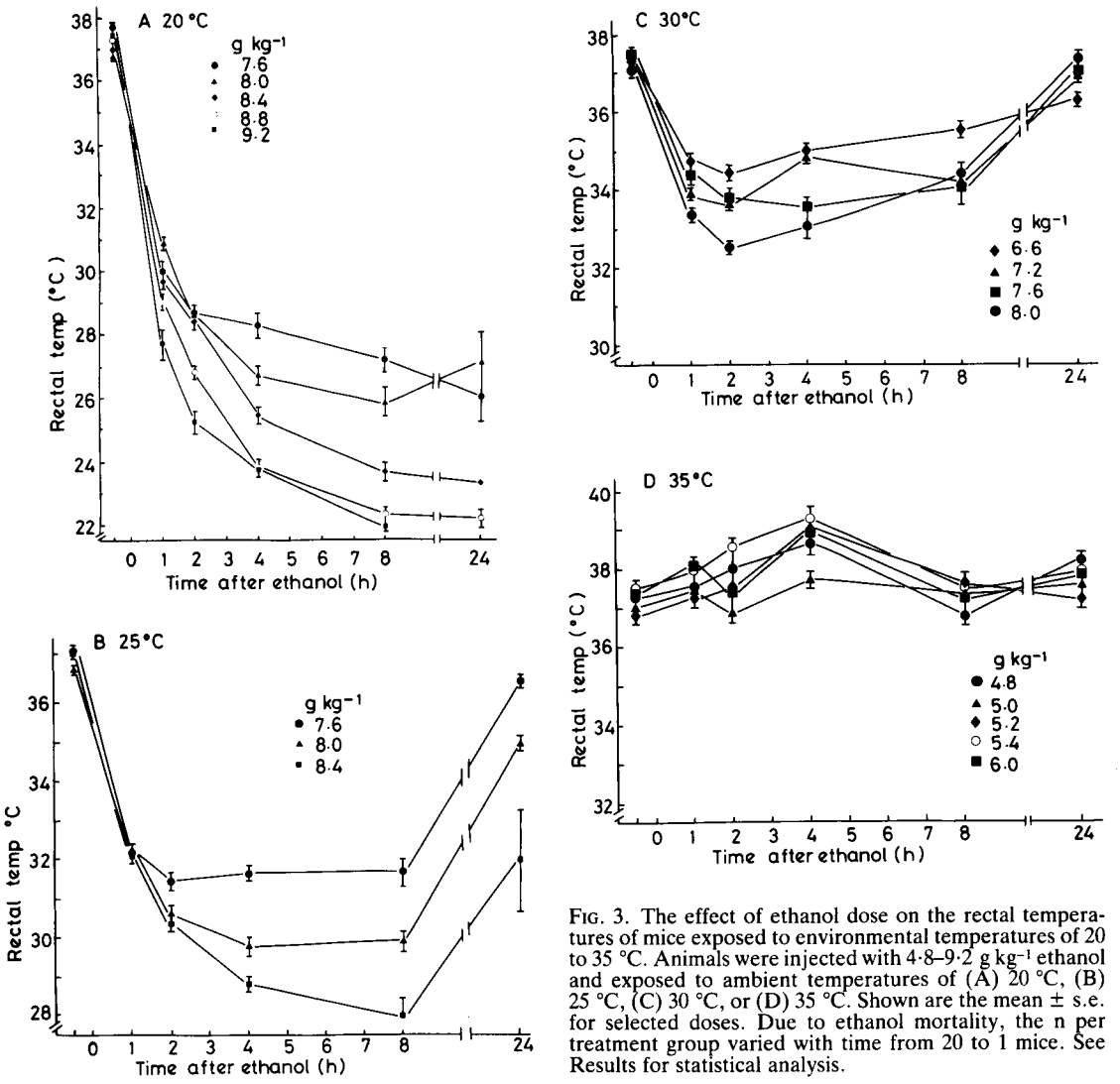

FIG. 3. The effect of ethanol dose on the rectal temperatures of mice exposed to environmental temperatures of 20 to 35 °C. Animals were injected with 4·8–9·2 g kg^{-1} ethanol and exposed to ambient temperatures of (A) 20 °C, (B) 25 °C, (C) 30 °C, or (D) 35 °C. Shown are the mean ± s.e. for selected doses. Due to ethanol mortality, the n per treatment group varied with time from 20 to 1 mice. See Results for statistical analysis.

ethanol (Malcolm & Alkana 1981; Pohorecky & Rizek 1981).

The present results extend and support previous studies that indirectly suggested a link between temperature and ethanol lethality, but were confounded by methodological difficulties or were limited to one experimental temperature point. Keplinger et al (1959) found an inverse relationship between approximate LD50 and ambient temperatures of 8, 26 and 36 °C but the small group sizes and injection of 100% ethanol precluded definitive conclusions. A study examining the 24 h rhythm of ethanol's effects in mice indicated that minimal lethality corresponded with maximal hypothermia (Haus & Halberg 1959). Similarly, Dinh & Gailis (1979) found that hypothermic mice had a higher LD50 than mice made hyperthermic. Grieve & Littleton (1979) also reported that mice exposed to a thermoneutral environment during intoxication died at blood ethanol concentrations that were not lethal to mice exposed to a lower ambient temperature.

The mechanism by which temperature influences ethanol lethality is unknown. Others have shown that neither acute cold exposure nor severe hypothermia induce the changes in ethanol absorption, distribution or clearance required to decrease toxicity (Dybing 1945; Platonow et al 1963; MacGregor et al 1965; Ferko & Bobyock 1978).

On the other hand, temperature-induced alterations in ethanol partition or in membrane structure represent two, possibly overlapping, means of explaining the relationship between temperature and ethanol lethality. In agreement with the present findings, partition or lipid solubility theories of anaesthesia predict that reductions in body temperature should decrease ethanol potency by reducing the portion of a given ethanol dose reaching the putative site of action in hydrophobic regions of brain membranes (Meyer 1899, 1901; Seeman 1969; Leo et al 1971). Similarly, membrane expansion-fluidization theories (Lever et al 1971; Miller et al 1973; Halsey et al 1978) predict that a decrease in body temperature, and hence brain membrane temperature, would reduce or offset the expanding-fluidizing action of ethanol that is thought to cause depression. Although recent work suggests that bulk membrane fluidization may not be the critical event in the action of ethanol (Franks & Lieb 1981; Kita et al 1981), the present findings could reflect an interaction between ethanol and temperature in discrete lipid or lipid-protein microenvironments.

Interestingly, the potentially lethal ethanol doses used in the present study did not totally block thermoregulatory capability since the degree of hypothermia was dose-related at ambient temperatures presenting a thermal gradient (20, 25 and 30 °C) (Fig. 3). This indicates that the dose-related impairment of thermoregulation accompanying intoxication extends beyond the sub-hypnotic and hypnotic dose ranges previously studied (Freund 1973; Ritzmann & Tabakoff 1976a; Lomax et al 1980; Myers 1981) and does not reach a ceiling.

The log dose-% mortality curve for the animals kept in the 20 °C environment, but not those exposed to higher ambient temperatures, shifted to the left between 8 and 24 h after ethanol (Fig. 1). The low rectal temperatures in these animals (31 to 22 °C, Fig. 3A) suggest that death during the 8 to 24 h period may have been mediated by prolonged and severe hypothermia, rather than by a more direct effect of ethanol (Hirvonen 1979).

The present study has important clinical implications. It suggests that current treatment protocols for ethanol overdose in humans may require modification. These typically ignore body temperature or view the attendant hypothermia as a complication requiring warming of the patient (Ritchie 1980; Czaha & Duffy 1980; Kline et al 1974). In contrast, the present findings suggest that holding body temperature at a sub-normal level, while avoiding severe hypothermia, may represent a simple, non-invasive means of enhancing existing supportive measures and further reducing mortality from ethanol overdose. Further studies are necessary to establish the efficacy of this treatment in humans and to determine whether temperature can influence the potency of other depressant drugs, alone or in combination with ethanol.

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