Ambient temperature and the development of functional tolerance to ethanol by mice

SUSAN J. GRIEVE, JOHN M. LITTLETON*, *Department of Pharmacology, King's College, London WC2R 2LS, U.K.*

Current theories of the mechanism of ethanol tolerance suggest that adaptations in cell membrane fluidity, analogous to those made in response to high ambient temperature by poikilotherms, underlie tolerance to the drug at the cellular level (see Hill & Bangham 1975). In mammalian cells in tissue culture, Li & Hahn (1978) have provided evidence which supports this view. Thus cells adapted to a high ambient temperature of the growth medium were also shown to be resistant to the toxic effects of ethanol. Conversely cells adapted to ethanol were resistant to high temperature. The acute presentation of high ambient temperature and ethanol showed additive toxicity to cells not previously exposed to either. It is of course difficult to reproduce this kind of experiment in the intact mammal, where body temperature is maintained within narrow limits, and where marked deviation from normal body temperature may have effects on integrative functions in the organism which might obscure effects at the cellular level. However, measurements of body temperature of mice, made during the development of functional tolerance to ethanol (Grieve & Littleton 1979b) suggested a possible approach. When ethanol is administered to mice by inhalation, some strains develop tolerance to the drug rapidly and this is associated with marked ethanol-induced hypothermia (Grieve & Littleton 1979b). These experiments were carried out at an ambient temperature of 23–25 °C. It seemed possible that performing identical experiments at thermoneutral ambient temperature (33–35 °C) might prevent the associated hypothermia and allow the development of tolerance to be studied in mice maintaining a normal body temperature. The hypothesis that links ethanol tolerance with adaptive change in cell membrane fluidity predicts that mice with a higher body temperature (i.e. in a thermoneutral environment) should be more susceptible to the central depressant and toxic effects of ethanol.

The development of tolerance to ethanol was investigated during inhalation of ethanol by mice as described previously (Grieve & Littleton 1979a, 1979b). Loss of righting reflex was used as the behavioural endpoint, rectal temperatures were obtained by thermistor probe, a temperature of 33–35 °C was maintained in and around the inhalation chamber, by an infrared lamp positioned above it. Three strains of mice (C57BL, TO Swiss and DBA2) were used because they show differing characteristics of development of ethanol tolerance (Grieve & Littleton 1979b). All animals were males aged 35–40 days and had no access to food or water during the period of an experiment.

* Correspondence.

First experiments were carried out on mice of the TO Swiss strain. The results are shown in Fig. 1. They demonstrate that when kept in a thermoneutral environment TO Swiss strain mice develop functional tolerance to ethanol normally, in the absence of any change in body temperature. There is also no significant difference in the concentration of ethanol in the bloodstream at which mice first lose the righting reflex at the different ambient temperatures. When experiments were performed on mice of the three different strains, essentially similar results to the TO strain were obtained with the C57BL strain. The C57BL mice develop ethanol functional tolerance very rapidly (Grieve & Littleton 1979b) and this was not affected by the higher ambient

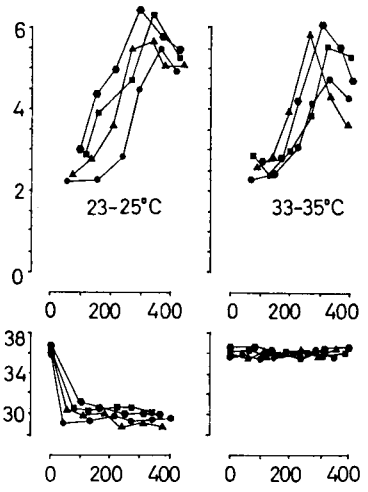

FIG. 1. The development of ethanol tolerance in mice of the TO Swiss strain at different ambient temperatures. The upper graphs show the concentration of ethanol in the blood in mg ml^{-1} at which the righting reflex was lost (abscissae). The lower graphs show the corresponding rectal temperature in °C (abscissae). The ordinates in both cases represent the time in minutes after beginning exposure to ethanol. Results obtained at normal room temperature (22–25 °C) are shown on the left; results at thermoneutral ambient temperature (33–35 °C) on the right. Connected symbols represent values obtained from an individual mouse. At 23–25° mice first lost the righting reflex at a concentration of ethanol in blood of 2·72 ± 0·15 mg ml^{-1}; at 33–35°C the equivalent value was 2·74 ± 0·08 (N.S. in Student's test). The highest values for blood ethanol concentration at loss of righting reflex were: at 23–25 °C, 6·15 ±0·20 mg ml^{-1}; at 33–35 °C, 5·73 ± 0·18 mg ml^{-1} ($P > 0·05$ in Student's test). Values given are mean ± s.e.m. of 4 estimations.

temperature (Fig. 2). When mice of the DBA2 strain were investigated for development of ethanol tolerance at the high ambient temperature, an unexpected finding was obtained. DBA2 mice normally show little development of ethanol tolerance during the administration of ethanol vapour for this period (Grieve & Littleton 1979b). However, they do not show mortality during assessment of tolerance to any greater extent than mice of the other strains. But when tested in a thermoneutral environment it proved impossible to obtain results for more than 2–3 h, as after this time in every experiment most animals died. This mortality was seen in the absence of any difference in rectal temperatures of these animals compared to controls (Fig. 2). Also ethanol-induced mortality occurred in the absence of any evidence of functional tolerance as assessed by righting reflex. In other words, these DBA2 mice were dying at concentrations of ethanol in blood no higher than those survived by mice of the same strain at the lower ambient and body, temperature (see Fig. 2 for representative results from a single experiment).

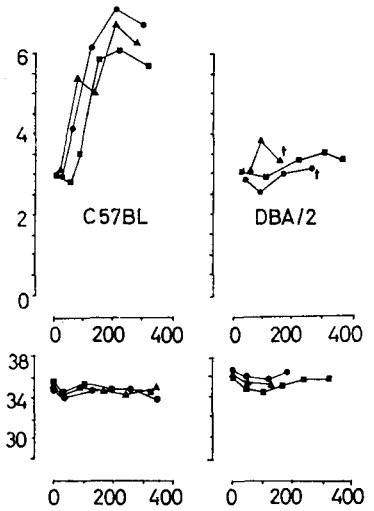

FIG. 2. Comparison of the development of ethanol tolerance in different strains of mice at thermoneutral ambient temperature. The upper graphs show the concentration of ethanol in blood in mg ml^{-1} at which the righting reflex was lost (abscissae). The lower graphs show the corresponding rectal temperatures (abscissae). The ordinates in both cases represent the time in minutes after beginning exposure to ethanol. Results obtained with C57BL mice are shown on the left; results from DBA/2 mice are on the right. A cross (+) on the upper graphs shows the point in the experiment at which death occurred. Connected symbols represent values obtained from an individual mouse.

These results provide some support for the concept that ethanol and increased temperature have additive effects on mammalian cells. Although mice of the strains that are able to adapt rapidly to ethanol retain this capacity when their body temperature is maintained within normal limits, mice of the slowly-adapting strain (DBA2 strain) show increased mortality when ethanol-induced hypothermia is prevented. It is conceivable that this result may have implications for the treatment of ethanol acute toxicity.

It is curious that, if ethanol and temperature are additive at the cellular level, no change in ethanol's effects were seen in the other two strains of mice. It is possible that if lethal concentrations of ethanol had been reached in these strains some difference might have been seen. It should also be recognized that the difference in body temperature of mice in the different ambient temperatures (4–6 °C) is slight when compared with experiments on membrane fluidity changes in poikilotherms (e.g. 20 °C—Cossins 1977). It may simply be that the difference in body temperature is not great enough to produce significant differences in ethanol sensitivity or cellular tolerance in these strains.

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