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Age and strain differences in the rate of development of functional tolerance to ethanol by mice

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The time course of development of functional tolerance to ethanol was investigated in mice during the inhalation of ethanol vapour, using loss of righting reflex as the behavioural endpoint. In male mice of the TO Swiss strain, weanling mice (18 days) showed no development of tolerance during continuous or repeated exposure to ethanol vapour for 6 h. Adolescent TO Swiss mice (35–40 days) showed rapid development of functional tolerance, reaching a state where 2× the original effective concentration of ethanol in blood was required to produce loss of righting reflex within 5 h. Older adult mice (150–200 days) showed some development of tolerance during continuous or repeated exposure to ethanol for 5 h but this was much less than that seen in adolescent mice. When adolescent males of the C57BL, TO Swiss and DBA2 strains were compared, marked differences were observed. C57BL mice showed very rapid development of functional tolerance to ethanol in which more than 2× the original effective dose was required to produce loss of righting reflex after about 3 h of ethanol exposure. TO Swiss mice showed somewhat slower development of ethanol tolerance. DBA2 mice showed little evidence of development of functional tolerance over the time course of these experiments. Evidence was also obtained that similar age and strain differences may exist with respect to tolerance to the hypothermic effects of ethanol. These results are discussed in relation to current concepts of ethanol sensitivity, tolerance and physical dependence.

Although reports exist of age differences (e.g. Lagerspetz 1972) and genetic differences (e.g. Riley & Lochry 1977) in the form of ethanol tolerance which develops slowly (i.e. over the course of days or weeks) little is known of such differences in rapid or 'acute' tolerance to ethanol which can reach a maximum in a few hours (Grieve & Littleton 1979a). There are two reasons why such differences may be important. First if differences of this kind do exist they should facilitate work on the biochemical basis of functional tolerance to ethanol. Second it may be possible to examine relationships between functional tolerance and physical dependence by comparing these states in animals of different age and genetic background. Most current theories of tolerance and dependence suggest that the physical withdrawal syndrome represents the exposure of the state of functional tolerance by removal of the drug (see Mendelson 1971). Since marked differences in the physical withdrawal syndrome of mice of different strain exist (Goldstein & Kakihana 1974; Griffiths & Littleton 1977) these may be mirrored by strain differences in functional tolerance. Similarly age differences in the time course of the physical withdrawal syndrome have been observed (Abu Murad et al 1977) these too may be reflected in age differences in the time course of development of tolerance to ethanol. In these experiments we have investigated the development of functional tolerance to ethanol in mice of differing age and strain during the inhalation of ethanol vapour.

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METHODS

Male mice of the TO Swiss strain were obtained from A. Tuck, Battlesbridge, Essex. Those described as 'weanlings' were 18 days old, and 8–10 g; 'adolescents' were 35–40 days old and 20–25 g; 'mature adults' were 150–200 days old and >40 g. Male mice, 20–25 g, of the DBA2 and C57BL strains were obtained from OLAC Ltd., Bicester.

In the first experiments ('group exposure') TO Swiss mice of different ages were placed in inhalation chambers (Grieve & Littleton 1979). Half the animals in each group received ethanol vapour (20 mg litre^{-1}) by inhalation, the others acted as controls. After 6 h all mice were removed from the chamber and the controls received ethanol, 2·0 g kg^{-1} i.p., and the 'ethanol exposed' group received the equivalent volume of isotonic saline i.p. All mice were then exposed to ethanol vapour, 40 mg litre^{-1}, in a modified inhalation chamber (Grieve & Littleton 1979) where they were tested for loss of righting reflex. On losing it they were removed from the chamber and blood ethanol concentration at this time estimated by extrapolation from values obtained for

ethanol in expired air obtained 15 min and 30 min after their removal from the chamber (see experimental design (b), Grieve & Littleton 1979a). Weanling mice rebreathed air in a chamber of volume 1·0 ml rather than the 3 ml chamber used for adults. The relation between expired air ethanol concentration and blood ethanol concentration (Grieve & Littleton 1979) was verified in mice of different ages on many occasions during these experiments.

In subsequent experiments ('individual exposure') individual mice of different age or strain were first injected intraperitoneally with ethanol at a dose (2–3 g kg^{-1}) found in preliminary experiments to produce marked ataxia in the particular age group or strain. Mice were then exposed to ethanol vapour, 40 mg $litre^{-1}$, in the modified chamber and tested for loss of righting reflex (as described under experimental design (c) in Grieve & Littleton 1979a). Rectal temperature was measured (insertion of thermistor probe to a depth of 10 mm, 5 mm in weanlings), immediately on removal of an animal from the chamber. Ambient temperature in and around the chamber was 23–25 °C. The relationship between concentration of ethanol in expired air and in blood was verified by direct gas-chromatographic measurement. Similarly, that ethanol elimination obeyed approximately zero order kinetics in each age group and strain was verified frequently. No significant differences in the rates of ethanol elimination between mice of different age and strain were observed in these experiments but measurement of elimination rate was over a short period only.

RESULTS

In the experiments involving group exposure of TO Swiss mice of different age only the adolescent and adult mice showed significant development of functional tolerance (Table 1), the adolescents showing greater development than old adults (Table 1).

In the experiments where individual mice were exposed to ethanol (Table 1) the same pattern was seen with TO Swiss mice of different age. Adolescent mice showed greater development of functional tolerance than did mature adults. Weanling mice showed no significant development of functional tolerance. Fig. 1 shows the upward trend in estimated blood ethanol concentrations in the adolescent, indicating the development of functional tolerance. This trend is absent from the results obtained from weanling animals and is much less distinct in the mature adults (Figure 1).

Further evidence of rapid development of functional tolerance in adolescent TO Swiss mice was provided by measurement of rectal temperatures (Fig. 2). In these animals the hypothermia produced by ethanol remained constant throughout repeated exposures to ethanol despite the fact that estimated concentrations in blood were increased at each sequential measurement of temperature. Rectal temperatures of mature adults also remained relatively constant during repeated exposures to ethanol, but in these animals estimated concentrations of ethanol in blood were not rising to the

Table 1. Development of tolerance to ethanol in mice of different age and strain

Development of functional tolerance is shown by an increase in the estimated concentration of ethanol in blood at which righting reflex is lost between the control or initial value and the test or maximum value. Mean time of exposure gives the time required for the maximum value to be reached. Means and standard errors of blood ethanol concentrations are shown and these have been compared by an unpaired Students *t*-test to give the P values in the final column.

Experiment, age and strain	n	Control or initial [EtOH] loss of RR (mg ml^{-1})	Mean at time of exposure (min)	Test or max [EtOH] at loss of RR (mg ml^{-1})	P
Group exposure					
Weanling TO	6	3·15 ± 0·30	360	3·11 ± 0·17	>0·1
Adolescent TO	4	3·68 ± 0·10	360	5·23 ± 0·23	<0·01
Mature TO	6	2·83 ± 0·09	360	3·32 ± 0·14	<0·05
Individual exposure					
Weanling TO	4	2·69 ± 0·09	245	2·90 ± 0·18	>0·05
Adolescent TO	4	2·90 ± 0·20	245	6·05 ± 0·22	<0·01
Mature TO	4	3·20 ± 0·08	275	3·93 ± 0·25	<0·05
Individual exposure					
Adolescent TO	4	3·04 ± 0·16	255	5·95 ± 0·20	<0·01
Adolescent C57	4	2·94 ± 0·06	187	7·03 ± 0·24	<0·01
Adolescent DBA	4	2·44 ± 0·14	253	3·65 ± 0·18	<0·05

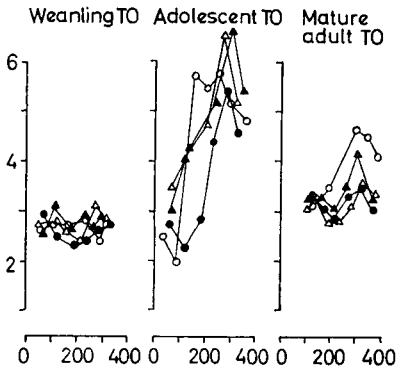

FIG. 1. The development of functional tolerance to ethanol in TO strain mice of different ages. Connected symbols represent values obtained for an individual animal. The abscissa shows the time in minutes after first beginning exposure to ethanol; the ordinate the concentration of ethanol in the blood in mg ml^{-1} at loss of righting reflex.

extent seen in adolescents. In weanlings there was a tendency for hypothermia to be greater on each removal from the chamber despite the fact that estimated concentrations in blood were not significantly altered (Fig. 2).

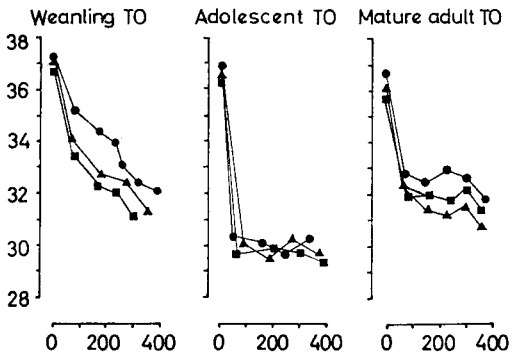

FIG. 2. The hypothermia developed during the induction of ethanol tolerance in TO strain mice of different ages. Connected symbols represent values obtained for an individual animal. The abscissa shows the time in minutes after first beginning exposure to ethanol; the ordinate the rectal temperature in °C.

When development of functional tolerance to ethanol was investigated in adolescent mice of different strains, the results shown in Table 1 and Fig. 3 were obtained. Mice of the C57BL strain showed very rapid development of tolerance so that more than 2× the original effective concentration of ethanol was required to produce loss of righting reflex after 2–3 h of exposure. TO Swiss mice showed the previously described, more modest response. DBA2 mice showed much less evidence of the development of tolerance over the time course of these experiments.

When the ethanol hypothermia produced was compared in the three strains it remained relatively constant after repeated ethanol administration. In the C57BL and TO Swiss strains, however, this constant degree of hypothermia was found at increasing estimated blood ethanol concentrations whereas in the DBA2 strain blood concentrations did not increase (Fig. 4). The results provide further evidence for the rapid development of functional tolerance to ethanol in the TO Swiss and C57BL strains.

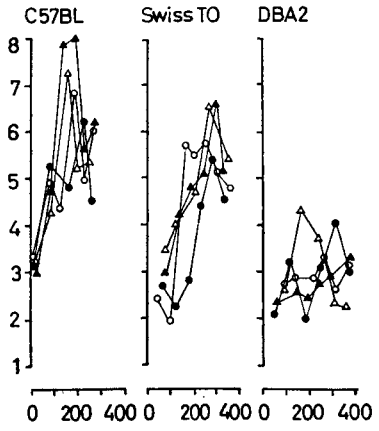

FIG. 3. The development of functional tolerance to ethanol in mice of different strains. Connected symbols represent values obtained for an individual animal. The abscissa shows the time in minutes after first beginning exposure to ethanol; the ordinate the concentration of ethanol in the blood in mg ml^{-1} at loss of righting reflex.

DISCUSSION

These experiments show that there are marked differences in the rapidity with which mice of different age and strain can develop functional tolerance to ethanol during repeated inhalation of the drug. In mice of the TO Swiss strain, weanling animals, of age 18 days, do not show appreciable functional tolerance during the 6 or 7 h period of drug administration. On the other hand, mice of age 35–40 days showed rapid development of functional tolerance which reached a maximum in which 2× the original concentration of ethanol was required to produce loss of righting reflex in 3–5 h. Mature adult TO Swiss mice of over 150 days showed much

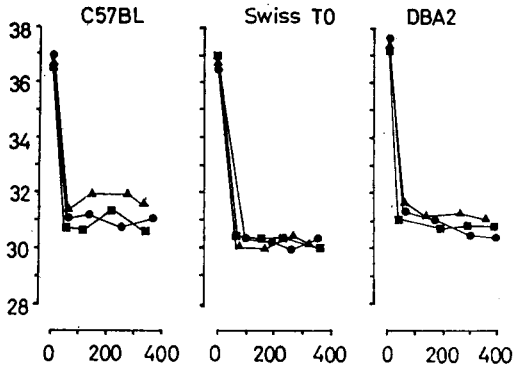

FIG. 4. The hypothermia developed during the induction of ethanol tolerance in mice of different strains. Connected symbols represent values obtained for an individual animal. The abscissa shows the time in minutes after first beginning exposure to ethanol; the ordinate the rectal temperature in °C.

less rapid development of cellular tolerance to ethanol. When adolescent mice of different strains were compared with respect to ethanol-induced loss of righting reflex only those of the C57BL and TO Swiss strains showed clearly rapid development of functional tolerance, which was more rapid and of greater magnitude in the C57BL strain. Mice of the DBA2 strain showed little tolerance over the 7 h time-course of these experiments. In subsequent experiments DBA2 mice have been observed to develop functional tolerance to ethanol if administration is prolonged. Although not specifically designed to investigate tolerance to the hypothermic effect of ethanol, the experiments described suggest that the time-course of tolerance to the effects of ethanol on body temperature may be similar to that on righting reflex. The hypothermia induced by ethanol may also be of relevance to the mechanism of functional tolerance (see Grieve & Littleton 1979b).

These results underline the fact that development of functional tolerance must be considered even when ethanol is administered acutely. Thus any test for the effects of ethanol in which more than a few minutes elapse between drug administration and measurement (e.g. hypothermia, ataxia, 'sleep-time', death) make it necessary to consider that difference in rapidity of development of tolerance could explain apparent differences between groups of subjects in terms of dose or tissue concentration of ethanol required to produce the effect. Thus the reported lower LD50 for ethanol in old rats (Wiberg et al 1970) could be due to impaired development of functional tolerance in animals of this age. Similarly the often noted low 'sensitivity' of mice of the C57BL strain to ethanol-induced central depression, based on measurements of 'sleep-time' (e.g. Kakihana et al 1966; Randall & Lester 1974), may be explained by the very rapid development of functional tolerance in this strain. It may be that there is little difference in initial sensitivity to the depressant effects of ethanol between animals, and that differences hitherto regarded as differences in 'sensitivity' in fact represent differences in the rapidity of development of functional tolerance. Results supporting this concept (and supporting the results on strain differences reported here) have recently been obtained by Tabakoff & Ritzmann (1979). Investigating acute tolerance to ethanol after i.p. injection, they have found that mice of the DBA2 and C57BL strains lose their righting reflex at the same point on the ascending curve of blood ethanol concentration (i.e. their 'sensitivity is similar), but that only C57BL animals regain their reflex at a higher point on the descending curve (i.e. show rapid or acute tolerance).

The experimental results obtained here also suggest a genetic relationship between the rapidity of development of functional tolerance and the duration and severity of the ethanol physical withdrawal syndrome. It has been reported (Goldstein & Kakihana 1974; Griffiths & Littleton 1977) that mice of the DBA2 strain show a more severe and prolonged physical withdrawal syndrome than do mice of the C57BL strain. Mice of the Swiss Webster or TO Swiss strains are intermediate. If, as suggested by many (e.g. see Mendelson 1971), the physical withdrawal syndrome represents exposure of the state of functional tolerance when ethanol is removed then its severity and duration should be limited by the rate at which functional tolerance can itself be removed. It might be expected that the rate at which functional tolerance could be removed would be related to the rate at which it can be instituted. This is borne out by the results. C57BL mice show very rapid cellular tolerance and a very mild and brief withdrawal syndrome. DBA2 mice are at the other end of the spectrum showing slow development of tolerance and a severe and protracted withdrawal syndrome. TO Swiss mice are intermediate in both respects.

The results also have implications for the mechanism by which functional tolerance develops in the mouse. It seems unlikely that a learned pattern of behaviour is responsible, since in the first experiments on grouped mice each animal was tested approximately the same number of times under the influence of ethanol. The ethanol-exposed group did not therefore have greater opportunity to learn the specific behavioural response although they did have more opportunity to practice motor co-ordination under the influence of ethanol than did the controls. If the development of functional tolerance cannot be fully explained by learned behaviour then it seems probable that some adaptive mechanism at the neuronal level must be invoked. This could be related to the physical effect of ethanol at the neuronal membrane (Chin & Goldstein 1977) and subsequent alterations in neuronal membrane lipid composition (Littleton & John 1977). Genetic (Littleton et al 1979) and age-related differences in brain (Houslay et al 1978) exist which could explain the findings reported here in the context of neuronal metabolism. The changes in body temperature associated with the action of ethanol might also be relevant and this will be considered subsequently (Grieve & Littleton 1979a).

In conclusion these experiments show marked age

and genetic differences in the rate with which functional tolerance to ethanol may develop in mice. These results have important implications for the concepts of ethanol 'sensitivity', tolerance and physical dependence.

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