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Metabolic and Pharmacodynamic Tolerance to Ethanol in Rats[1]

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WOOD, J. M. AND R. LAVERTY. *Metabolic and pharmacodynamic tolerance to ethanol in rats* PHARMAC. BIOCHEM. BEHAV. 10(6) 871–874, 1979.—The development of tolerance to ethanol was studied in rats fed nutritionally adequate liquid diets containing ethanol or sucrose for up to 5 weeks. Tolerance was shown to be due to both metabolic and pharmacodynamic factors. Tolerance began to develop after 3 days of ethanol intake, reached a plateau by 16 days and persisted for up to 22 days after stopping the ethanol intake. The rate of onset and decay of both components of tolerance was similar.

Ethanol Alcohol Tolerance Metabolic tolerance Pharmacodynamic tolerance

CHRONIC ethanol intake produces tolerance to the intoxicating effects of the drug and at least two different factors may contribute independently to this. Repeated ethanol administration may result in an increased rate of ethanol elimination, i.e., metabolic tolerance may develop. Adaptive changes at the site of drug action may also be induced, resulting in a reduced response to a given tissue concentration of the drug, i.e., pharmacodynamic tolerance may develop.

It is widely believed that the tolerance that develops to ethanol is largely pharmacodynamic and due to adaptive changes within the central nervous system. There are considerable discrepancies among previous studies as to whether metabolic tolerance also develops. Chronic ethanol administration has been reported to increase [3, 4, 6, 10, 13] decrease [11] or have no effect [5,8] on the rate of ethanol elimination in the rat. Similar conflicting observations have been reported in man [17].

Certain nutritional factors have been shown to affect the rate of ethanol elimination and may account for some of the conflicting results. Both food deprivation [1, 9, 12, 15] and maintenance on a low protein diet [2,14] have been reported to decrease ethanol elimination rates. Samson *et al.* [11] have shown that food deprivation for 24 hours results in a 30% reduction in the blood ethanol elimination rate of control rats but only a 10% reduction in the rate of ethanol-dependent rats. They suggest that this factor alone accounts for the higher elimination rates of the ethanol-dependent rats compared to controls reported in studies where rats have been fasted overnight before assessing elimination rates.

In this study, in order to avoid any possible effects of nutritional variations on blood ethanol elimination rates, tolerance to ethanol was induced in rats by feeding them on nutritionally adequate, completely liquid diets containing ethanol. Control rats were maintained on equal quantities of a nutritionally equivalent diet and the rats were not fasted before assessing tolerance. The relative contributions of metabolic and central nervous system adaptive changes to the development of tolerance were investigated. The time course of the acquisition and loss of these adaptive changes was also studied.

METHOD

Animals

Random-bred, male albino rats of Wistar origin, initially weighing 120 ± 5 g and aged 6–7 weeks, were used. Rats were housed in groups of 2–3 animals.

Assessment of Tolerance to Ethanol

Seventeen rats were fed on a balanced liquid diet (Complan, Glaxo Laboratories, N.Z. Ltd.) (200 g/l) containing 95% v/v ethanol (70 ml/l), for 14 days. Rats fed on the ethanol-containing diet (ethanol-treated) were weight-matched with 17 control rats which were fed the same basic liquid diet except that sucrose (90 g/l) was isocalorically substituted for the ethanol. Control rats were "pair-fed" with the ethanol-treated rats, i.e. on each day control animals received the same quantity of diet as the ethanol-treated rats had consumed on the previous day.

After 14 days, the ethanol-treated rats were taken off the ethanol-containing diet. Tolerance to ethanol was assessed 16 hours later. During this period both the control and ethanol-treated rats were given equivalent quantities of the control diet. This allowed for elimination of any remaining ethanol from the blood of the ethanol-fed rats. Tolerance was

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assessed by measuring the duration of sleeping time at 28°C after a standard single dose of ethanol (87 mmol/kg). Ethanol, 30% v/v in 9 g/l NaCl solution (saline), was infused over 8 minutes into a jugular vein. The rats were maintained under light ether anaesthesia during the insertion of the needle into the vein. Sleeping time was taken as the time from the end of the infusion until the rat regained its righting reflex.

The development of metabolic tolerance was assessed by comparing blood ethanol elimination rates of control and ethanol-treated rats. Five blood samples were collected from each rat at 1- to 2-hour intervals after 30 minutes up to 10 hours after the standard dose of ethanol. The samples were collected in 50 μl heparinized capillary tubes from small incisions in the lateral tail vein. Blood ethanol concentrations were measured in duplicate on diluted blood samples (1:20 in 4 mmol/l *t*-butanol as internal standard) by gas chromatography. A Pye series 104 gas chromatograph with a flame ionization detector was used. The glass column (1 m × 4 mm i.d.) was packed with Porapak Q. The column temperature was 190°C and the carrier gas (N_2) flow rate was 50 ml/min. The line of best fit for the blood ethanol concentration-time data from each rat was obtained by least squares linear regression analysis. The slope of the best-fit line was used as a measure of the blood ethanol elimination rate. The elimination line for each rat was extrapolated back to zero-time to obtain an estimate of the zero-time blood ethanol concentration.

The development of pharmacodynamic tolerance was assessed by comparing blood and brain ethanol concentrations at the time of waking in control and ethanol-treated rats. After the standard dose of ethanol, tail venous blood samples were collected from 10 treated and 10 control rats at the time when the rats regained their righting reflex. Blood ethanol concentrations were measured as described above. Seven treated and 7 control rats were decapitated on waking and their brains were removed for the estimation of waking brain ethanol concentrations. Each brain was weighed, then homogenized in ice-cold 0.4 N perchloric acid containing *n*-propanol (13 mmol/l) as an internal standard. The homogenates were centrifuges at 9800 G for 10 min. The ethanol concentrations of the supernatants were measured by gas chromatography using the same column and conditions as for the blood samples.

Time Course of the Acquisition and Loss of Tolerance

Twelve rats were fed on the ethanol-containing liquid diets for 35 days. Another twelve control rats were "pair-fed" on the sucrose-containing diet. Using the method described above, tolerance to ethanol was assessed in 6 of the ethanol-treated and 6 of the control rats after 3 and 16 days on the liquid diets. Tolerance was assessed in the other 6 ethanol-treated and 6 control rats after 7 and 35 days on the diets. After 35 days all the rats were taken off the liquid diets and fed laboratory pellets and water ad lib. Tolerance was assessed in one group of 6 control and 6 treated rats 7 days after stopping the liquid diet administration and in the other 6 treated and 6 control rats 22 days after stopping the liquid diet administration.

Student's *t*-test was used to test the significance of differences between ethanol-treated and control rats.

RESULTS

The average daily ethanol intake of rats maintained on the ethanol-containing liquid diet was 280 mmol/kg. Table 1 gives the results of the assessment of tolerance to the standard dose of ethanol in rats that had been fed the liquid diets for 14 days The mean sleeping time of the ethanol-treated rats was significantly shorter than the sleeping time of the controls ($p<0.001$).

Ethanol elimination approximated most closely to zero-

TABLE 1

SLEEPING TIME, BLOOD ETHANOL ELIMINATION RATE, WAKING BRAIN ETHANOL CONCENTRATION, WAKING BLOOD ETHANOL CONCENTRATION AND ZERO-TIME BLOOD ETHANOL CONCENTRATION AFTER STANDARD DOSE OF ETHANOL (87 mmol/kg, IV)

	Control	Ethanol-treated§
Sleeping time (hr) (N=10)	4 1 ± 0 3	2 1 ± 0.2‡
Blood ethanol elimination rate (mmol/l/hr) (N=10)	9.8 ± 0.4	12 4 ± 0.9*
Waking brain ethanol concentration (mmol/kg) (N=7)	56 ± 2	66 ± 2†
Waking blood ethanol concentration (mmol/l) (N=10)	70 ± 2	82 ± 2‡
Zero-time blood ethanol concentration (mmol/l) (N=10)	110 ± 2	108 ± 2

Values are mean ± SE §Ethanol intake 280 mmol/kg daily for 14 days
*$p<0.05$ †$p<0.01$: ‡$p<0$ 001, significance of difference from control value (Student's *t*-test)

order kinetics over the time course studied: least squares linear regression analysis of the blood ethanol-time data gave regression coefficients greater than 0.98 in each case. The mean blood ethanol elimination rate of the ethanol-treated rats was significantly ($p<0.05$) higher than the mean elimination rate of the controls (Table 1). The mean brain and blood ethanol concentrations on waking were significantly ($p<0.01$ and $p<0.001$, respectively) higher in the ethanol-treated rats than in the controls (Table 1). There was no significant difference in the mean estimated zero-time blood ethanol concentrations of the control and treated rats (Table 1).

Figure 1(a) shows the difference in mean sleeping times of control and treated rats produced by the standard dose of ethanol after 3, 7, 16 and 35 days on the liquid diets and 7 and 22 days after cessation of ethanol administration. The means of the sleeping times of the ethanol-treated rats were significantly shorter than those of the controls at all times of testing. The mean sleeping time of the ethanol-treated rats was reduced to approximately 70% of the controls on Days 3 and 7 and had fallen to approximately 50% after 16 days. There was no further reduction after 35 days. Seven days after stopping the ethanol intake the mean sleeping time of the treated rats had increased to approximately 70% of that of the controls but the difference was still significant. After 22 days the difference was slightly less but still significant.

Figure 1(b) shows the difference in blood ethanol elimination rates of control and ethanol-treated rats over the same time course as described above. After 3 days of ethanol intake the mean elimination rate of the treated rats was slightly higher than that of the controls but the difference was not statistically significant. The difference was significant after 7 days and had reached a maximum after 16 days. One week after stopping the continuous ethanol intake, the elimination rate of the ethanol-treated rats was still significantly raised. After 22 days the mean rate of the treated rats was raised but the difference was no longer statistically significant.

Figure 1(c) shows the difference in waking blood ethanol concentrations of control and ethanol-treated rats. After 3 days of ethanol consumption the mean waking blood ethanol concentration of the ethanol-treated rats was slightly higher than that of the controls but the difference was not statistically significant. After 7 days the difference was significant and remained stable for up to 35 days of ethanol intake. Seven days after stopping the ethanol administration the difference remained significant but was no longer significant after 22 days.

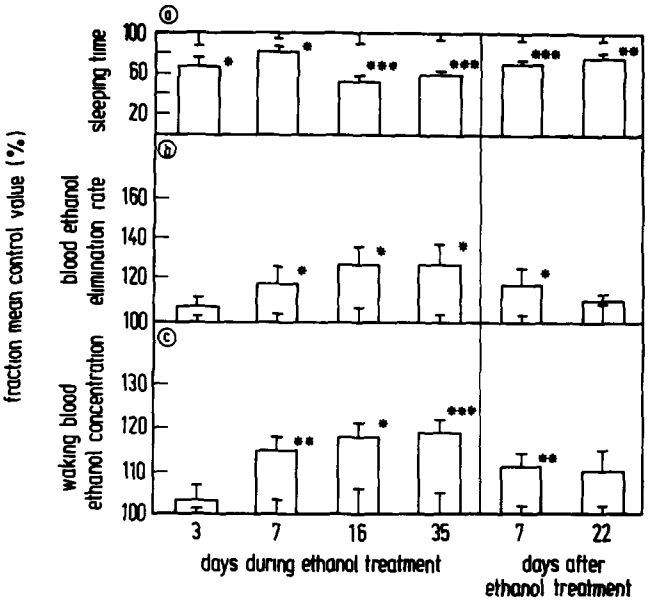

*FIG. 1. Difference in (a) sleeping time (b) blood ethanol elimination rate and (c) waking blood ethanol concentration between control (N=6) and ethanol-treated (N=6) rats produced by a single standard dose of ethanol (87 mmol/kg, IV) after 3, 7, 16 and 35 days of ethanol treatment and 7 and 22 days after the ethanol treatment was stopped The bars represent the mean values for the ethanol-treated rats expressed as a percentage of the mean control values Error bars are SEM Error bars at 100% are SEM for the controls. Analysis of the significance of differences was performed on raw data using Student's t-test. *$p<0.05$; **$p<0.01$, ***$p<0.001$*

DISCUSSION

The results show that considerable tolerance to ethanol develops in rats after prolonged consumption of an ethanol-containing diet. One factor contributing to the observed tolerance was a significant increase in the blood ethanol elimination rate. Because the age, weight and nutritional status of the ethanol-treated and control rats were kept strictly equivalent, the difference in elimination rates cannot be attributed to any of these factors. The difference in elimination rates cannot be due to the differential effect of fasting proposed by Samson *et al.* [11], as these rats were not fasted overnight before testing tolerance. Since the extrapolated zero-time blood ethanol concentrations of control and tolerant rats were the same, none of the tolerance observed was due to a difference in the volume of distribution of ethanol.

Failure to demonstrate an increased ethanol elimination rate in other studies may have been due to strain differences or to differences in the methods used to achieve chronic ethanol consumption. In some studies rats were undernourished during the period of chronic ethanol administration [11] or the dietary intake of the control and ethanol-treated rats was not kept strictly equivalent [5,8]. Continuous ingestion of high doses of ethanol may be necessary to induce metabolic tolerance to ethanol: in contrast to the methods of ethanol administration used in many other studies, this liquid diet method produces a high and relatively continuous consumption of ethanol [18].

As well as metabolic tolerance, considerable pharmacodynamic or CNS tolerance to ethanol developed: ethanol-tolerant rats woke with significantly higher brain ethanol concentrations than controls. Tail venous blood samples taken on waking reflected the changes in brain ethanol concentrations. Thus, measurement of waking blood ethanol concentrations appears to be a suitable method of assessing pharmacodynamic tolerance. The obvious advantage of measuring blood, rather than brain, ethanol concentrations is that animals need not be sacrificed.

These results demonstrate that the tolerance which develops in this strain of rat is due to both metabolic and pharmacodynamic factors. The relative contributions of these factors to the total tolerance observed after the standard dose of ethanol can be estimated as follows: Fig. 2 shows mean blood ethanol elimination curves for control and ethanol-tolerant rats plotted from the mean elimination rates

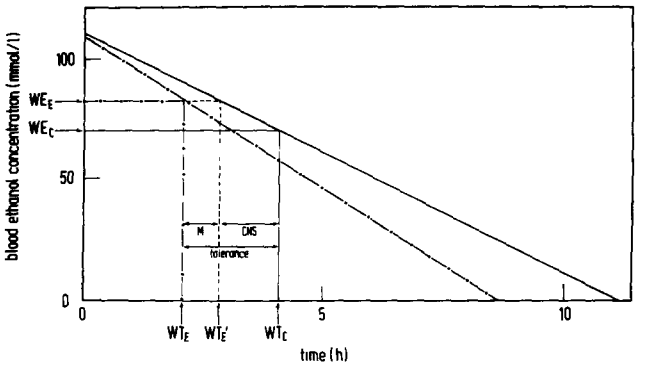

FIG. 2. Relative contributions of metabolic (M) and pharmacodynamic (CNS) changes to total tolerance after the standard dose of ethanol (87 mmol/kg, IV). Mean elimination lines for the control (——) and ethanol-tolerant rats (–·–·–) were drawn using the mean values for elimination rate and zero-time blood ethanol concentration given in Table 1. WE_C and WE_E are the mean waking blood ethanol concentrations of the control and ethanol-tolerant rats respectively (Table 1). WT_C and WT_E are the corresponding mean waking times. The broken line (---) indicates the estimated mean waking time of the ethanol-tolerant rats ($WT_{E'}$) if the metabolic component of tolerance was abolished.

and extrapolated zero-time blood ethanol concentrations given in Table 1. If the mean waking blood ethanol concentration of the ethanol-tolerant rats is placed on the curve for the control rats, the mean sleeping time for the ethanol-tolerant rats in the absence of metabolic tolerance ($WT_{E'}$) can be estimated. Thus, in these experiments, and after this particular dose of ethanol, metabolic factors account for approximately 40% of the tolerance observed. Pharmacodynamic factors would account for the remainder.

Tolerance had begun to develop after 3 days of ethanol intake. Both metabolic and pharmacodynamic changes contributed to this rapid onset of tolerance. Tolerance reached a maximum after about 16 days of continuous ethanol intake and remained constant over 35 days of ethanol intake. The time course of the acquisition of maximum tolerance is similar to that reported by others [5, 7, 16]. It is also very similar to the time course of the onset of physical dependence on ethanol in this strain of rat [18]. After stopping the prolonged ethanol administration, tolerance became less but was still detectable after 22 days. The metabolic and pharmacodynamic changes decayed at a similar rate. Tolerance persisted longer than has been reported in most previous studies [5, 10, 16]. This is probably because the dose and the period of ethanol consumption were greater than in many other studies.

In summary, these experiments show that tolerance to ethanol may be due to both metabolic and pharmacodynamic factors. Tolerance may develop rapidly and persist for a considerable period after the cessation of ethanol administration.

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