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Tolerance to Ethanol in Rats Bred on Essential Fatty Acid Deficient Diets[1]

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JONES, A. W., C. ALLING, W. BECKER AND E. ÄNGGÅRD. *Tolerance to ethanol in rats bred on essential fatty acid deficient diets.* PHARMACOL BIOCHEM BEHAV 19(1) 115–119, 1983.—We bred three generations of Sprague-Dawley rats on a diet deficient in essential fatty acids, low-EFA (0.3 energy %), whereas age matched controls received normal-EFA (3.0 energy %). Subgroups (N=6) of female rats were given daily IP injections of ethanol (3.0 g/kg) or isocaloric glucose for 23 consecutive days. On days 1 and 22 blood-ethanol profiles, rates of ethanol metabolism and ethanol-induced impairment of motor coordination were measured after the challenge dose of 3.0 g/kg. Rats exposed to ethanol ate and drank more than controls and gained more body weight over the 23 days. Low-EFA rats were slightly more impaired than normal EFA rats after an acute dose of ethanol even though the peak blood ethanol concentrations reached were about the same at 2.9 mg/ml (63 mmol/l). After chronic ethanol treatment a functional tolerance developed in both dietary groups but the degree of tolerance was less clearcut in low EFA rats. Metabolic tolerance was confirmed after chronic treatment in both dietary groups as shown by steeper slopes (19–27%) of the ethanol elimination curves. But no significant differences in the development of metabolic tolerance were apparent in rats on low EFA and normal EFA diets.

Alcohol Essential-fatty-acids Ethanol Impairment Metabolism Rats Tolerance

ETHANOL interacts with the metabolism of essential fatty acids to change the composition of phospholipid acyl groups in the lipidbilayer of cell membranes [1, 10, 18]. Chronic intake of ethanol increases the ratio of saturated to unsaturated fatty acids and this change in structure has been linked with the development of tolerance [13, 16, 17]. Interindividual differences in tolerance to ethanol could depend on the relative proportion of unsaturated fats built into the membrane lipid and therefore on dietary sources of essential fatty acids. Moreover, mammals might be able to adjust their membrane lipid as a biological response to the disordering effect of ethanol [11,12], a corollary to the lipid adaptation seen in micro-organisms to changes in environmental temperature [8].

We have investigated the effects of chronic ethanol treatment in rats bred through three generations on a diet deficient in essential fatty acids. Our main focus in this paper concerns the development of metabolic and functional tolerance to chronic treatment with ethanol.

METHOD

Animals and Diets

Sprague-Dawley rats were bred through three generations on a nutritionally adequate diet except for a deficient content of essential fatty acids, being 0.3 energy % (low-EFA). Age matched controls were fed an identical diet but the content of essential fatty acid was optimal at 3.0 energy % (normal-EFA [2]. The ratio between fatty acids in the linoleic (n-6) and the linolenic (n-3) series was approximately 7:1 in the 0.3 energy % EFA group and 4:1 in the 3 energy % group [7].

Female rats 45 days old were selected at random from batches of low-EFA and normal-EFA animals and were allocated into subgroups (N=6). At the start of treatment the mean body weight of low-EFA rats was 110 g (range 101–117) and normal-EFA rats weighed 137 g (range 126–146). Throughout the study the rats were housed in metabolic cages in an animal room maintained at 23°C and relative humidity 60% with a 12 hr light/dark cycle. Water and diet (crushed pellets) were available ad lib. Body weights, intake of food and water and urine volumes were recorded daily.

Ethanol Treatment

The test animals were injected intraperitoneally with 3.0 g/kg ethanol at 09.00 for 23 consecutive days. Rats in the control groups were injected with isocaloric glucose solutions. The ethanol solution was 15% w/v in 0.9% w/v NaCl and the glucose was 13% w/v in tap-water. During the first 9 days of the experiment the alcohol groups were offered a 5% w/v solution of ethanol instead of water and the controls were given 3% w/v sucrose. Because of an unacceptably low intake of fluid in the alcohol groups, this protocol was abandoned and water was offered ad lib to all animals thereafter. The experiment was ended after 23 days.

[1]Essential fatty acids (EFA) are designated by chain lengths and number of double bonds; (n–6) denotes that the first double bond from the methyl group occurs after the sixth carbon atom, the methyl group being counted as carbon number one.

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Blood Samples and Determination of Ethanol

Blood samples (10 μl) were taken in duplicate from a cut made in the tip of the tail at 30 min intervals for the first 2 hr and then every hour up to 8 hr. Each sample was taken into a capillary microcap (Drummond Scientific Co., USA) and diluted immediately with 1 ml of 0.2% w/v sodium fluoride in an Auto-analyzer cup. The dilutions were rapidly mixed and the bloods stored at 4°C pending analysis. During the period of chronic ethanol treatment blood samples were taken at 60 min after the daily injections of ethanol to monitor the peak concentrations reached.

The concentration of ethanol in blood was determined with an automated enzymatic method as previously described in detail [9]. The standard deviation of the assay increases with the concentration of ethanol and at 1.0 mg/ml (21.7 mmol/l) the coefficient of variation was about 2.0%.

Blood Ethanol Parameters

From the blood-ethanol concentration time course of each rat the distribution volume and rate of metabolism of ethanol were calculated by the methods introduced by Widmark [19]. The slopes of the linear portions of the disappearance curves were used as an index of the rate of ethanol metabolism. To assess metabolic tolerance following chronic ethanol treatment the intra-individual changes in rate of metabolism were used.

Ethanol-Induced Impairment

Ethanol-induced impairment of motor coordination in rats was assessed on day 1 and 22 after a dose of 3.0 g/kg ethanol. The test of coordination was made immediately before the blood samples were taken for up to 8 hr. The device used was an automated version of the tilting plane [5]. Each rat was placed on the rough-side of a surface made of hardboard and the board was tilted from 0–90° within 8 sec at a constant speed. At a certain angle of elevation the rats slide backwards down the board to pass a photocell by means of which the motor and thus the tilting is stopped. Impaired rats slide down the plane at lower angles. The rats were given several training sessions before the ethanol treatment so as to establish consistent basal values. The pre-treatment tilt scores were not significantly different among different animals as shown by analysis of variance. Moreover, the sliding angles after ethanol treatment were always compared with the pre-treatment result within the same rat. Each animal was tilted three times within about 2 min and the mean tilt score was used in all the calculations.

Statistical Analysis

Conventional methods were used to evaluate the statistical significance of results. Student's *t*-test for both inter- and intra-individual differences were used to compare group means. Ethanol elimination rate for individual rats was worked out by regression analysis with at least 4 variates being included in the straight line. The tilt-scores after ethanol treatment (A) were expressed as percentages of pre-treatment basal values (B) by computing the difference between logarithmic values i.e., $\log(A) - \log(B) = \log(A)/(B)$. The antilogarithm × 100 allows percent ethanol-induced impairment to be calculated. Confidence limits and tests of significance are given from the variances in logarithm units. This transformation device is useful in biological statistics when handling ratio variables because it tends to stabilize variance so that the assumptions underlying the use of normally distributed variables are still valid.

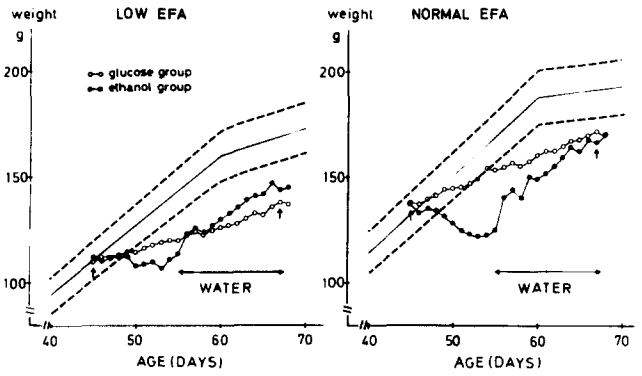

FIG. 1. Body weight changes of rats during chronic treatment with ethanol (3.0 g/kg IP) or isocaloric glucose for 23 consecutive days. The solid and dashed diagonal lines represent growth curves (mean±2 SD) for rats of the same age and strain under free-feeding unrestrained conditions. Low EFA=0.3 energy %, Normal EFA=3.0 energy %. For the first 9 days the rats injected with ethanol were also given 5% w/v as drinking fluid but this was switched to tap-water thereafter.

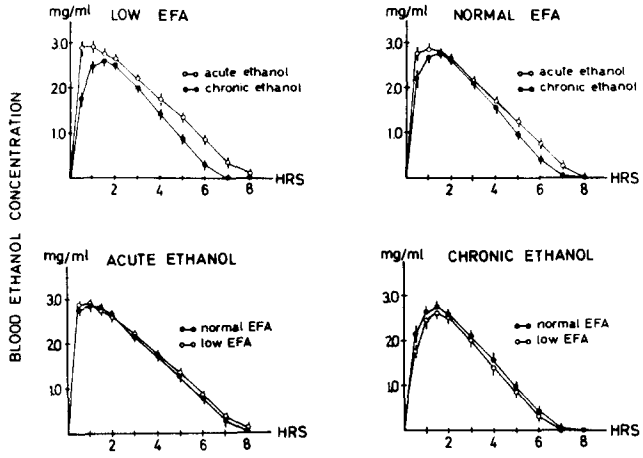

FIG. 2. Blood ethanol profiles after 3.0 g/kg IP on day 1 (acute treatment) and after 22 consecutive daily injections of 3.0 g/kg (chronic treatment). Low EFA=0.3 energy %, Normal EFA=3.0 energy %. At each sampling point mean±S.E. (N=6) is plotted.

RESULTS

Weight Gain, Food Consumption and Fluid Balance

Figure 1 shows changes in body weight of rats treated with ethanol and glucose and fed on low-EFA and normal-EFA diets. For comparison standard weight curves are shown for unrestrained rats fed the same diets. The initial weight-loss in the ethanol treated groups was reversed when the 5% w/v ethanol solution given as drinking fluid was switched to tap water. The ethanol treated rats in both dietary groups gained weight at a faster rate thereafter ($p<0.001$).

TABLE 1

FOOD INTAKE, GAIN IN WEIGHT AND FLUID BALANCE IN RATS FED LOW EFA AND NORMAL EFA DIETS*

Dietary group	Treatment	Food consumed (g/day)	Gain in weight (g/day)	Water intake (ml/day)	Urine output (ml/day)	Urine output / Water intake
Low EFA	Ethanol	14.0 ± 0.39†	2.5 ± 0.17†	42.2 ± 2.39†	22.6 ± 1.89†	0.54 ± 0.054
Low EFA	Glucose	11.5 ± 0.19	1.4 ± 0.15	23.2 ± 0.73	12.9 ± 0.61	0.55 ± 0.031
Normal EFA	Ethanol	15.1 ± 0.37†	2.9 ± 0.34†	50.2 ± 2.04†	30.7 ± 1.66†	0.61 ± 0.041
Normal EFA	Glucose	13.0 ± 0.20	1.5 ± 0.15	21.6 ± 0.71	12.6 ± 0.54	0.58 ± 0.031

*Rats were injected (IP) with 3.0 g/kg ethanol at 09.00 for 23 consecutive days. Low EFA=0.3 energy %. Normal EFA=3.0 energy %. Control rats were injected with an isocaloric glucose solution. The results presented are average values (mean ± S.E., N=6) between days 14 and 21 of the experiment.

†$p<0.001$ between ethanol and glucose groups.

TABLE 2

BLOOD-ETHANOL PARAMETERS IN RATS FED ON LOW-EFA (0.3 ENERGY %) AND NORMAL-EFA (3.0 ENERGY %) DIETS*

Blood-ethanol parameter	Low-EFA		Normal-EFA	
	Acute	Chronic	Acute	Chronic
Peak BAC§ (mg/ml)	2.94 ± 0.04	2.64 ± 0.03‡	2.88 ± 0.06	2.79 ± 0.04
Time to peak BAC (min)	55 ± 5	90 ± 9†	54 ± 6	85 ± 5†
Time to zero BAC, min_0 (min)	475 ± 16	389 ± 17†	452 ± 12	402 ± 9‡
Estimated BAC at time zero, C_0 (mg/ml)	3.55 ± 0.04	3.66 ± 0.08	3.59 ± 0.06	3.79 ± 0.06†
Elimination from blood (mg/ml/hr)	0.45 ± 0.013	0.57 ± 0.027†	0.48 ± 0.010	0.57 ± 0.019‡
Volume of distribution (L/kg)	0.84 ± 0.009	0.82 ± 0.020	0.84 ± 0.015	0.79 ± 0.011†
Turnover of ethanol (g/kg/hr)	0.38 ± 0.012	0.47 ± 0.019†	0.40 ± 0.011	0.45 ± 0.011‡

*The dose of ethanol was 3.0 g/kg given IP before (acute treatment) and after 22 consecutive daily injections of 3.0 g/kg (chronic treatment).

†$p<0.05$, ‡$p<0.01$ between acute and chronic treatments. No significant differences between the two dietary groups. The figures given are mean ± S.E. (N=6).

§BAC=Blood alcohol concentration. The parameters C_0 and min_0 were computed by extrapolating the rectilinear elimination phase to the ordinate (y-intercept, time zero) and to the abscissa (x-intercept, zero BAC) respectively. Rate of ethanol metabolism is given by $C_0/min_0 \times 60$ (mg/ml/hr) and volume of distribution = dose (g/kg)/C_0 (mg/ml).

Table 1 gives the average daily food consumption, water intake and urine output between day 14 to 21 of the experiment. This represents a stable period with rats having adjusted to the stressful test conditions. Intake of food and water was significantly higher in the ethanol-treated rats ($p<0.001$). This is in line with the faster gain in body weight of rats chronically treated with ethanol. Ratios of urine output to water intake were lower in the low EFA groups.

Blood Ethanol Parameters

The time courses of blood-ethanol concentration after acute and chronic treatments are shown in Fig. 2 as average curves for both dietary groups. Table 2 gives the underlying parameters that reflect disposition and metabolism of ethanol. No significant differences were evident between the low EFA and normal EFA rats either after acute or chronic treatment. But statistically significant increases in the rate of ethanol metabolism occurred after chronic exposure in both dietary groups. This was shown from the steeper slopes of the linear portions of the elimination curves, increasing by 19–27% on average. An increase in turnover of ethanol and a shorter time to reach zero BAC supports the development of metabolic tolerance in chronically treated animals. In low

TABLE 3

ETHANOL INDUCED IMPAIRMENT IN RATS FED ON LOW EFA (0.3 ENERGY %) AND NORMAL EFA (3.0 ENERGY %) DIETS*

Time after ethanol (min)	Low-EFA		Normal-EFA	
	Acute	Chronic	Acute	Chronic
30	32.6 ± 1.20‡	17.9 ± 0.83§	26.3 ± 2.31	14.5 ± 0.73§
60	26.8 ± 0.63‡	13.4 ± 0.92§	16.4 ± 2.73	12.9 ± 0.57
90	16.3 ± 2.31	11.7 ± 0.61	12.5 ± 1.10	11.8 ± 0.59
120	9.6 ± 1.10	10.9 ± 0.76	10.3 ± 0.92	12.3 ± 1.00
180	5.9 ± 1.21	9.9 ± 1.1	7.6 ± 0.70	11.0 ± 0.80§

*A challenge dose of ethanol (3.0 g/kg IP) was given before (acute treatment) and after 22 consecutive daily injections of 3.0 g/kg (chronic treatment). The figures shown are mean values (±SE, N=6) and indicate an animal's performance decrement in percent of pre-treatment score before giving the dose of ethanol. After 180 min tilting angles were not significantly different from control groups of rats not given ethanol.

‡$p<0.05$ compared with the acute treatment in normal EFA rats.

§$p<0.01$ between acute and chronic treatments within each dietary group.

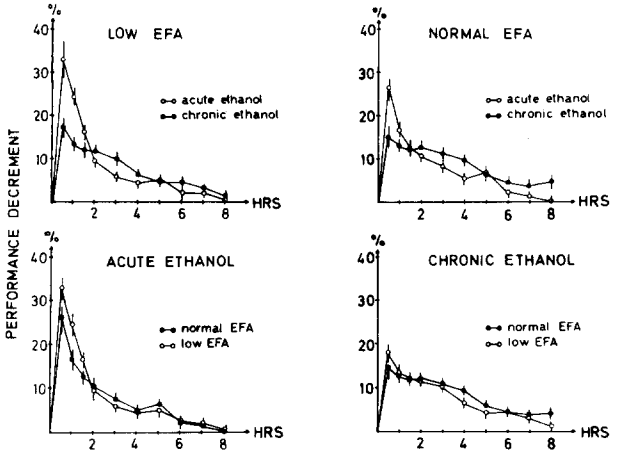

FIG. 3. Effects of ethanol on motor coordination measured with a tilting plane device after 3.0 g/kg IP on day 1 (acute treatment) and after 22 consecutive daily injections of 3.0 g/kg (chronic treatment). Low EFA=0.3 energy %, Normal EFA=3.0 energy %. The figures plotted are mean±S.E. (N=6).

EFA rats the peak BAC was lowered and the time required to reach the peak was longer after chronic treatment. Peak blood ethanol concentrations of about 3.0 mg/ml (65 mmol/l) were reached daily and remained elevated above zero levels for about 8 hr each day.

Ethanol-Induced Impairment

Figure 3 shows the degree of ethanol-induced impairment recorded as performance decrement on the tilting plane. The trends seen were similar in all groups of rats. First, a maximum impairment score develops, corresponding to the peak concentration of ethanol in the blood, second a sharp recovery occurs when the acute stage of intoxication begins to ware off after 60–90 min and third, a more or less curvilinear phase emerges as the blood-alcohol curve begins to decline and ethanol becomes metabolised. After about 3–4 hr there were no significant differences in the performance decrement between different groups and the tilt scores were close to those of control rats not given ethanol.

A significant degree of impairment was obvious at 30, 60, 90 and 120 min after a dose of 3.0 g/kg ethanol in both dietary groups in acute tests and after chronic treatment ($p<0.01$). Both dietary groups showed less impairment after chronic exposure. Rats on low EFA diets were somewhat more impaired than normal EFA rats after the acute treatment but this difference disappeared after chronic administration of ethanol. Table 2 gives a statistical summary of the percent impairment scores seen in these experiments.

DISCUSSION

In this study, Sprague-Dawley rats were fed a low dietary level of essential fatty acid (0.3 energy %) through three generations even though the total dietary fat was nutritionally adequate at 20 energy % in both low EFA and normal EFA groups [7]. The 0.3 energy % EFA is near to the limits necessary to maintain fertility and avoid overt symptoms of EFA malnutrition.

Several new findings have emerged from our experiments. First, chronic ethanol treatment caused the rats on low EFA and normal EFA diets to increase their intake of the diet. Second, low EFA rats were made more impaired than normal EFA rats after an acute dose of ethanol but this difference was no longer evident after chronic treatment. Third, the development of metabolic tolerance was seen in both dietary groups but there were no apparent differences between the two groups.

The effect of ethanol on growth rate was unfortunately confounded during the first part of the study because the rats refused to drink a 5% ethanol solution as their sole drinking fluid. On switching to tap-water ad lib rats in both dietary groups quickly gained weight and when the experiment was terminated they weighed more than control rats treated with isocaloric glucose. The faster rate of growth in the ethanol treated rats is best attributed to their higher consumption of food. It is tempting to speculate that chronic ethanol treat-

ment might increase the turnover of EFA which the rats try to compensate for by eating more dietary EFA. But instead there may have been a depression of food intake and a loss in body weight only during the initial stages of drug action. The accelerated recovery seen later on may simply indicate the development of tolerance [14].

The intake of fluid and output of urine were considerably higher after chronic ethanol treatment. This probably results from ethanol-induced inhibition of the release of vasopressin which causes a water diuresis and the rats counteract for this by drinking more [6]. Note that rats on low EFA diets had lower ratios of urine output to water intake (Table 1) implying a greater insensible loss of body water. This supports the notion that rats in the low EFA group were on the borderline for overt EFA-deficiency. An increased insensible loss of water is typical of this syndrome and so a humid environment is essential for the rats to thrive.

Our low EFA rats were somewhat more sensitive to an acute dose of ethanol although other workers have shown that ethanol-induced sleep-time in mice with an EFA deficiency remained unchanged from controls [15]. It seems likely that motor coordination and sleep-time measure two functionally different aspects of ethanol tolerance where parallels cannot be drawn. The loss of righting reflex and impairment of neuromuscular function in small animals belong to different dose-response curves associated with tolerance [14].

The exposure of rats to ethanol for 23 days caused a functional tolerance in both low EFA and normal EFA dietary groups that cannot be accounted for by differences in peak blood-ethanol concentrations because these were not radically changed by the chronic treatment. But a question still remains about the degree of functional tolerance achieved for low EFA rats on chronic treatment. The peak BAC was lower in this group on final testing ($p<0.05$). Metabolic tolerance was not significantly influenced by differences in dietary EFA between the two groups. A slower absorption of ethanol seen after chronic treatment in both dietary groups may reflect vascular or other changes not directly related to ethanol metabolism.

In a recent biochemical study with EFA-deficient rats we showed that chronic ethanol treatment enhanced markers of EFA deficiency in various tissue phospholipids [4]. In particular, the levels of arachidonic acid (20:4, n-6), an essential fatty acid and a precursor of prostaglandins (PG), were depleted but the concentration of $PGF_{2\alpha}$ excreted in urine was increased. This decreased formation and increased utilization of 20:4, n-6 suggests that chronic ethanol treatment, among other things, may lead to a malfunctioning PG-system.

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