Biochemical Pharmacology, Vol. 28, pp. 2027–2030.

0006-2952/79/0701-2027 $02.00/0

COMPARISON OF ACUTE AND CHRONIC ETHANOL ADMINISTRATION ON RATES OF ETHANOL ELMINATION IN THE RAT *IN VIVO**

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(*Received* 29 *September* 1978; *accepted* 15 *December* 1978)

Abstract—Rats were treated with ethanol employing both acute (one 6.5 g/kg dose or three 5.0 g/kg doses) and chronic (40 days treatment with the Porta diet) models. The magnitude of the increase in the rate of ethanol elimination with all three methods of treatment was similar (52–79 per cent). Furthermore, the increased rate persisted after removal of ethanol for the same time intervals irrespective of the method of treatment. The half-time of the decline was between 20 and 24 hr with all methods. The data are consistent with the hypothesis that increases in ethanol elimination due to acute as well as chronic treatment with alcohol are mediated via similar mechanisms.

An adaptive increase in alcohol metabolism as a result of chronic ethanol intake was described in man and experimental animals over a decade ago [1, 2]. Subsequently, this phenomenon has been studied extensively in liver slices [3, 4] and the perfused liver [5, 6]. In these chronic models, alcohol treatment produces a metabolic alteration which leads to an increase in oxygen uptake by the liver. This, in turn, oxidizes NADH faster, thereby stimulating alcohol dehydrogenase-dependent ethanol oxidation by accelerating the dissociation of the alcohol dehydrogenase–NADH complex [5].

Two recent observations suggest that stimulation of both oxygen uptake and ethanol metabolism may occur very rapidly. First, when one large (6.5 g/kg) dose of ethanol was given to rats *in vivo*, alcohol dehydrogenase-dependent ethanol oxidation was activated [7]. Second, oxygen uptake of the perfused rat liver nearly doubled 2–3 hr following a similar treatment [8, 9].

The purpose of the present study, therefore, was to compare the magnitude and the duration of the increase in ethanol metabolism produced by acute and chronic ethanol treatments. A single dose (6.5 g/kg) of ethanol, three doses (5.0 g/kg) over a 24-hr period, and 40 days of chronic treatment with a diet containing ethanol as the sole source of liquid were compared. The data indicate that all three methods of treatment produce: (1) increases in ethanol metabolism of similar magnitude which (2) persist for the same time intervals when ethanol is removed. This indicates clearly that alcohol can rapidly activate its own metabolism, and suggests that a repeated acute rather than a chronic action of ethanol is responsible for the increase in ethanol metabolism following chronic treatment with ethanol.

* This investigation was supported, in part, by a grant from the National Institute on Alcohol Abuse and Alcoholism (AA-03264) and a grant from the North Carolina Alcoholism Research Authority (ARA-7704).

† Recipient of Research Scientist Career Development Award 2K02-AA 00033.

METHODS

Treatment of animals. Well-fed, female Sprague–Dawley rats (200–300 g) were treated with ethanol by one of three methods. First, rats received a single (6.5 g/kg) large dose of ethanol intragastrically. After blood levels declined to less than 50 mg/100 ml, animals were rechallenged with 2.5 g/kg of ethanol intragastrically, and the rate of elimination (see below) was determined. With the second method, a 5.0 g/kg dose was given intragastrically three times over a 24-hr period prior to a rechallenge with 2.5 g/kg as above. The third method involved treating rats with a diet of 25% (w/v) sucrose containing 20% (w/v) ethanol (the Porta diet) as the only fluid source for 3–5 weeks [10]. Control animals received 25% (w/v) sucrose, and all groups had free access to laboratory chow.

Blood sampling techniques. Duplicate blood samples were collected in heparinized 25 μl capillary pipets from the tip of the tail at times indicated in Fig. 1. The samples were then delivered into 25-ml Erlenmeyer flasks containing 1.0 ml of 25 mM thiourea in 0.6 N perchloric acid and were immediately stoppered with a rubber septum.

Gas chromatographic determination of blood ethanol. The Erlenmeyer flasks containing the blood samples were then incubated in a water bath at 37° for at least 45 min. Subsequently, 1.0 ml of the vapor phase was withdrawn through the rubber septum with a Hamilton gas tight syringe and injected into a Hewlett Packard model 5720A gas chromatograph equipped with a flame-ionization detector. The apparatus contained a 6 ft by $\frac{1}{8}$ in. Poropack Q column. Operating parameters were as follows: oven 175°; detector 250°; injection port 200°; carrier gas flow 40 ml/min. A peak corresponding to ethanol with a retention time of about 2.5 min was compared with ethanol standards.

RESULTS

Comparison of the magnitude of increased ethanol elimination with three methods of ethanol treatment.

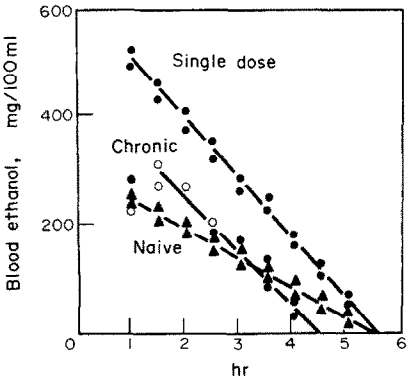

Fig. 1. Effect of acute and chronic pretreatment with ethanol on the ethanol elimination curve in the rat *in vivo*. At time zero, a naive rat or one which had received the chronic diet (Porta) for 40 days was given 2.5 g/kg of ethanol by gastric intubation [20% (w/v) ethanol]. Another rat received 6.5 g/kg of ethanol intragastrically (single dose). Duplicate blood samples were then collected every 30 min for the gas chromatographic determination of ethanol.

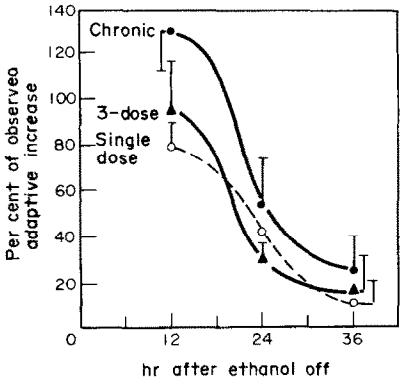

Fig. 2. Loss of accelerated ethanol metabolism following termination of acute or chronic pretreatment with ethanol. Ethanol elimination rates were determined on 48 naive rats. Three groups of 16 were then treated with ethanol to produce the maximal increase in elimination rate by acute (single dose; three doses) or chronic methods (Porta diet). The rate of elimination of each group was then subsequently determined 12, 24 and 36 hr later. Zero time data were set equal to 100 per cent increase over the naive rate (8.5 ± 0.7).

Examples of some of the types of typical data obtained in this study are shown in Fig. 1. The ethanol elimination curve for a typical naive (i.e. the rat had never received ethanol before) rat following 2.5 g/kg of ethanol is depicted. Augmented rates were determined with 2.5 g/kg of ethanol given to animals which had been pretreated with the chronic diet. In addition, rates were also determined following a single dose of 6.5 g/kg (Fig. 1). With both the short-term and long-term treatments, a steeper slope of ethanol elimination was observed (Fig. 1). The increase in the rate of ethanol elimination observed was of similar magnitude with both methods of ethanol treatment (Fig. 1). Although some variability in the rate of ethanol elimination in naive rats (6.9 to 9.5 m-moles/kg/hr) was observed (Table 1), all three methods of treatment produced an increase in the rate of ethanol elimination, ranging from 52 per cent in animals treated with the chronic diet to 79 per cent following one large dose of ethanol (Table 1). The increases produced by the three methods of ethanol administration employed in this study were not statistically different.

In another series of experiments, following the determination of the rate of ethanol elimination in naive rats, a treatment with the chronic diet was carried out for 4 weeks. Subsequently, the rate of ethanol elimination was determined following a dose of 2.5 g/kg (Table 2). After a 3-day rest, the rats were divided into two groups: one received a single 6.5 g/kg dose of ethanol while the other received three 5.0 g/kg doses. When ethanol was eliminated from the blood, both groups

Table 1. Effects of acute and chronic treatment with ethanol on rates of ethanol elimination in rats *in vivo**

Treatment	n	Rate of ethanol elimination (m-moles/kg rat/hr)	Per cent of control
None	30	7.8 ± 0.7†	
24-hr		14.0 ± 1.5	179
None	7	6.9 ± 0.5	
Single dose		11.2 ± 1.6	162
None	14	9.5 ± 1.2	
Chronic		14.5 ± 1.9	152
None	12	8.1 ± 0.5	
Chronic vehicle		7.1 ± 1.8	88

* Rats labeled none received 2.5 g/kg of ethanol intragastrically, and the rate of ethanol elimination was determined. They were treated with ethanol as described in Methods. Chronic vehicle-treated animals received 25% (w/v) sucrose in water as the only source of fluid for 3–5 weeks. After treatments, rates of ethanol elimination were redetermined following a 2.5 g/kg dose of ethanol. None of the increases produced by the various ethanol treatments were statistically significantly different from each other when compared by Student's *t*-test. However, all were different from their respective controls at the 0.01 level. Rates were calculated from data similar to that shown in Fig. 1.

† Mean ± S.E.M.

Table 2. Effects of acute ethanol treatments on elimination of ethanol following prior chronic treatment with ethanol*

Treatment	n	Rate of ethanol elimination (m-moles/kg rat/hr)	Per cent of control
Naive	12	8.8 ± 0.5†	
Chronic	12	14.3 ± 0.8	163‡
Single dose§	5	14.0 ± 0.9	159‡
Three doses§	7	14.0 ± 1.5	159‡

* Rates of ethanol elmination (naive) were determined on twelve rats following a 2.5 g/kg dose of ethanol. All animals were then placed on the Porta diet for 4 weeks. Rates of ethanol elimination were then redetermined with 2.5 g/kg of ethanol (chronic). Animals were subsequently maintained on chow and water for 72 hr. Five rats (single dose) received one dose of 6.5 g/kg ethanol, and the rate of ethanol elimination was determined. The other seven rats (three doses) received the three-dose treatment as described in Methods. They were then retested with a 2.5 g/kg dose of ethanol, and the rate of ethanol elimination was determined.

† Mean ± S.E.M.

‡ Not statistically significantly different from each other; however, all three treatments produced statistically significant changes when compared with the control group by Student's *t*-test ($P < 0.01$).

§ Acute experiments were initiated 72 hr after analysis on the chronic model was completed (see Fig. 2).

were rechallenged with 2.5 g/kg of ethanol, and the elimination rate was redetermined. Neither treatment with a single dose nor three doses of ethanol produced an increase in ethanol metabolism which was different from the increase produced by the chronic treatment (Table 2). In all cases, the increase observed was between 59 and 63 per cent.

Decay of accelerated rate of ethanol elimination due to treatment with ethanol. In another experimental series, rats were given the three different treatments to maximally increase rates of ethanol elimination. Subsequently, each group was subdivided into four sets. The rate of ethanol elimination was then determined 12, 24 and 36 hr after termination of the respective treatment. As can be seen in Fig. 2, the decay of the increased rate of ethanol metabolism was similar irrespective of the method of treatment. With all three methods, the half-time of the decline in rate of elimination following termination of treatment with ethanol was between 20 and 24 hr (Fig. 2).

DISCUSSION

Whether rats were treated for 6 hr (single dose), 24 hr (three doses) or 960 hr (chronic) with ethanol, the "adaptive increase" subsequently measured was of the same order of magnitude (e.g. between 52 and 79 per cent, Tables 1 and 2). When the single dose was compared with the 4-week treatment in the same rats (Fig. 3), an excellent fit via regression analysis was observed ($r = 0.98$; $r^2 = 0.96$). In addition, high correlations (r = greater than 0.9) were also observed when the 24-hr treatment was compared with the single dose, or when the chronic model was compared with the 24-hr treatment. Thus, it is concluded that the "adaptive increase" [3, 5] in ethanol metabolism due to chronic pretreatment with ethanol and the "concentration effect" (i.e. the increase in rates of ethanol metabolism observed at high concentrations of ethanol; [7, 11] observed after one large dose of ethanol may have similar mechanisms. Moreover, these data also suggest that the increases observed in the chronic models are due to repeated acute, rather than chronic, actions of ethanol.

These conclusions are further supported by the observation that the decay in increased ethanol metabolism was also rapid ($t_{\frac{1}{2}}$ = 20–24 hr) and essentially identical with the three methods of treatment studied (Fig. 2). In support of these findings, Mezey [12] showed that the "adaptive increase" was essentially totally reversed 48 hr after the termination of chronic ethanol treatment.

In a number of *in vitro* preparations of liver, including slices [13], isolated cells [14] and perfused organs [11], an acceleration of the rate of ethanol elimination at high ethanol concentrations has been observed. Recently, Wendell and Thurman [7] demonstrated that this swift increase in alcohol metabolism (SIAM) also exists *in vivo*, a finding which has been confirmed here

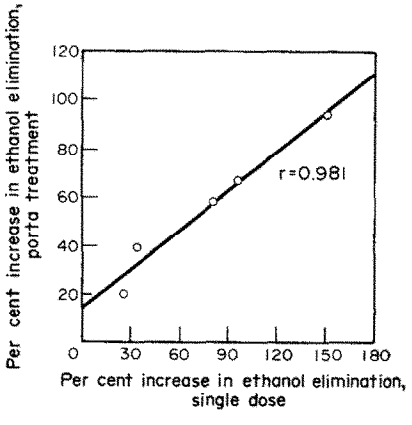

Fig. 3. Correlation between acute and chronic methods of increasing ethanol elimination rate. Data points represent per cent increases for individual rats treated with chronic followed by single dose methods as described in Methods. Polynomial linear regression analysis was performed on a Hewlett Packard model 9830A computer.

(Tables 1 and 2). Because of the speed of this phenomenon (a few hours), it is unlikely that cytochrome P-450, alcohol dehydrogenase or catalase–H_2O_2 levels are altered under these conditions. However, as soon as 2–3 hr after one large dose of ethanol was given to the rat, the oxygen uptake of the ethanol-free perfused liver was nearly doubled [8, 9]. This rapid increase in respiration allows NADH to be reoxidized faster and thereby stimulates the dissociation of the alcohol dehydrogenase–NADH complex. This, in turn, leads to an acceleration of ethanol oxidation. The observation that a large portion of the increase in ethanol metabolism due to a single dose of ethanol can be abolished by the inhibitor of alcohol dehydrogenase, 4-methylpyrazole, is consistent with this hypothesis [7].

Increases in rates of ethanol elimination following chronic treatment with ethanol also appear to involve a similar mechanism since (a) oxygen uptake of liver slices [3, 4] and perfused liver is increased by chronic ethanol treatment [6, 7], (b) the accelerated ethanol uptake is sensitive to 4-methylpyrazole [5, 6] and (c) the phenomenon is observed at low ethanol (1 mM) concentrations, values which are at least one order of magnitude below the K_m of ethanol observed for catalase–H_2O_2 or microsomes [6]. Thus, it appears that the increase in ethanol metabolism following both acute and chronic ethanol treatments involves alcohol dehydrogenase. This suggests that accelerated reoxidation of NADH is responsible for both the "concentration effect" (SIAM) and the "adaptive increase" in ethanol metabolism due to chronic pretreatment with ethanol. Because chronic treatments by their very nature lead to non-specific changes, the acute single dose or 24-hr treatment described in this paper may provide convenient models to examine the mechanism of the ethanol-induced increase in hepatic oxygen uptake.

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