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IN VIVO ETHANOL ELIMINATION IN MAN, MONKEY AND RAT: A LACK OF RELATIONSHIP BETWEEN THE ETHANOL METABOLISM AND THE HEPATIC ACTIVITIES OF ALCOHOL AND ALDEHYDE DEHYDROGENASES.

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Summary.

The in vivo ethanol elimination in human subjects, monkeys and rats was investigated after an oral ethanol dosage. After 0.4 g. ethanol/kg of body weight, ethanol elimination was much slower in human subjects than in monkeys. In order to detect a rise in monkey plasma ethanol concentrations as early as observed in human subjects, ethanol had to be administered at a dose of 3 g/kg body weight. Ethanol metabolism in rats was also much faster than in human subjects. However, human liver showed higher alcohol dehydrogenase activity and higher low Km aldehyde dehydrogenase activity than rat liver. Thus, our data suggest a lack of relationship between hepatic ethanol-metabolizing activities and the in vivo ethanol elimination rate.

The principal site for ethanol elimination in the body is the liver (1, 2). Ethanol is metabolized there by enzymatic conversion into acetaldehyde and subsequently to acetate. When the ethanol concentration is below 20 mM, it is mainly metabolized by alcohol dehydrogenase (E.C. 1.1.1.1) and aldehyde dehydrogenase (E.C. 1.2.1.3).

Previous observations from our laboratory have shown that in subjects displaying alterations of hepatic alcohol dehydrogenase activity, the ethanol elimination rate is unchanged (3). In addition, the ethanol elimination rate is lowered in suckling rats, even when the levels of alcohol dehydrogenase activity are indistinguishable from adult rats (4). In toto, these data cast some doubts on the hypothesis that alcohol dehydrogenase activity might be rate-limiting in the metabolism of ethanol, and, in fact, suggest that in vivo ethanol metabolism is not limited by alcohol dehydrogenase activity.

In the present study, we have investigated the pattern of in vivo ethanol elimination in human subjects, monkeys and rats. We report the existence of large differences as far as their capacity to metabolize ethanol is concerned. In addition, we provide further experimental evidence for a lack of relationship between the hepatic activities of alcohol dehydrogenase and the ethanol elimination rate when these parameters are compared in healthy subjects and rats.

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Methods.

Subjects.

This study was performed on 3 female and 10 male Spanish Caucasian subjects. For determination of alcohol and aldehyde dehydrogenase activities, samples were studied from fresh human liver biopsies taken for diagnosis during abdominal surgery (cholecystectomy) from 9 patients, who were later proved to be histopathologically unaffected. Ethanol administration studies were performed in 4 healthy volunteer controls that had fasted overnight.

Animals.

Wistar female rats from our own colony weighing 180-200 g. were used. Animals were kept in a light cycle- and temperature-controlled room and fed ad libitum with Purina Chow pellets. Adult old World monkeys from our animal headquarters comprising 2 females Macaca sylvanus (barbary macaque), 2 females Macaca mulatta (Rhesus macaque) and 3 males Macaca fascicularis (cynomolgus monkeys) were also used in some studies. Animals were fasted overnight in all studies in order to reduce the first pass metabolism of ethanol (5, 6).

Ethanol administration studies.

In rats, 0.4 g/kg body weight of ethanol (4% w/v, 1 ml/100 g body weight) was given intragastrically by intubation. In men, 0.4 g/kg body weight of ethanol (10% w/v, 4 ml/kg body weight) was given perorally and drinking time was 5 minutes. In monkeys, either 0.4 g/kg body weight ethanol (10% w/v, 4 ml/kg body weight) or 3 g/kg body weight of ethanol (20% w/v, 15 ml/kg body weight) was given by gastric intubation under light pentothal anesthesia and administration time was 5 minutes. Following the ingestion of ethanol to healthy humans, monkeys or rats, blood samples were collected to determine the circulating concentrations of ethanol and acetaldehyde. Blood samples were collected in tubes containing 300 ul of 100 mM chloral hydrate. Ethanol and acetaldehyde determinations were always done in fresh blood samples on the same day they were collected. The von Wartburg and Ris' method (7) was followed with some modifications (3, 8). Immediately after placing blood aliquots in chloral hydrate, proportions were adjusted to 1:1 (vol/vol) by weighing the tubes and adding the appropriate amount of chloral hydrate. After being thoroughly mixed at 4°C and centrifuged at 1,000 g for 15 min, 150 ul of supernatant aliquots were placed in 1 ml glass vials containing 300 ul saline, 500 ul of 1.5 mM 1-propanol (internal standard) and 50 ul of 60% perchloric acid after which the vials were hermetically sealed. External standard vials, containing plasma from untreated individuals or animals supplemented with chloral hydrate and saline and different amounts of ethanol and acetaldehyde, were always run in parallel with blank vials. All vials were subjected to head space gas chromatography performed with a Perkin-Elmer Sigma 15 apparatus and a Carbowax 1540 column. Temperatures were 60°C for the sample thermostat and 150°C for the injector and detector block. With this procedure, the amounts of ethanol and acetaldehyde recovered from fresh plasma samples were respectively 106% and 98%, and detection limit for acetaldehyde was above 1 umol/l. The artifactual formation of acetaldehyde from ethanol present in samples was totally prevented by rapid removal of erythrocytes. The possible loss of acetaldehyde from samples was prevented by early addition of chloral hydrate that inhibits any oxidation catalyzed by erythrocyte aldehyde dehydrogenase during the separation process.

Determination of alcohol dehydrogenase and aldehyde dehydrogenase activities.

Immediately after excision, human and rat liver biopsies were placed in ice-cold 50 mM sodium phosphate buffer at pH 7.4. Samples were homogenized (ratio 1:1,

weight:volume) with a teflon pestle in a glass Potter vessel placed in ice and then sonicated in a MSE sonifier (set at 12 u, for 1 min). This method is effective in disrupting mitochondria and releasing enzymes from the mitochondrial matrix to the medium. Final homogenates were spun at 56,000 g for 20 min at 4°C. The supernatants were then centrifuged at 143,000 g for 60 min, and final supernatants were immediately used for enzymatic assays. Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) activities were assayed on the same day following spectrophotometric methods described by Von Wartburg et al (9) and by Blair and Bodley (10), with a few modifications (3, 11).

Alcohol dehydrogenase was assayed at 25°C in cuvettes containing either 67 mM sodium phosphate (pH 7.4) or 100 mM glycine-NaOH buffers (pH 8.8 or 10.5), 1.3 mM NAD^+ and 0.8-1.0 mg of protein (20 ul of sample) in 3 ml. The reaction was initiated by adding ethanol up to a final concentration of 17 mM. Aldehyde dehydrogenase assay was performed at 37°C in cuvettes containing 100 mM glycine-NaOH, 130 mM KCl, 1 mM pyrazole and 1 mM NAD^+, pH 8.8, and 0.8-1.0 mg of protein in 1 ml. The reaction was started by adding acetaldehyde 0.05, 0.3 or 20 mM (final concentration).

In all the assays, measurements were made with a Beckman DU-8B (Kinetics II) spectrophotometer at 340 nm. One unit of enzymatic activity corresponded to 1 umol of substrate transformed per min. Proteins were determined by the Lowry method (12). Results were expressed as mean $\pm$ SE and statistical comparison among the groups was done by Student's t test.

Results.

Ethanol Metabolism in Human Subjects Compared to Monkeys and Rats.

We initially investigated the ethanol elimination pattern after a single oral administration of 0.4 g ethanol/kg body weight in healthy humans, monkeys (macaques) and rats after 12 hours' fasting (Figure 1). The pattern observed in human subjects was characterized by low ethanol concentrations at 5 min after administration (close to 1 mM) that progressively increased peaking at 30 min after ethanol dosage. Later on, ethanol concentrations decreased slowly and as a result of this, ethanol concentrations were still 5 mM 3 hours after ethanol administration. This pattern was initially compared to monkeys that had also ingested 0.4 g of ethanol per kg of body weight (Figure 1 A). In monkeys, ethanol increased progressively in blood as in human subjects and the maximal levels were obtained at 60 min after ethanol administration. However, this peak was much lower than in healthy human subjects (Figure 1 A). Ethanol levels decreased with time and at 180 min they were close to 0.7 mM and therefore much lower than in human subjects. In these studies, circulating acetaldehyde concentrations were undetectable at all the times studied (data not shown). In order to provide further evidence for faster ethanol metabolism in monkeys than man, 3 g of ethanol per kg of body weight were administered to monkeys and the results were compared with the administration of 0.4 g/kg body weight in human subjects (Figure 1B). Under these conditions, the rise in ethanol concentrations during the first 30 minutes was similar in both groups. However, the ethanol levels in monkeys increased continuously and peaked (approximately 45 mM) 180 min after ethanol administration, whereas in humans they decreased after the 30 min peak (approximately 13 mM).

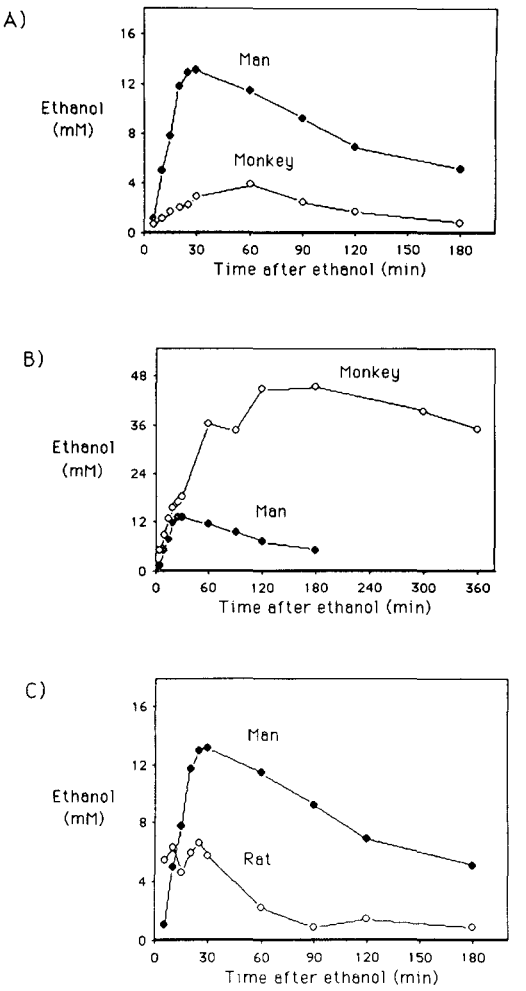

FIGURE 1

CHANGES IN BLOOD ETHANOL CONCENTRATIONS FOLLOWING ORAL INGESTION OF ETHANOL IN HUMAN SUBJECTS, MONKEYS AND RATS.

Results are means±SE of 4-6 observations per group. Human subjects (◆ , A; ◆ , B; ◆ , C), monkeys (◇ , A) and rats (◇ , C) were administered with 0.4 g of ethanol per kg of body weight. Monkeys (◇ , B) also ingested 3 g of ethanol per kg of body weight. A) Differences between human and monkey ethanol levels were statistically significant ($P< 0.05$) at all times investigated except at min 5. B) Differences between human and monkey ethanol levels were statistically significant ($P< 0.05$) at 60, 90, 120 and 180 min of ethanol administration. C) Differences between human and rat blood ethanol concentrations were statistically significant ($P< 0.05$) at all times investigated with the exception of min 10 and 15 after ethanol administration.

The pattern of in vivo ethanol elimination obtained in man was also compared to that found in the rat, the classic animal model in research studies (Figure 1C). In rats, circulating ethanol concentration was high shortly after ethanol administration (higher than 5 mM at 5 min) and stayed constant during the first 30 min after administration. Thereafter, ethanol levels decreased attaining levels of 0.8 mM at 90 min after the ethanol was administered. Rates of in vivo ethanol elimination were calculated from the data shown above by dividing the ethanol dose given by the time required for its elimination. The latter was obtained by extrapolating the linear part of the decay curve to the abcisa. Rates of ethanol elimination were greater in rats, as compared to Macacca and human subjects (Table 1) In fact, rates of ethanol metabolism were the slowest in human subjects.

TABLE 1

RATES OF ETHANOL ELIMINATION FOLLOWING ORAL INGESTION OF ETHANOL IN HUMAN SUBJECTS, MONKEYS AND RATS.

	mg ethanol consumed. kg -1. h-1
Human Subjects	119 ± 12
Monkeys	157 ± 10 *
Rats	266 ± 20 * +

Results are means ± SE of 4-6 observations per group. Human subjects, monkeys and rats were administered with 0.4 g of ethanol per kg of body weight. Rates of ethanol consumption were calculated in individual curves (see Figure 1) by dividing the dose given by the time required for its elimination. The latter was obtained by extrapolation of the linear part of the decay curve to the abscisa. *, indicates a significant difference with the human group, at $P < 0.05$. +, indicates a significant difference between values of monkeys and rats, at $P < 0.05$.

Hepatic alcohol and aldehyde dehydrogenase activities in man and rat.

In order to investigate whether the results of in vivo ethanol metabolism were caused by decreased ethanol-metabolizing capacity, the alcohol and aldehyde dehydrogenase activities were determined in liver biopsies obtained from human subjects and from rats (Table 2). Alcohol dehydrogenase from human subjects and rats was progressively increased by raising the pH from 7.4 to 10.5. At all the pH's investigated not only was alcohol dehydrogenase greater in man than in rats, but the increase in the activity was also quantitatively greater.

TABLE 2

HEPATIC ALCOHOL DEHYDROGENASE ACTIVITY IN HUMAN SUBJECTS AND RATS.

pH	Human Subjects	Rats
	(Units/g. tissue)	
7.4	1.54 ± 0.17	1.00 ± 0.15 *
8.8	4.79 ± 0.55	1.63 ± 0.14 *
9.6	5.46 ± 0.45	2.00 ± 0.36 *
10.5	6.24 ± 0.56	2.72 ± 0.21 *

Results are means ± SE of 5-8 observations per group. Alcohol dehydrogenase activity was assayed in liver extracts at 25 °C, different pH and 17 mM ethanol. * denotes a significant difference between values of human subjects and rats, at $P < 0.05$.

Hepatic aldehyde dehydrogenase activity was also measured in hepatic biopsies from human subjects and rats (Table 3). Aldehyde dehydrogenase was dependent of pH, and activity increased when pH was raised from 7.4 to 8.8. However, a further increase from 8.8 to 9.6 caused a decrease in activity both in human and in rat aldehyde dehydrogenase. Aldehyde dehydrogenase activity was assayed at 0.05 mM acetaldehyde to determine the high affinity component, and at higher acetaldehyde concentrations that resulted in maximal aldehyde dehydrogenases activities (0.3 mM in human liver and 20 mM in rat liver), to assess the low affinity component. Under these conditions, aldehyde dehydrogenase activity, assayed with 0.05 mM acetaldehyde, was greater in human subjects than in rats, and the differences were significant at pH 7.4 (Table 3). Contrarily, maximal aldehyde dehydrogenase activity was significantly greater in livers from rats as compared to human subjects.

Discussion.

The results of the present study demonstrate that the in vivo ethanol elimination rate in man is much slower than that observed in Old-World monkeys and laboratory rats. Thus, after an oral ethanol gavage, blood ethanol remains at greater concentrations and for longer periods of time in humans as compared to the other species studied. In monkeys, ethanol concentrations were always much lower than in man, and in fact much higher doses of ethanol (3 g/kg body weight) were required in order to provoke an initial rise in ethanol comparable to that found in man after 0.4 g/kg. The pattern observed in rats was different; shortly after an intragastric ethanol dosage (5 min), circulating levels of ethanol were much higher than in man, and they stayed relatively stable during 30 minutes before decreasing. Curiously enough, the rate of ethanol elimination in human subjects is comparable to rates previously reported in baboons (13), a species in which ethanol causes hepatic damage. On the contrary, rats and Macacca are species characterized by greater rates of ethanol elimination, in which investigators have failed to show an ethanol-induced severe liver injury.

TABLE 3

HEPATIC ALDEHYDE DEHYDROGENASE ACTIVITY IN HUMAN SUBJECTS AND RATS.

	Human Subjects		Rats	
	Acetaldehyde		Acetaldehyde	
pH	0.05 mM	0.3 mM	0.05 mM	20 mM
	(Units/g. tissue)			
7.4	0.65 ± 0.07	0.87 ± 0.12	0.42 ± 0.05 *	1.99 ± 0.17 *
8.8	1.09 ± 0.14	1.58 ± 0.21	0.84 ± 0.05	2.60 ± 0.14 *
9.6	0.93 ± 0.10	1.18 ± 0.15	0.65 ± 0.12	1.91 ± 0.22 *

Results are means ± SE of 4-13 observations per group. Aldehyde dehydrogenase activity was assayed in liver extracts at different pH, 37 °C and acetaldehyde concentrations of either 0.05 and 0.3 or 20 mM acetaldehyde, in order to differentiate between high and low affinity components. * denotes a significant difference between values from human subjects and rats, at $P < 0.05$.

Therefore, in light of these large differences in the in vivo ethanol metabolism between humans and rats or monkeys, caution must be taken when extrapolating results obtained in experimental animals to the corresponding processes in man.

Ethanol is a lipid-soluble nonelectrolyte molecule that is rapidly absorbed into the circulation by diffusion across the gastric and intestinal mucosa. Therefore, it is difficult to explain the large differences among species in the in vivo ethanol elimination rate on the basis of their intestinal absorption rate. However, it is widely accepted that the major part of ethanol elimination is mediated via the hepatic alcohol dehydrogenase (14). Nevertheless, alcohol dehydrogenase activity was greater in human subjects as compared to rats, as expressed in units of enzymatic activity per gram of tissue. Since the ratio of liver weight/body weight is not very different between humans and rats (15), it seems that there is no correlation between the in vivo ethanol elimination rate and the hepatic enzymatic capacity for ethanol metabolism (that is, alcohol dehydrogenase activity). Whether the microsomal ethanol oxidizing system (MEOS) (16, 17) contributes to any extent to the differences in ethanol elimination rates in man, monkey and rat, remains unknown.

Alcohol dehydrogenase activity per se might not be rate-limiting for human subjects or rats in the ethanol metabolism pathway. Thus, alcohol dehydrogenase activity is already saturated at 10 mM ethanol in human subjects and accounts for more than 220 mg of ethanol consumed per kg of body weight and hour; that is approximately 2-fold greater than the in vivo ethanol elimination rate (Table 1). Previous reports support the concept that alcohol dehydrogenase activity is not rate-limiting in the oxidation of ethanol. Thus, modification of alcohol

dehydrogenase activity in the human liver, as found in atypical alcohol dehydrogenase-bearing subjects, in alcoholic hepatitic patients, or in drinking alcoholics does not lead to parallel alterations in ethanol elimination kinetics (3, 18, 19). In addition, ethanol elimination is decreased in suckling rats, when alcohol dehydrogenase activity is like adult levels (4). It is also known that metabolites which enhance NADH reoxidation in the liver, such as fructose, increase the rate of ethanol metabolism both in vitro and in vivo (20, 21). This has led to the hypothesis that the NADH-oxidizing capacity of the liver might be the rate-limiting factor for ethanol oxidation (14).

In conclusion, results from the present study: a) demonstrate a slow rate of in vivo ethanol elimination in man as compared to monkeys or rats, and b) provide further evidence for the hypothesis that hepatic alcohol dehydrogenase activity is not rate-limiting for the metabolism of ethanol, since there is no correlation between alcohol dehydrogenase activity and the in vivo ethanol metabolism rate in species like humans or rats.

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