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Sex differences, alcohol dehydrogenase, acetaldehyde burst, and aversion to ethanol in the rat: a systems perspective

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[1]*Molecular and Clinical Pharmacology Program, Institute of Biomedical Sciences, Faculty of Medicine;* [2]*Department of Pharmacological and Toxicological Chemistry, Faculty of Chemical and Pharmaceutical Sciences;* [3]*Department of Chemical Engineering and Biotechnology, Centre for Biochemical Engineering and Biotechnology, University of Chile, Santiago, Chile; and* [4]*Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, Pennsylvania*

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Quintanilla ME, Tampier L, Sapag A, Gerdtzen Z, Israel Y. Sex differences, alcohol dehydrogenase, acetaldehyde burst, and aversion to ethanol in the rat: a systems perspective. *Am J Physiol Endocrinol Metab* 293: E531–E537, 2007. First published May 8, 2007; doi:10.1152/ajpendo.00187.2007.—Individuals who carry the most active alcohol dehydrogenase (ADH) isoforms are protected against alcoholism. This work addresses the mechanism by which a high ADH activity leads to low ethanol intake in animals. Male and female ethanol drinker rats (UChB) were allowed access to 10% ethanol for 1 h. Females showed 70% higher hepatic ADH activity and displayed 60% lower voluntary ethanol intake than males. Following ethanol administration (1 g/kg ip), females generated a transient blood acetaldehyde increase ("burst") with levels that were 2.5-fold greater than in males ($P < 0.02$). Castration of males led to *1*) an increased ADH activity (+50%, $P < 0.001$), *2*) the appearance of an acetaldehyde burst (3- to 4-fold vs. sham), and *3*) a reduction of voluntary ethanol intake comparable with that of naïve females. The ADH inhibitor 4-methylpyrazole blocked the appearance of arterial acetaldehyde and increased ethanol intake. Since the release of NADH from the ADH·NADH complex constitutes the rate-limiting step of ADH (but not of ALDH2) activity, endogenous NADH oxidizing substrates present at the time of ethanol intake may contribute to the acetaldehyde burst. Sodium pyruvate given at the time of ethanol administration led to an abrupt acetaldehyde burst and a greatly reduced voluntary ethanol intake. Overall, a transient surge of arterial acetaldehyde occurs upon ethanol administration due to *1*) high ADH levels and *2*) available metabolites that can oxidize hepatic NADH. The acetaldehyde burst is strongly associated with a marked reduction in ethanol intake.

male; female; orchidectomy; arterial; pyruvate; limited access

ETHANOL IS METABOLIZED IN THE LIVER by alcohol dehydrogenase (ADH) into acetaldehyde, a metabolite that is subsequently oxidized into acetate by aldehyde dehydrogenase (ALDH2). Humans who carry a dominant negative mutation of the gene that codes for mitochondrial aldehyde dehydrogenase (33) display a marked elevation in blood acetaldehyde levels following ethanol intake, which curtails further alcohol use (15). Individuals carrying this allele (*ALDH2*2*) are protected by 66 to 99% against alcohol abuse and alcoholism (7, 8, 30, 31). Although it has been suggested that acetaldehyde generated in the brain may be a rewarding metabolite (20), high levels of blood acetaldehyde are aversive, as shown by the marked inhibition of ethanol intake in animals in which peripheral ALDH2 activity is reduced and blood acetaldehyde levels are increased by antisense drugs that do not penetrate the blood-brain barrier (6).

Individuals carrying an *ADH* allele *(ADH1B*2)* that codes for an isoform that is one order of magnitude more active than that coded by the usual *ADH1B*1* (9) are also protected against heavy alcohol use and alcoholism (26; see also meta-analysis, Ref. 34). The highly active ADH is found in some East Asians and Ashkenazi Jews. The steady-state rate of ethanol metabolism in individuals carrying the *ADH1B*2* allele is only 8–13% greater than that of subjects carrying *ADH1B*1* (18), indicating that in humans the maximal activity of ADH is far from fully expressed in vivo and showing that other factors limit its full expression. Since there have been no reports on acetaldehyde levels being elevated in *ADH1B*2* allele carriers, the mechanism of protection against alcoholism of the fast ADH(s) is not clear. The same uncertainty applies for two other fast ADH alleles (*ADH1C*1* and *ADH1B*3*) that are also protective against alcoholism and found in some Caucasians and Africans, respectively (34).

It should be noted that the amount of acetaldehyde leaving the liver into the blood reflects the difference between the rate of acetaldehyde generation minus that of the oxidation of this metabolite rather than the absolute rate of ethanol elimination. Furthermore, acetaldehyde appearing in blood constitutes only a minor fraction of metabolized ethanol. In vitro studies have shown that, under saturating concentrations of NAD^+, the generation of acetaldehyde by ADHs is limited not only by the amount of enzyme but also by the rate of dissociation of reduced nicotinamide adenine dinucleotide (NADH) from the enzyme·NADH complex (4, 29). Since prior to ethanol ingestion the levels of NADH in the liver are low, the maximum catalytic activity of ADH and the maximal rate of acetaldehyde generation are expected to occur shortly after ethanol intake. Thus, the release of acetaldehyde into the blood will depend on the ability of ALDH2 to metabolize such an acetaldehyde surge before it escapes into the blood. Unlike ADH activity, ALDH2 activity is not limited by the release of NADH from the enzyme (17).

In addition to the existence of low hepatic NADH levels prior to ethanol intake, other peripheral organs release NADH-oxidizing substrates (e.g., pyruvate) that are made available to the liver via the blood and can lower hepatic NADH levels (e.g., via lactate dehydrogenase). We propose that a transient surge of blood acetaldehyde (burst) will occur when the max-

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imum initial activity of ADH significantly exceeds that of ALDH2. It has been reported (21) that a low-activity ALDH2 leads to a transient burst in arterial acetaldehyde shortly after ethanol administration, leading to a low voluntary ethanol intake. In line with the above, we hypothesize that *1*) a high ADH activity will result in a blood acetaldehyde burst in pre-steady-state conditions, *2*) an acetaldehyde burst can be induced by the administration of NADH-oxidizing metabolites (e.g., pyruvate), and *3*) the acetaldehyde burst leads to an aversion to ethanol.

Rats of the Wistar strain and of some Sprague-Dawley lines (3, 23, 27) show marked sex differences in the levels of hepatic ADH activity, being up to 100% higher in females than in males (27). The studies described here, performed in naïve Wistar-derived rats selectively bred as high ethanol drinkers, show that the high hepatic ADH activity observed in females leads to an arterial acetaldehyde burst shortly after ethanol administration, a burst that is strongly associated with a low voluntary ethanol intake. An acetaldehyde burst and reduced ethanol drinking can also be elicited in male rats subjected to castration. These studies provide a possible explanation for the lower consumption of ethanol in individuals who present the high-activity ADH isozymes. Humans carrying the fast ADH alleles can become alcoholics following prolonged alcohol intake. In line with the above, we show that tolerance to the aversive effect is indeed experienced by animals following chronic intake. From a systems biology perspective, our studies further suggest that the capacity of extrahepatic organs to make NADH-oxidizing substrates available to the liver play a role in developing the acetaldehyde burst and an aversion to ethanol intake.

MATERIALS AND METHODS

Animals. Alcohol-naïve 12- to 13-wk-old male and female rats of the high-alcohol-drinking UChB line were used. This line, derived from the Wistar strain, has been bred at the University of Chile for more than 70 generations (22). The animals were housed in individual cages under a 12:12-h light-dark cycle and had free access to water and food. All studies were conducted with naïve animals. No animals were removed from the study, and no mortality occurred. The number of animals varied in different experiments aiming at a high statistical significance (number of animals is described in the figure legends). The protocols were approved by the Institutional Review Board (Ethics Committee for Experiments with Laboratory Animals) of the University of Chile Medical School (Protocol CBA no. 087, FMUCH) and the Commission of the Science and Technology Research Council.

Orchidectomy. Castration was performed in 4- to 5-wk-old male UChB rats. Animals were anesthetized with ethyl ether, and a small median incision of ~1 cm was made at the tip of the scrotum. A smaller, 5-mm incision was made into each sac at its tip, vessels were ligated, and both testes were removed. Each incision was closed with a single suture before the skin was closed with one or two small skin clips. The rats were returned to their individual cages for 8 wk before the experiments were conducted.

Limited-access drinking. Alcohol consumption in the 1-h limited access condition was assessed in adult (12- to 13-wk-old) naïve males, castrated males, and naïve females of the UChB line. Rats were weighed and placed in individual cages where food and water from a graduated tube were always available. The limited-access availability was conducted in the light phase between 2 and 3 PM by offering an alcohol solution in 10-ml Richter-like tubes. Animals had a 1-h choice between 10% ethanol and water every day as indicated. The amount of alcohol consumed over the 1-h access period was recorded to the nearest 0.1 ml. In initial studies it was noted that, during the 1-h ethanol drinking session, rats consumed only a negligible amount of water, which made its determination so unreliable that it was not registered.

Determination of ethanol consumption after 4-methylpyrazole administration. Alcohol-naïve female UChB rats were given an intraperitoneal dose of 10 mg/kg of 4-methylpyrazole (4MP; Sigma-Aldrich, Atlanta, GA) from a 4 mg/ml solution 30 min before the 1 h of limited access to ethanol began.

Determination of ethanol consumption after pyruvate administration. Alcohol-naïve male UChB rats were given 600 mg/kg of sodium pyruvate intraperitoneally (Sigma-Aldrich) from a 60 mg/ml solution, adjusted to pH 7.4, 5 min before the beginning of the ethanol-drinking session.

Blood acetaldehyde levels. To determine the arterial acetaldehyde levels, ethanol (20% solution in saline) was administered intraperitoneally at a dose of 1 g/kg. Acetaldehyde levels were measured at 5, 10, 15, and 30 min after ethanol administration in samples of whole blood obtained from the carotid artery of rats anesthetized with a 60 mg/kg dose of ketamine hydrochloride (Laboratorio Richmond, Buenos Aires, Argentina) administered intramuscularly to animals sedated with 2 mg/kg acetopromazine (Drug-Pharma Invetec, Santiago, Chile). The blood samples (0.1 ml) were collected without anticoagulants and were immediately added to 0.9 ml of distilled water at 4°C in glass vials sealed with Mininert valves (Supelco, Bellefonte, PA). Samples were kept on ice for ≤15 min before determination of acetaldehyde, for which the diluted blood samples were incubated 15 min at 60°C, and 1.0 ml of the gas phase was removed and analyzed by head space gas chromatography (PerkinElmer SRI 8610). Nitrogen was used as the carrier gas at 65 ml/min through a stainless steel column packed with 5% Carbowax 20M on 60/80 Carbopack at an oven temperature of 75°C and detected by flame ionization. At no time were samples frozen nor proteins precipitated. Care was taken not to denature (foam) hemoglobin. Control solutions (0.1 ml) containing either 100 or 200 mg/dl ethanol in water were added to 0.9 ml of 1:9 diluted blood samples of animals that did not receive ethanol in vivo (thus the highest concentration of ethanol in the head space vial was 20 mg/dl), kept up to 45 min at 4°C, and then analyzed by head space as indicated above. No acetaldehyde was detected in the blood of naïve animals when ethanol was added in vitro prior to the gas chromatography analysis. Standard curves were conducted using analytical grade acetaldehyde (Merck, Darmstadt, Germany).

Subcellular fractionation of rat liver. Subcellular fractionation of rat liver was performed as previously reported (12), with some modifications. In brief, adult alcohol-naïve male, castrated male, and female UChB rats were killed by decapitation and the livers removed immediately. Livers were homogenized in 5 volumes of 0.25 M sucrose, 5 mM Tris, 0.5 mM EDTA, and 2 mM 2-mercaptoethanol (pH 7.2). All steps were carried out at 0–4°C. The homogenate was centrifuged for 10 min at 1,000 *g* and the supernatant further centrifuged for 10 min at 10,000 *g*, discarding both the nuclear and mitochondrial pellets.The supernatant was centrifuged at 48,000 *g* for 1 h, and the resultant supernatant comprising the cytosolic fraction was used for ADH determinations. Protein concentration of the cytosolic fraction was determined in duplicate by the method of Lowry, using bovine serum albumin as a standard.

Alcohol dehydrogenase assay. The activity of hepatic ADH was determined spectrophotometrically by the increase in absorbance at 340 nm of NADH generated from NAD^+ (Sigma-Aldrich). The assay was performed in duplicate in a final volume of 3.0 ml at 37°C in glycine buffer (0.1 M glycine, adjusted to pH = 9.5 with NaOH) containing NAD^+ (0.625–5 mM for K_m and V_{max} determinations) and the cytosolic fraction corresponding to 1 mg of protein. After 5 min of equilibration, the reaction was initiated by the addition of 2.5 mM ethanol. No differences in K_m were observed in males vs. females or in castrated males. A blank cuvette containing 1 mM 4MP instead of

ethanol was run simultaneously, and the results were corrected for blank reactions. It is noted that the supernatant employed to determine ADH activity was prepared with 2 mM mercaptoethanol. The final concentration of mercaptoethanol in the ADH reaction mixture was 0.09 mM. We have shown that, within the limits of error of the method (±5%), this concentration of mercaptoethanol does not alter the ADH activity recorded for either male or female rats.

Determination of ethanol concentration in tail blood. Samples of tail blood (0.1 ml) were collected from the tip of the tail after 5, 10, 15, 30, 60, 120, and 180 min following the intraperitoneal administration of ethanol (1 g/kg ip). Ethanol (Merck, Darmstadt, Germany) was determined by head space gas chromatography using *n*-propanol (Merck, Darmstadt, Germany) as internal standard.

Statistical analyses. Data are expressed as means ± SE. When required, significance of the difference was analyzed by ANOVA with repeated measures for the time factor.

RESULTS

Figure 1 shows the activity of liver ADH in male and female Wistar-derived (UChB) rats. Females displayed a 67% ($P < 0.001$) higher liver ADH activity than males. Castration of males led to marked increases in hepatic ADH ($P < 0.001$) to levels that were virtually identical to those in naïve females (Fig. 1). Upon the administration of ethanol (1 g/kg ip), naïve female rats showed a marked transient increase (burst) in blood acetaldehyde levels, which were 2.5-fold higher than those observed in males, mainly 5, 10, and 15 min ($P < 0.001$) after ethanol administration (Fig. 2). This marked difference was observed despite the fact that, at these early times, blood ethanol levels were virtually identical for both sexes, whereas differences in the rate of ethanol elimination were observed only at later times (Fig. 3). Arterial acetaldehyde levels remained slightly elevated in females, in line with a greater rate of ethanol metabolism (Fig. 2). When male and female rats of this alcohol-preferring line were offered a 10% ethanol solution for 1 h, males consumed two to three times more ethanol than females (ANOVA $P < 0.001$), showing a relative aversion for ethanol in the latter (Fig. 4). After 14 days of daily ethanol intake, tolerance to ethanol is seen in females such that alcohol intake was similar in males and females (see DISCUSSION).

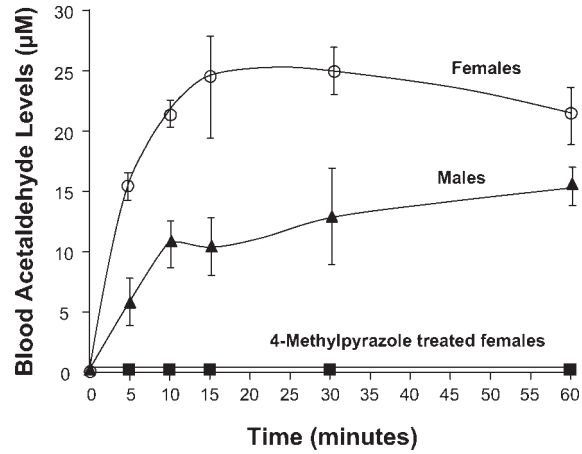

Fig. 2. Sex differences in arterial blood acetaldehyde levels following the administration of ethanol (1 g/kg ip) in alcohol-naïve male, female, and 4-methylpyrazole-treated female rats. Each point represents the mean ± SE of 5 animals/group. Differences between male and female alcohol-naïve rats: $F(1,29) = 38.29$, $P < 0.001$. Difference between female and 4-methylpyrazole-treated female rats: $P < 0.001$.

We further determined whether the ADH inhibitor 4MP, which fully blocked any increase in blood acetaldehyde, increased alcohol intake in females. As seen in Fig. 5, administration of 4MP increased two- to threefold the intake of 10% ethanol in female rats ($P < 0.002$). Arterial acetaldehyde levels in female rats treated with 4MP and given 1 g ethanol/kg (ip) were zero at 15, 30, 45, and 60 min (see Fig. 2).

As shown earlier (Fig. 1), castration increased hepatic ADH activity in males to levels comparable to those in females; thus, it was expected that an "acetaldehyde burst" would be generated in castrated males upon ethanol administration and that voluntary ethanol intake would also be reduced by castration. Data in Figs. 6 and 7 are in line with such a postulate. Following ethanol administration, acetaldehyde levels increased three- to fourfold in castrated males ($P < 0.01$), an effect seen most clearly 5–10 min following the administration of ethanol (Fig. 6). Castration also led to a 60% reduction in

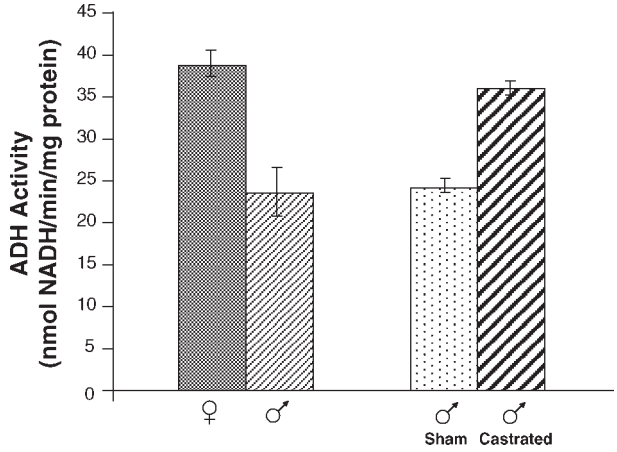

Fig. 1. Liver alcohol dehydrogenase (ADH) activity in naïve female and male rats (*left*) and sham-operated male and castrated male rats (*right*). Each point represents the mean ± SE of 4 animals/group. Difference between males and females and difference between castrated males and sham-operated males: $F(3,15) = 28.96$, $P < 0.001$.

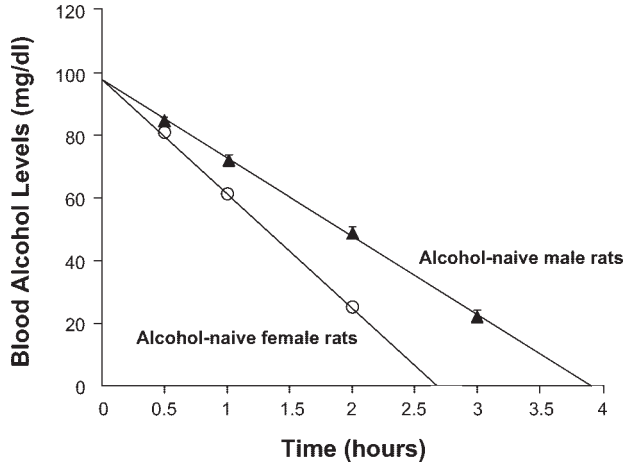

Fig. 3. Blood ethanol concentrations in alcohol-naïve male and female rats following the administration of 1 g/kg ethanol. Each point represents the mean ± SE. Rates of ethanol metabolism were 243.0 ± 3.1 $mg \cdot kg^{-1} \cdot h^{-1}$ for males and 366.5 ± 5.9 for females ($n = 4$ animals/sex).

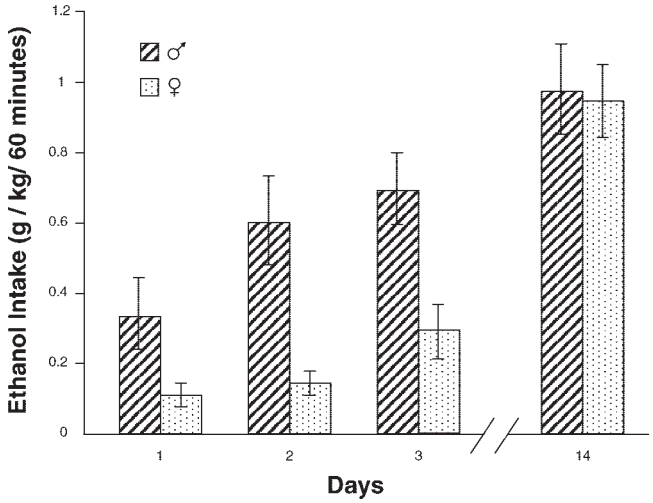

Fig. 4. Sex differences in alcohol intake during the initial days of daily 1-h access to a 10% (vol/vol) alcohol solution and subsequent tolerance. Each point represents the mean ± SE of 13 animals/sex [F(1,77) = 27.65, $P <$ 0.001] for *days 1–3* and 9 animals for *day 14* (not significant).

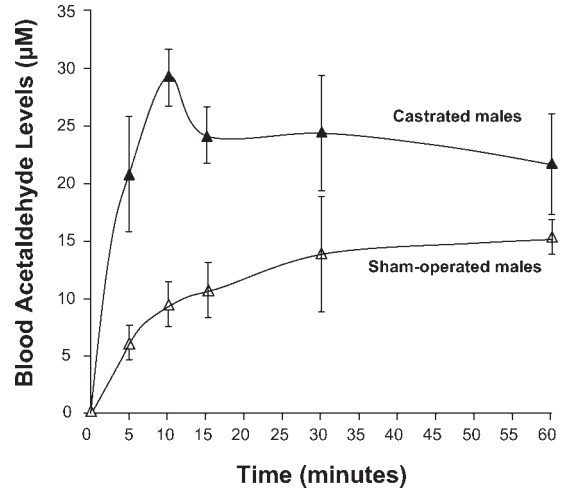

Fig. 6. Effect of castration on blood acetaldehyde levels following the administration of a dose of ethanol (1 g/kg ip) in male rats. Each point represents the mean ± SE of 5 animals/group. Overall difference between castrated (n = 5) and sham operated males (n = 5): F(1,49) = 23.89, $P < 0.01$. Values at 60 min are not significantly different.

alcohol preference in males ($P <$ 0.01; Fig. 7) to levels comparable with those in females.

Several compounds able to oxidize hepatic NADH circulate in blood and can enter hepatocytes; one of these is pyruvate, which in the presence of NADH is readily transformed into lactate by the action of lactate dehydrogenase. The equilibrium and the rate of this reaction greatly favor the oxidation of NADH by pyruvate and the generation of lactate and NAD^+ (16, 25). Thus, we tested the hypotheses that pyruvate administration would lead to an acetaldehyde burst upon ethanol administration even in males, having low ADH activity, and that the effect would lead to a reduction in ethanol consumption in these animals. Data in Fig. 8 show that a marked, short-lived acetaldehyde burst developed in males that were administered sodium pyruvate (600 mg/kg, equivalent to 5.45 mmol/kg) given virtually simultaneously (5 min earlier) with ethanol (1 g/kg equivalent to 21.7 mmol/kg). Administration of pyruvate in the absence of ethanol did not yield acetaldehyde (data not shown). Pyruvate administered 5 min prior to the 1-h access to the alcohol solution rendered ethanol greatly aversive, as seen by a marked reduction (−85%, $P <$ 0.001) in ethanol intake (Fig. 9).

DISCUSSION

The acetaldehyde burst. The major finding presented in these studies is that a high ADH activity augmented by the oxidized initial condition of the liver results in a short-lived increase in arterial acetaldehyde levels after ethanol administration. Such an effect, referred to as an acetaldehyde burst, indicates that, in the pre-steady-state period of ethanol oxidation, acetaldehyde generation markedly exceeds its removal. We propose that the greater hepatic oxidation of ethanol into acetaldehyde in the pre-steady-state condition occurs because

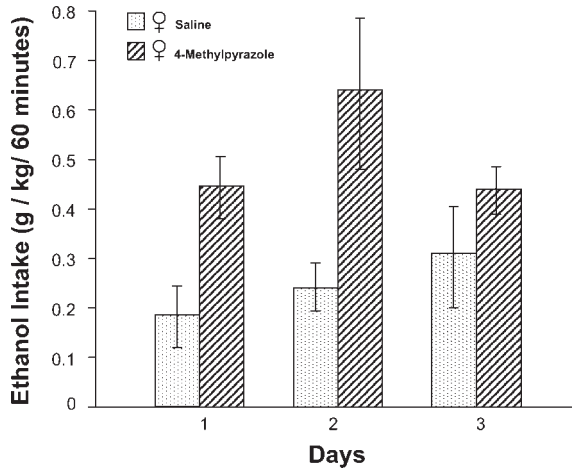

Fig. 5. Effect of 4-methylpyrazole (10 mg/kg ip) on alcohol intake during the 1-h access to 10% (vol/vol) ethanol in alcohol-naïve female rats. Each point is the mean ± SE of 9 animals for the pyrazole group and 5 saline-treated animals. Differences between saline and 4-methylpyrazole-treated female rats: F(1,44) = 8.31, $P <$ 0.006.

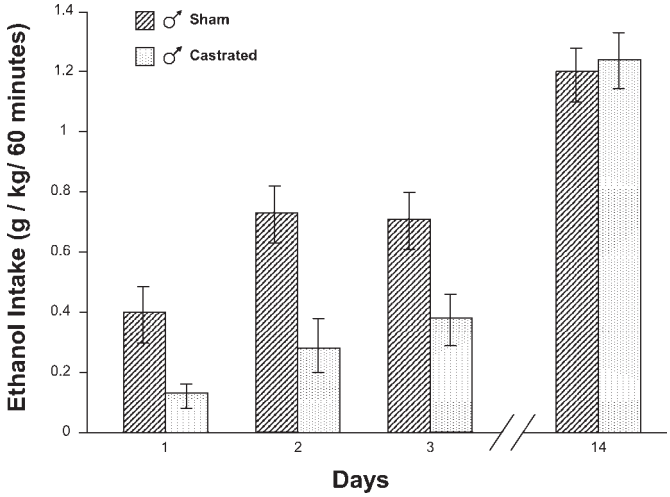

Fig. 7. Effect of castration on ethanol intake during the 1-h access to 10% (vol/vol) alcohol. Each point represents the mean ± SE of 5 animals/group. Differences (*days 1* to *3*) between castrated male and sham-operated male rats: F(1,29) = 8.61, $P <$ 0.01.

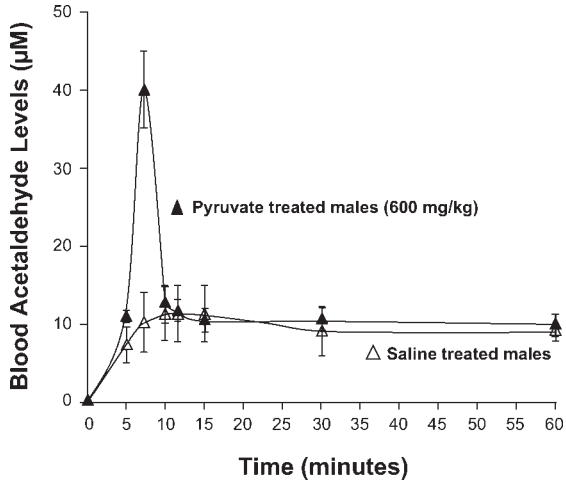

Fig. 8. Effect of pyruvate (600 mg/kg ip) on arterial blood acetaldehyde levels induced by a dose of ethanol (1 g/kg ip) in male rats. Sodium pyruvate was administered 5 min before ethanol. Significant difference in the acetaldehyde burst at 7 min between control and pyruvate-treated animals ($n = 4$/group): t-test, $P < 0.001$.

hepatic NADH levels are initially low, which allows the maximal catalytic activity of ADH. This postulate is supported by the fact that pyruvate, which can readily reoxidize NADH, generates an acetaldehyde burst even in males in which ADH levels are low. Pyruvate administered exogenously is quickly metabolized by many tissues; thus, its effect is expected to be short lived. Both endogenous pyruvic and lactic acids, being weak acids, cross membranes and reach the liver and other tissues. The normal concentration of pyruvate in serum is 50–100 μM (11), exceeding that normally found for acetaldehyde. The pyruvate plus NADH reaction is one order of magnitude more displaced toward the oxidation of NADH than the acetaldehyde plus NADH reaction (25, 28). Furthermore, the activity of liver cytosolic lactate dehydrogenase exceeds by several orders of magnitude that of ADH (1, 16). Even in the presence of ethanol, which dampens glycolysis, the glycolytic flux and thus the availability of pyruvate exceeds the rate of ethanol metabolism (1, 13). Thus, from a systems biology point of view, the alcohol burst should depend not only on the pyruvate generated in the liver itself but also on the ability of other organs to contribute pyruvate (and other NADH-oxidizing metabolites) to the liver. Indeed, upon ethanol metabolism, the lactate/pyruvate ratio of body tissues and plasma increases by 100 to 400% (10, 32). Clearly, ethanol metabolism imposes a reduced state not only in the liver but also in other organs in the body by the export of the reduced substrates (e.g., lactate) from the liver to the periphery. Thus, part of the oxidizing capacity of the liver to metabolize ethanol likely involves both the pre-steady-state levels of pyruvate as well as the subsequent capacity of different organs to reconvert lactic acid into pyruvate and to export it back to the liver and is therefore dependent on the overall metabolic activity of the animal. A mathematical description will be presented separately.

Data in the present study show that when hepatic ADH levels are low, as in males, the capacity of acetaldehyde removal exceeds the high pre-steady-state generation of acetaldehyde. However, when males were administered pyruvate exogenously, a marked, short-lived acetaldehyde burst was seen upon ethanol administration. An acetaldehyde burst in males in the presence of the endogenous physiological levels of NADH-oxidizing metabolites was also observed following castration, which increased liver ADH activity. Conversely, in females the administration of 4MP, which inhibits ADH activity, blocked any appearance of arterial acetaldehyde. Data also indicate that the administration of pyruvate preferentially activated the generation of acetaldehyde over its oxidation into acetate. This is understandable, since the release of NADH from the ADH·NADH complex constitutes the rate-limiting step of ADH turnover (4, 29), whereas it is not the case for ALDH2, for which the rate-limiting step is the release of the acyl group from the ALDH·acetate complex (17). We have also observed that the K_i of NADH for ADH is not different in male and female rats (data not shown).

We wish to emphasize several aspects with regard to the sampling of blood and the method used to analyze acetaldehyde in the blood samples. First, arterial rather than venous blood was extracted. Venous blood, after having perfused several tissue beds, may have greatly reduced or no acetaldehyde levels (24). When sampling from the carotid artery, the only major organ of acetaldehyde metabolism or exchange is the lung. Second, the method we used avoids the artifactual formation that occurs when hemoglobin is denatured and iron is oxidized by acid precipitation and vigorous mixing with air (24). Furthermore, when 4MP was administered to animals given ethanol (1 g/kg), the acetaldehyde in blood was zero (vide infra), thus again showing that spurious in vitro formation of acetaldehyde does not exist in our experimental conditions. Our analyses of arterial acetaldehyde were conducted within 30 min of sampling, and blood samples were neither frozen nor precipitated.

The acetaldehyde burst as deterrent of ethanol intake. From the above, an acetaldehyde burst is proposed to occur when ADH turnover is temporarily higher than the capacity of ALDH2 to remove this metabolite. This can occur when ADH activity is increased but also, as shown earlier, when ALDH2 activity is marginally reduced (21). In the present study it was

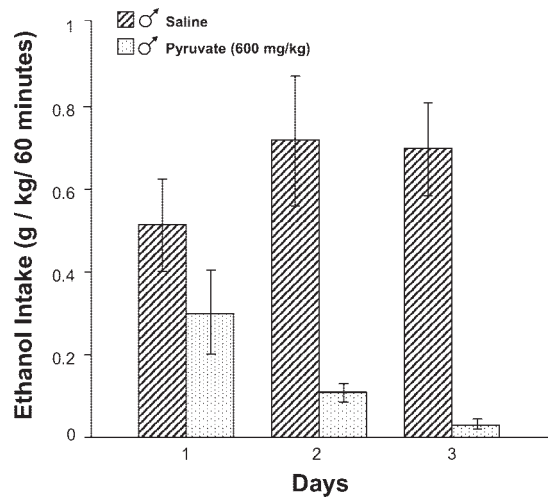

Fig. 9. Effect of pyruvate on ethanol intake during 1-h access to ethanol 10% (vol/vol) in alcohol-naïve male rats. Each point is the mean ± SE of 4 animals/group. Sodium pyruvate (600 mg/kg ip) was administered 5 min before ethanol access. Significant differences between pyruvate-treated and control male rats: $F(1,23) = 26.11$, $P < 0.001$.

shown that the acetaldehyde burst can be changed according to *1*) sex, *2*) castration, *3*) administration of an NADH-oxidizing agent, or *4*) administration of an inhibitor of ADH, all in directions consistent with the above postulate. In all conditions an acetaldehyde burst was directly related to a lower ethanol intake, strongly suggesting that the high systemic acetaldehyde in the burst generates or initiates a relative aversion to ethanol. In this context, the term aversion is used to reflect the acetaldehyde-mediated mechanism responsible for the reduction in ethanol ingestion, much as the term "taste aversion" reflects the deterrence mechanism that leads an animal to reduce the intake of a certain fluid or food.

Although the mechanism by which a fast ADH activity in humans protects against alcoholism is not clear, a mechanism similar to the one reported here is conceivable. Whereas studies agree that in the rat, under steady-state conditions, 50–60% of the maximal capacity of ADH is used to oxidize ethanol (2, 19), in humans the maximal activity of ADH does not appear to constitute a major rate-limiting step in the metabolism of ethanol. This is seen in well-conducted studies showing that the rate of ethanol metabolism is only minimally changed or is unchanged in individuals who carry the *ADH1B*2* allele (5, 18), encoding an ADH that in vitro has a maximum activity that is one order of magnitude greater than that encoded by *ADH1B*1* (9). From the present studies in animals, a higher ADH activity is expected to result in a greater pre-steady-state acetaldehyde burst. The mechanism in humans and rats may, however, be different while leading to the same overall effect. In humans partially protected against alcoholism by the ADH1B*2 variant, the single amino acid change generates an ADH having a 10-fold higher K_m for NAD^+ and a 30-fold lower K_i (K_d) for NADH than those for the more common ADH1B*1 (9). Thus, for individuals carrying the *ADH1B*2* allele, NAD^+ availability to ADH rather than NADH removal from the ADH·NADH complex is more likely to be rate limiting in the pre-steady-state condition. Nevertheless, the oxidation reaction of NADH to NAD^+ by endogenous pyruvate is expected to occur for both ADH1B*1 and ADH1B*2, likely leading to a higher acetaldehyde burst with the latter. In line with this view, it has been reported recently (5) that the effects of ethanol are perceived more strongly shortly after ethanol administration by humans carrying the *ADH1B*2* allele than by individuals carrying the *ADH1B*1* allele.

A finding we wish to leave on record for further exploration is the observation that, following prolonged (15 days) daily exposure to ethanol on the limited access paradigm, female rats developed tolerance to ethanol and consumed as much ethanol as males. It should be noted that the protection against alcoholism conferred by the *ADH1B*2* allele does not generally exceed 50% and that individuals who carry this allele and consume ethanol in excess do become alcoholics. In the alcohol-seeking animal line we studied in the present work, acute central nervous system (CNS) tolerance elicited by the single acute administration of ethanol predicts subsequent increases in ethanol intake when ethanol is available on a daily basis (see Ref. 22). Whether the tolerance observed is due to a direct CNS effect or is initiated by peripheral changes deserves further investigation. To our knowledge, this is the first demonstration that a pre-steady-state elevation in arterial acetaldehyde levels resulting from an elevated ADH activity is associated with deterrence from ethanol intake.

Overall, data presented herein present convergent evidence showing that the generation of an acetaldehyde burst depends both on ADH activity and on the time-limited capacity of the liver to oxidize NADH and is strongly associated with an aversion to ethanol.

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