The UChA and UChB rat lines: metabolic and genetic differences influencing ethanol intake

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ABSTRACT

Ethanol non-drinker (UChA) and drinker (UChB) rat lines derived from an original Wistar colony have been selectively bred at the University of Chile for over 70 generations. Two main differences between these lines are clear. (1) Drinker rats display a markedly faster acute tolerance than non-drinker rats. In F_2 UChA × UChB rats (in which all genes are 'shuffled'), a high acute tolerance of the offspring predicts higher drinking than a low acute tolerance. It is further shown that high-drinker animals 'learn' to drink, starting from consumption levels that are one half of the maximum consumptions reached after 1 month of unrestricted access to 10% ethanol and water. It is likely that acquired tolerance is at the basis of the increases in ethanol consumption over time. (2) Non-drinker rats carry a previously unreported allele of aldehyde dehydrogenase-2 (*Aldh2*) that encodes an enzyme with a low affinity for Nicotinamide-adenine-dinuclectide (NAD^+) ($Aldh2^2$), while drinker rats present two *Aldh2* alleles ($Aldh2^1$ and $Aldh2^3$) with four- to fivefold higher affinities for NAD^+. Further, the ALDH2 encoded by $Aldh2^1$ also shows a 33% higher Vmax than those encoded by $Aldh2^2$ and $Aldh2^3$. Maximal voluntary ethanol intakes are the following: UChA $Aldh2^2/Aldh2^2 = 0.3$–0.6 g/kg/day; UChB $Aldh2^3/Aldh2^3 = 4.5$–5.0 g/kg/day; UChB $Aldh2^1/Aldh2^1 = 7.0$–7.5 g/kg/day. In F_2 offspring of UChA × UChB, the $Aldh2^2/Aldh2^2$ genotype predicts a 40–60% of the alcohol consumption. Studies also show that the low alcohol consumption phenotype of $Aldh2^2/Aldh2^2$ animals depends on the existence of a maternally derived low-activity mitochondrial reduced form of nicotinamide-adenine-dinucleotide (NADH)-ubiquinone complex I. The latter does not influence ethanol consumption of animals exhibiting an ALDH2 with a higher affinity for NAD^+. An illuminating finding is the existence of an 'acetaldehyde burst' in animals with a low capacity to oxidize acetaldehyde, being fivefold higher in UChA than in UChB animals. We propose that such a burst results from a great generation of acetaldehyde by alcohol dehydrogenase in pre-steady-state conditions that is not met by the high rate of acetaldehyde oxidation in mitochondria. The acetaldehyde burst is seen despite the lack of differences between UChA and UChB rats in acetaldehyde levels or rates of alcohol metabolism in steady state. Inferences are drawn as to how these studies might explain the protection against alcoholism seen in humans that carry the high-activity alcohol dehydrogenase but metabolize ethanol at about normal rates.

Keywords Alcohol consumption, ethanol intake, UChA and UChB rats, acetaldehyde, rat lines, genetic differences.

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INTRODUCTION

Numerous studies have shown that the development of alcoholism is influenced by permissive and protective genetic factors. Among the genes associated with high alcohol intake in animals appear those that reduce the tone or the synthesis of dopamine (El-Ghundi *et al.* 1998; Thanos *et al.* 2001), serotonin (Engel, Lyons & Allan 1998), endorphin (Hall, Sora & Uhl 2001), γ-amino butyric acid (Blednov *et al.* 2003) and neuropeptide Y (Thiele *et al.* 1998). In contrast, only two genes have been shown to have a strong protective effect in humans: (1) the gene coding for a slow form of aldehyde dehydrogenase (ALDH2); and (2) that coding for a fast form of alcohol dehydrogenase (ADH) (Thomasson *et al.* 1991; Higuchi 1994; Tu & Israel 1995; Chen *et al.* 1999; Chambers *et al.* 2002; Neumark *et al.* 2004). It was recently reported that the gene for ALDH2 also modulates alcohol intake in animals (Quintanilla *et al.* 2005a,b).

At the outset, it is important to indicate that animal lines selectively bred over many generations as

high-drinking lines will *both* accumulate 'permissive' genes and exclude 'protective' genes. Conversely, non-drinker animals will accumulate protective genes and exclude permissive genes. Thus, it is unlikely that a single gene will fully determine the difference between two related lines of drinker and non-drinker animals. Another important aspect is that some genetic polymorphisms irrelevant to alcohol intake may seem to be associated to a drinker or non-drinker line because they may have traveled along with genes that do influence alcohol consumption as genes that are too close to each other will seldom be separated by the process of crossing-over. Thus, the mere existence of genetic differences or of different phenotypes should not *per se* be associated with ethanol volition until such a difference is found in an F_2 generation in which genes of the two extreme ethanol consumption phenotypes are randomly segregated ('shuffled'). Even in such cases the proximity of two genes may mislead the experimenter and mechanistic studies are therefore required.

The development of the UChA and UChB lines

Selective breeding of rats that differ in alcohol consumption began in 1950 at the University of Chile (UCh) with the development of a low alcohol-drinking line (UChA) and a high alcohol-drinking line (UChB; 'B' for '*Bebedoras*' or 'Bibulous') (Mardones & Segovia-Riquelme 1983). The foundation stock from which both lines were derived was an outbred colony of Wistar rats from the Instituto Bacteriológico de Chile. In 1949, during the course of experiments on the influence of nutritional conditions on the appetite for alcohol, Mardones and his collaborators began to study the spontaneous voluntary consumption of alcohol by Wistar rats and found wide individual variations: some rats consistently drank large amounts while others consistently drank little or no alcohol. It occurred to Mardones that this difference might be genetically determined, and he and his group at the University of Chile began the genetic selection.

In the selective breeding process, the voluntary alcohol drinking behavior of these rats was assessed by a continuous access paradigm in which the animals were permanently allowed to choose between two bottles, one containing a 10% (v/v) ethanol solution and the other distilled water (the two-bottle free choice paradigm). From the animals tested in this manner for alcohol preference, a single pair with low preference was mated and a single pair with high preference was mated in order to start the UChA and UChB lines, respectively. Selective breeding was continued by mating of males and females with the lowest preference in the UChA line and by mating of males and females with the highest ethanol preference in the UChB line on each succeeding generation. Definitive evidence of a genetic transmission of the preference for ethanol was obtained in 1953 when Mardones, Segovia-Riquelme & Hederra (1953) calculated the heritability of this phenotype by using the selection data from the third (F_3) to the seventh (F_7) generations, which overall provided 365 offspring from the mating of parents of the same alcohol preference. Even at that early selection stage, the heritability of alcohol preference was highly significant ($r = 0.416$; $P < 0.001$) indicating that the spontaneous choice behavior is strongly inherited by the offspring from their parents (as will be seen, segregation is complete at F_{87} for UChA and F_{78} for UChB). The assessment of voluntary drinking behavior has remained essentially the same through the years. Rats are housed individually after the onset of puberty (60 days old) in temperature- and humidity-controlled rooms with a 12 hours light/12 hours dark cycle. Animals are given continuous access to both 10% (v/v) ethanol and water from graduated tubes for 8 weeks. Food is provided *ad libitum* and the volumes of water and ethanol solution consumed are recorded daily. Ethanol intake is calculated for each rat as the average amount of ethanol consumed during the last 2 weeks of preference testing and is expressed as grams of ethanol per kilogram of body weight per day (Mardones & Segovia-Riquelme 1983).

In the early 1980s, Mardones & Segovia-Riquelme (1983) reported the alcohol consumption for UChA and UChB rats in a free-choice condition, i.e. not preceded by a habituation period of forced alcohol consumption. Figure 1 shows the voluntary ethanol consumption of the F_{55} generation of UChA rats and the F_{45} generation of UChB rats. On the first day of free-choice, there were no major differences in alcohol intake between the lines, as upon ethanol presentation UChB rats had only a weak alcohol preference. The amount of alcohol consumed by

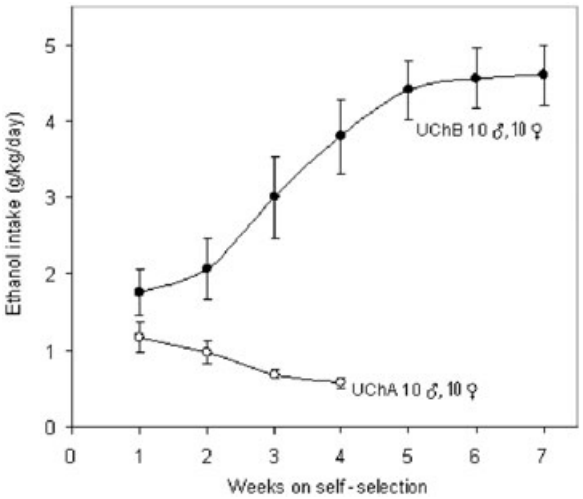

Figure 1 Voluntary ethanol consumption of UChA and UChB rats in the course of time. Animals had free choice between water and a 10% (v/v) ethanol solution. Adapted from Mardones & Segovia-Riquelme (1983)

UChB rats increased as the period of ethanol availability increased, reaching a plateau by approximately day 28 while alcohol intake of UChA rats decreased asymptotically by day 28. The line differences were highly significant ($\chi^2 = 77.83$; $P < 0.001$) (Tampier *et al.* 1984), but there were no significant differences in ethanol intake between males and females of the same line. It should be noted that these conclusions refer to alcohol intake when ethanol is present for 24 hours and has been consumed for many days or weeks (at plateau).

In December 1982, the commercial diet Fabalim® fed to our rats changed and a marked decrease in alcohol intake was observed for both lines (Mardones & Segovia-Riquelme 1984). This diet had been used for years in our rat colony without showing such an effect. A reasonable explanation for this drop was the presence of a cyanamide-like substance in the diet, as reported by Marchner & Tottmar (1976), originating from the high temperature calcination of bones in the fish meal present in rat diets. In fact, a significant inhibition of aldehyde dehydrogenase was demonstrated in UChB rats that had been fed the modified diet, resulting in high acetaldehyde levels (Tampier, Quintanilla & Mardones 1985). No selection of rats was performed during this period of 2 months. After this observation, our rats received only commercial diets devoid of animal products and the alcohol intakes promptly returned to their previous levels.

REVIEW AIMS

The main objective of this communication is to review the recent progress made in the search for behavioral and genetic differences underlying the low or high ethanol drinking behavior of the UChA and UChB rat lines, respectively. As will be shown, the non-drinker (UChA) and drinker (UChB) lines show two main differences in the handling of ethanol: (1) the degree of intoxication reached upon ethanol administration, differences based primarily on the degree of acute tolerance achieved by each animal line; and (2) the metabolism of acetaldehyde and the existence of an 'acetaldehyde burst', based on mutations in the mitochondrial aldehyde dehydrogenase gene and on the mitochondrial capacity to oxidize reduced form of nicotinamide-adenine-dinucleotide (NADH). Thus, both central nervous system (CNS) and metabolic differences are the bases of the phenotypic distinction of low and high alcohol consumption between the University of Chile rat lines. These make an important part of the variance, while other possible differences remain to be investigated.

General metabolism

As rats were originally selected for ethanol consumption to study the influence of nutritional conditions on the 'appetite' for alcohol, differences in carbohydrate metabolic pathways were studied in the UChA and UChB lines during the first decade of their development. The results of these studies using ^{14}C-labeled substrates were reviewed by Segovia-Riquelme *et al.* (1970) who reported that, under steady-state conditions, the oxidation rates of the following metabolites were not significantly different in UChA and UChB rats: acetate, pyruvate, butyrate, citrate, ribose, glycerol, sorbitol, glucose and galactose (or ethanol metabolism, see below). With regard to the daily total energy intake, considering both the energy value of solid food and that of ethanol, no differences were found between both lines (116.8 kJ/100 g body weight in UChA rats and 117.6 kJ/100 g body weight in UChB rats), suggesting that drinking is not related to energy consumption (Tampier *et al.* 1984). Indeed, rats of the two lines, UChA and UChB, grow at similar rates (Tampier & Quintanilla, unpubl. data). Thus, the metabolic mechanisms involved in determining alcohol volition in these lines are likely to be specific rather than general.

Ethanol elimination rate

UChA rats of the F_{11} generation and UChB rats of the F_{10} generation were used in the first study of ethanol metabolism. In this experiment, no significant line differences in the rate of recovery of radioactive label from administered 1-^{14}C-ethanol (2 g/kg body weight) in expired CO_2 were found (Segovia-Riquelme *et al.* 1956). More recently, from experiments in which UChA and UChB rats were administered a standard dose of ethanol either orally or intraperitoneally, it was shown that the rate of ethanol metabolism, measured by following the disappearance of ethanol from blood by gas chromatography, was not different between these two lines (Quintanilla, Sepúlveda & Tampier 1993; Tampier, Quintanilla & Mardones 1999a). The ethanol elimination rate is 330 ± 18 mg/kg/hour for UChA rats and 334 ± 20 mg/kg/hour for UChB rats, which is equivalent to an elimination rate of about (0.33×24) 8 g/kg/day. The amount of alcohol consumed by the UChB rats, 4–8 g/kg/day, is close to the ethanol elimination rate. Thus, the ability to metabolize ethanol may partly limit the ethanol intake in UChB rats. However, the amount of alcohol consumed by UChA rats (0.2–2 g/kg/day) is not limited by the ability to metabolize ethanol. These results suggest that the blood ethanol concentrations are not responsible for the line difference in alcohol intake.

Differences in the response to ethanol: acquired tolerance

There are wide differences in the response to ethanol among various species of rodents. An association between high alcohol intake and the development of alcohol tolerance, as well as between high alcohol intake and low initial sensitivity to alcohol, has been reported in

various lines and strains of rodents (Tabakoff & Ritzmann 1979; Erwin, McClearn & Kuse 1980; Waller *et al.* 1983; Kurtz *et al.* 1996). It has been proposed that a neuroadaptive response (tolerance) may be an important factor contributing to the progression of a drug-seeking behavior (Koob, Sanna & Bloom 1998). Alcohol tolerance has been divided into three categories: (1) *acute tolerance*, that can develop within minutes following a single exposure to ethanol (Mellamby 1919); (2) *rapid tolerance*, which can be observed up to at least 24 hours after a single administration of alcohol (Crabbe *et al.* 1979; Khanna, Chau & Shah 1996) and in which the effect of a second dose is attenuated; and (3) *chronic tolerance*, that appears after repeated administration of alcohol for days to months. In research comparing naïve UChB and UChA animals, we observed that high alcohol-drinking UChB rats develop acute tolerance to the motor impairment effects induced by a dose of ethanol (2.3 g/kg) more rapidly than the low alcohol-drinking UChA rats (Tampier & Mardones 1999) (Fig. 2). In these experiments, which were based on the tilting plane test, the two lines of rats did not differ in the initial depressant actions of ethanol but UChB rats exhibited a significant reduction in motor impairment within the same session. Moreover, UChB rats regain motor activity at significantly higher blood ethanol levels. An acute tolerance may explain the fact that when rats were offered the 10% ethanol solution in a free choice paradigm UChB rats increased ethanol consumption gradually and after 28 days reached their highest consumption ranging between 4 and 8 g/kg/day (Fig. 1). At such time, however, acute tolerance remained only correlational with ethanol intake (see below). These data suggest that in UChB rats the development of tolerance to ethanol may allow increases in ethanol intake.

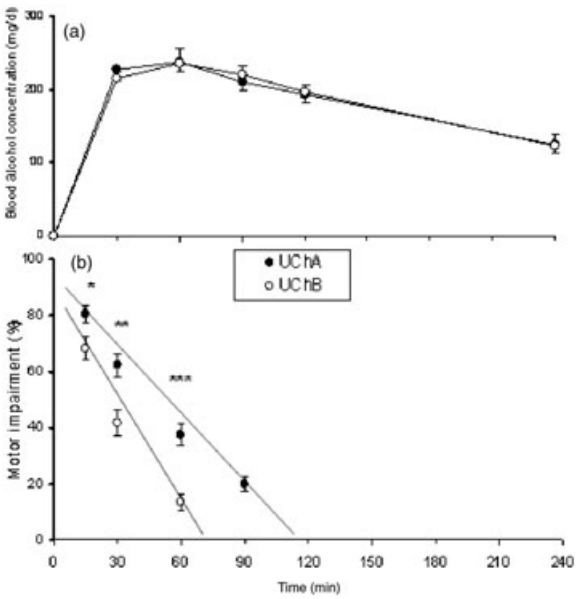

Figure 2 Results obtained with alcohol naïve UChA (●) and UChB (○) rats, after the administration of ethanol (2.3 g/kg intraperitoneally). (a) Whole ethanol blood levels (mg/dl) in rats of both sexes. (b) Regression line for data on motor impairment, measured by the tilting-plane test. The slope of the lines were significantly different ($P < 0.01$) ($b = -0.796 \pm 0.061$ for UChB versus $b = -1.18 \pm 0.12$ for UChA rats) but not the intercept ($a = 88.95 \pm 3.44$ for UChB versus $a = 82.65 \pm 4.83$ for UChA rats). Significance of differences between UChA and UChB lines: ***$P < 0.001$, **$P < 0.005$, *$P < 0.05$

In order to obtain more meaningful genetic correlations between alcohol preference and acute tolerance to motor impairment, we studied these traits in a segregating population, the F_2 generation of a cross between rats of the low ethanol consumption line (UChA) and rats of the high ethanol consumption line (UChB). This study gave strong support to the idea that both parameters, ethanol intake and acute tolerance, are related as they clustered together in the (UChA × UChB) F_2 generation (Tampier, Quintanilla & Mardones 2000). Further, the fact that a greater number of F_2 rats descending from the cross between UChB females and UChA males consumed larger amounts of ethanol compared with those descending from the initial cross between UChA females and UChB males suggested that an X-linked inheritance trait is associated with the development of this type of tolerance. Overall, these results led us to speculate that if we could reduce the development of acute tolerance to ethanol in UChB rats we might reduce their ethanol consumption. In fact, we have observed that bromocriptine, a dopaminergic agonist that decreases voluntary ethanol consumption in UChB rats (Mardones & Quintanilla 1996), also reduces the development of acute tolerance to the motor impairment induced by ethanol (Tampier *et al.* 1999b). Naltrexone, an opiate antagonist that reduces ethanol consumption, decreases both acute tolerance development and ethanol consumption in UChB rats (Quintanilla & Tampier 2000). Moreover, other drugs such as diltiazem (a calcium channel blocker) and ketamine (an *N*-methyl-D-aspartate receptor antagonist) that have been shown to decrease ethanol consumption in rodents also decrease the development of acute tolerance in UChB rats (Tampier & Quintanilla, unpubl. data). From the above observations it is concluded that genes that are responsible for CNS tolerance appear to be engaged in the greater alcohol intake of the UChB high-drinker line. We have not conducted studies to elucidate which are the participating genes but there are many in the dopaminergic, enkephalinergic, glutamatergic and second messenger systems that may be involved.

As rats that rapidly develop acute tolerance to the depressant effects of ethanol drink more ethanol, voluntary ethanol consumption may be increased by prior treatment with ethanol. Given that prior ethanol exposure promotes more acute tolerance, according to the

hypothesis of Radlow (1994), we investigated the effect of a single acute administration of ethanol on both acute tolerance and drinking pattern. For this purpose, we used rats of the UChB line that drank the lowest amount of ethanol (3–4 g ethanol/kg/day at the time of these studies). Rats received a single dose of ethanol of 2.3 g/kg intraperitoneally. After 1 week we tested both their acute tolerance to the depressant effects of a dose of ethanol and their voluntary ethanol consumption (Tampier & Quintanilla 2002a). The intraperitoneal dose of ethanol induced a greater development of acute tolerance in UChB rats and also a long-lasting higher preference for ethanol reaching the maximal capacity of the rat to drink ethanol. In contrast, the dose of ethanol of 2.3 g/kg administered intraperitoneally to UChA rats did not change their ethanol consumption nor their acute tolerance (Tampier & Quintanilla 2002a).

Agabio *et al.* (1996), investigating the drinking behavior of Sardinian alcohol-preferring rats, put forth the hypothesis that voluntary ethanol intake in Sardinian alcohol-preferring rats is sustained by the search for specific pharmacological effects of ethanol and is regulated by a central set-point mechanism that promotes or limits ethanol intake on the basis of the (positive and negative) perception of those effects. Thus, a possible mechanism by which ethanol leads to an increased consumption is the development of an acute tolerance that may limit aversion.

A pattern of increasing ethanol intake over a period of days for the UChB rats is consistent with the hypothesis that tolerance to the aversive effects of ethanol is acquired. We have observed that UChB rats, when compared with UChA rats, have less sensitivity to, or a higher threshold for, alcohol's aversive effects (Quintanilla, Callejas & Tampier 2001). Moreover, UChB rats appear to be comparatively insensitive to the aversive effects of large doses of acetaldehyde, at the same brain concentrations of acetaldehyde that produce a conditioned taste aversion in UChA rats (Quintanilla, Callejas & Tampier 2002).

Systemic acetaldehyde and the rate of acetaldehyde elimination

Acetaldehyde is produced mainly in the liver by oxidation of ethanol, primarily by ADH, with Nicotinamide-adenine-dinucleotide (NAD^+) as the co-factor. Blood levels of acetaldehyde following alcohol administration, in humans and in rats, are in the low micromolar (μM) range because most of the acetaldehyde produced hardly escapes the liver as it is efficiently metabolized to acetate by the low Michaelis–Menten constant (K_m) mitochondrial ALDH2, which uses NAD^+ as co-factor. In the liver, different ALDH isozymes have been found in mitochondria, microsomes and the cytosol (Marjanen 1972; Tottmar, Pettersson & Kiessling 1973; Horton & Barret 1975; Koivula & Koivusalo 1975). It is generally accepted that both in humans and in rats the ALDH located in the mitochondrial matrix (ALDH2), which has the lowest K_m for acetaldehyde, plays the major role in the oxidation of this metabolite (Parrilla *et al.* 1974; Eriksson, Marselos & Koivula 1975).

The rapid accumulation of acetaldehyde in blood following alcohol ingestion is aversive and is believed to play a protective role against alcoholism (Eriksson 2001). Accumulation of acetaldehyde may conceivably occur through either its faster production by ADH or its slower removal by ALDH2. Both mechanisms may act synergistically to produce higher systemic concentrations of acetaldehyde and therefore more protection (Tu & Israel 1995; Chen *et al.* 1999; Chambers *et al.* 2002; Neumark *et al.* 2004).

A lower rate of acetaldehyde metabolism may constitute the mechanism by which UChA rats consume less alcohol (0.2–2 g ethanol/kg/day) than that consumed by UChB rats (4–8 g ethanol/kg/day). This possibility was suggested in the middle 1990s by *in vitro* studies showing that UChA and UChB rats differ in their relative K_m for NAD^+, without changes in the V_{max}, of mitochondrial aldehyde dehydrogenase (Quintanilla & Tampier 1995; Tampier, Sánchez & Quintanilla 1996). The relative K_m for NAD^+ is fivefold higher in the low alcohol-drinking UChA rats (96–126 μM) than in the high alcohol-drinking UChB rats (21–23 μM). Despite having an ALDH2 with lower affinity for NAD^+, no increases in the steady-state levels of blood acetaldehyde, measured 30–90 minutes after alcohol administration, were found in UChA rats when compared with UChB rats (Tampier *et al.* 1996). This result is in line with the equal rate of alcohol elimination in UChA and UChB rats (Fig. 2).

Fairly recently, however, an *in vivo* study supported the hypothesis that UChA rats might display a lower rate of acetaldehyde metabolism (Quintanilla *et al.* 2002). Thus, the slopes of the descending limbs of the blood acetaldehyde concentration curves obtained after the intraperitoneal administration of a standard dose of exogenous acetaldehyde (50, 100 or 150 mg/kg) to UChA and UChB rats clearly showed that the low alcohol-drinking UChA rats display a slower elimination rate of acetaldehyde than UChB rats. The apparent contradiction between this finding and equal steady-state levels of acetaldehyde could not be explained at the time but was solved later (see below).

Genetic differences between UChA and UChB rats: identification of three allelic variants of mitochondrial aldehyde dehydrogenase

Because UChA and UChB rats display differences in the relative K_m of ALDH2 for NAD^+, we investigated whether there are differences in the coding regions of the ALDH2

cDNA in these lines and whether the *Aldh2* genotype (lower case nomenclature for rodent genes) predicts the phenotype of alcohol consumption and the K_m of ALDH2 for NAD^+ (Sapag *et al*. 2003). UChA rats of the F_{81} and F_{82} generations and UChB rats of the F_{72}, F_{73} and F_{74} generations were used in these studies. Because these rats are not inbred (T.-K. Li, pers. comm.), we initially characterized 10 UChA and 10 UChB rats for their alcohol consumption phenotype and determined their K_ms for NAD^+ of liver ALDH2. Subsequently, liver RNA was extracted, and *Aldh2* cDNA was prepared by reverse transcriptase-polymerase chain reaction (RT-PCR) techniques, cloned and sequenced. Genotyping of a larger group of animals was conducted by genomic DNA amplification and restriction enzyme digestion.

When compared with $Aldh2^1$ of Sprague–Dawley (the term $Aldh2^1$ is used as it was the first allele reported in the literature), 97% of the UChA (low-drinker) rats ($n = 61$) presented a mutation that changed Gln^{67} to Arg^{67} in the mature enzyme (allele referred to as $Aldh2^2$). In UChB (high-drinker) rats ($n = 69$), we found that 69% presented the $Aldh2^1$ allele (Gln^{67}, Glu^{479}), while 53% presented the Gln^{67} to Arg^{67} change plus a second mutation that changed Glu^{479} to Lys^{479} (allele $Aldh2^3$). Table 1 shows the allelic and genotype frequencies of $Aldh2^1$, $Aldh2^2$ and $Aldh2^3$ in the UChA and UChB rats. It is worth noting that the $Aldh2^2$ allele is present only in UChA rats and absent from UChB rats while the $Aldh2^3$ allele is present only in UChB rats and absent from UChA rats.

The genotypes highly predicted the phenotypic differences for both ethanol consumption (g/kg/day; mean ± SEM: $Aldh2^1/Aldh2^1 = 5.7 \pm 0.2$, $Aldh2^2/Aldh2^2 = 0.9 \pm 0.2$ and $Aldh2^3/Aldh2^3 = 4.6 \pm 0.2$; $P < 0.01$ versus $Aldh2^1/Aldh2^1$) and K_ms for NAD^+ (43 ± 3 μM, 132 ± 13 μM and 41 ± 2 μM, respectively; $Aldh2^2$ versus $Aldh2^1$ or $Aldh2^3$, $P < 0.001$ for both phenotypes). V_{max} of ALDH2 measured in mitochondria under saturating substrate concentrations were: $Aldh2^1/Aldh2^1 = 36 \pm 2$; $Aldh2^2/Aldh2^2 = 28 \pm 1$ and $Aldh2^3/Aldh2^3 = 27 \pm 2$ nmol NADH/mg protein/minute. It should be noted that the V_{max} of ALDH2 of $Aldh2^3/Aldh2^3$ animals is 25% lower ($P < 0.05$) than that of $Aldh2^1/Aldh2^1$ rats, which is consistent with a significantly lower voluntary alcohol intake in the $Aldh2^3/Aldh2^3$ animals than in $Aldh2^1/Aldh2^1$ rats (Fig. 3). Overall, these data show that the genotype of *Aldh2* and the kinetic properties of the enzymes encoded (K_m and V_{max} for ALDH2) are strongly associated with the phenotype of ethanol consumption. Further studies with F_2 animals showed that with an overall mixed genetic background the *Aldh2* genotypes indeed predict alcohol volition.

The *Aldh2²* allele and low voluntary alcohol consumption

As the above data showed an association between the $Aldh2^2$ allele and a low voluntary alcohol consumption, it was important to study whether the $Aldh2^2$ gene segregates together with a low alcohol consumption in animals of the F_2 generation descending from the cross between UChA ($Aldh2^2$) and UChB rats (both $Aldh2^1$ and $Aldh2^3$) in which all the other genes present in these two lines are randomly 'shuffled'. We studied F_2 generations derived from: (1) crosses between UChA rats that were homozygous $Aldh2^2/Aldh2^2$ and UChB rats that were homozygous $Aldh2^3/Aldh2^3$ (alleles $Aldh2^2$ and $Aldh2^3$ being present only in the UChA and UChB lines, respectively); and (2) crosses between UChA rats that were homozygous $Aldh2^2/Aldh2^2$ and UChB rats that were homozygous $Aldh2^1/Aldh2^1$.

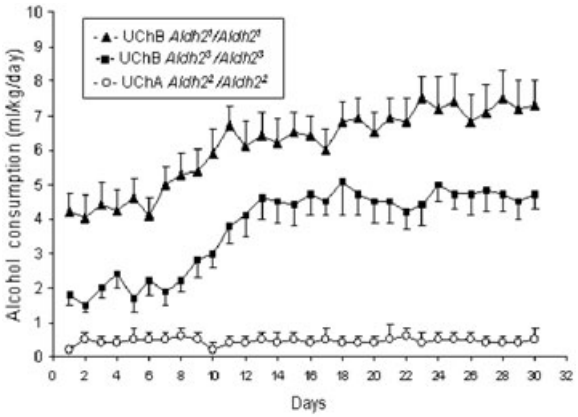

Figure 3 Voluntary ethanol consumption of UChA homozygous $Aldh2^2$ UChB homozygous $Aldh2^1$ and UChB homozygous $Aldh2^3$ rats in the course of time. Animals had choice between water and a 10% (v/v) ethanol solution. Ethanol consumption is expressed as milliliter of 10% (v/v) ethanol per kilogram body weight per day

Table 1 *Aldh2* allele frequencies and genotype frequencies.

	Allele frequency			Aldh2 *genotype frequency*					
Group	$Aldh2^1$	$Aldh2^2$	$Aldh2^3$	1/1	2/2	3/3	1/2	1/3	2/3
UChA ($n = 61$)	0.06	0.94[a]	0	0.03	0.92	0	0.05	0	0
UChB ($n = 69$)	0.58	0[b]	0.42	0.46	0	0.30	0	0.23	0

[a]*UChA*: *Aldh2* frequency versus $Aldh2^1$ and $Aldh2^3$ ($P < 0.0001$). [b]$Aldh2^2$ frequency in UChA versus $Aldh2^2$ in UChB ($P < 0.0001$).

F_2 *hybrids derived from UChA (*Aldh22*) and UChB (*Aldh2^3*): interaction between mitochondrial oxidation of NADH and the* Aldh2 *genotype*

The voluntary ethanol consumption of the F_2 *Aldh2*^{2}/*Aldh2*^{2} hybrid rats derived from homozygous *Aldh2*^{2} UChA rats and homozygous *Aldh2*^{3} UChB rats (Quintanilla *et al.* 2005a) indicated that there is a marked maternal influence on voluntary alcohol consumption (Fig. 4). Homozygous F_2 *Aldh2*^{2}/*Aldh2*^{2} animals derived from low-alcohol-drinking F_0 females and high-alcohol-drinking F_0 males (group 1) showed markedly lower ($P < 0.005$) ethanol consumption (1.8 ± 0.4 g/kg/day) than F_2 homozygous *Aldh2*^{2}/*Aldh2*^{2} rats derived from high-alcohol-drinking F_0 females and low-alcohol-drinking F_0 males (group 2) (3.9 ± 0.5 g/kg/day). The maternal origin did not influence voluntary alcohol intake of *Aldh2*^{3}/*Aldh2*^{3} animals. It should be recalled that ALDH2 is inserted in the mitochondrion, an organelle known to be mainly derived from the maternal line (Strachan & Read 1999), which must reoxidize the NADH generated in the oxidation of acetaldehyde into NAD^+ via the NADH-ubiquinone oxidoreductase. If mitochondria are responsible for the interaction with *Aldh2*, they only influence the consumption phenotype of animals displaying the lower-affinity ALDH2 for NAD^+ (*Aldh2*^{2} homozygous) and not that of animals with the higher-affinity ALDH2 (*Aldh2*^{3} homozygous).

Further studies addressed the NADH oxidizing ability of mitochondrial complex I from the UChA and UChB lines. Acetaldehyde was used as a substrate for ALDH2 in intact mitochondria to provide NADH to complex I. Glutamate was also used as a substrate for mitochondria to generate NADH and thus enter electrons at complex I of the respiratory chain. Results showed that mitochondria of F_2 rats derived from high-alcohol-drinking females were more active in oxidizing substrates that generate NADH for complex I than were mitochondria derived from low-alcohol-drinking females, leading in the former to higher rates of acetaldehyde metabolism and to a reduced aversion to ethanol. Succinate, a substrate that enters electrons at site II, was oxidized at the same rate by the mitochondria of both UChA and UChB animals. The rate of disappearance from blood of acetaldehyde administered *in vivo* was also greater in UChB than in UChA animals.

Overall, these studies (Quintanilla *et al.* 2005a) show that differences in the activity of complex I from F_2 rats either allow or fully abolish the expression of the *Aldh2*^{2}/*Aldh2*^{2} genotype *in vivo* on phenotypic traits such as acetaldehyde metabolism, acetaldehyde oxidation by intact mitochondria and voluntary alcohol consumption.

F_2 *hybrids derived from UChA (*Aldh2^2*) and UChB (*Aldh2^1*)*

The voluntary ethanol consumption of F_2 hybrid rats having the *Aldh2*^{2}/*Aldh2*^{2} genotype derived from homozygous *Aldh2*^{2} UChA rats and homozygous *Aldh2*^{1} UChB rats was also studied (Quintanilla *et al.* 2005b). In all cases, the crosses incorporated the mitochondria of the UChA line. Rats of the F_2 generation carrying the *Aldh2*^{1}/*Aldh2*^{1} genotype voluntarily consume 65% more alcohol ($P < 0.01$) than F_2 *Aldh2*^{2}/*Aldh2*^{2} animals (Fig. 5). However, the two groups did not display significant differences in the overall rate of ethanol metabolism (Quintanilla *et al.* 2005b).

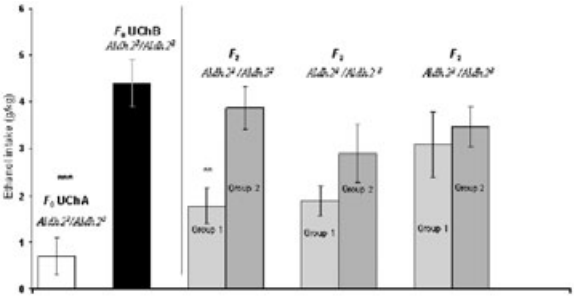

Figure 4 Ethanol consumption of F_0 (UChA *Aldh2*^{2}/*Aldh2*^{2} and UChB *Aldh2*^{3}/*Aldh2*^{3}) and F_2 rats according to their *Aldh2* genotype. Bars represent means ± SEM of ethanol consumption (g ethanol/kg body weight/day). Group 1: F_2 from an F_0 low-consumption UChA maternal line (and an F_0 high-consumption UChB father). Group 2: F_2 from an F_0 high-consumption UChB maternal line (and an F_0 low-consumption UChA father). The maternal line greatly influenced ($P < 0.005$) alcohol consumption of *Aldh2*^{2}/*Aldh2*^{2} animals but did not influence the consumption of *Aldh2*^{3}/*Aldh2*^{3} rats. The maternal line did not significantly alter ethanol consumption of heterozygous *Aldh2*^{2}/*Aldh2*^{3} rats (**$P < 0.005$; ***$P < 0.001$)

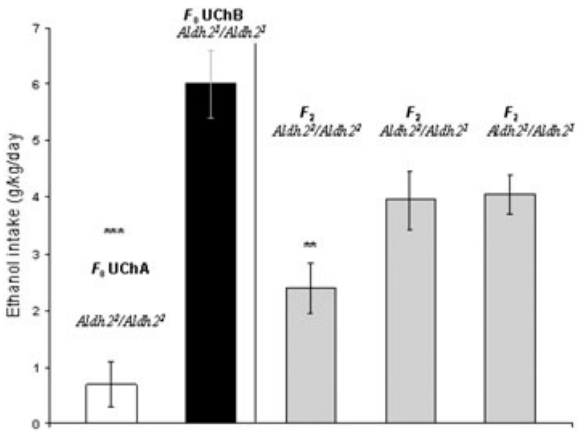

Figure 5 Ethanol consumption of F_o (UChA *Aldh2*^{2}/*Aldh2*^{2} and UChB *Aldh2*^{1}/*Aldh2*^{1}) and F_2 rats according to their *Aldh2* genotype. Bars represent the mean ± SEM of ethanol consumption expressed as g ethanol/kg body weight/day (F_o low drinkers: UChA, F_o high drinkers: UChB; ***$P < 0.001$). Homozygous *Aldh2*^{1}/F_2 rats consumed 65% more ethanol than both homozygous *Aldh2*^{2} (**$P < 0.01$) or heterozygous *Aldh2*^{1}/*Aldh2*$^2F_2$ rats (**$P < 0.05$)

Previous studies (Tampier *et al.* 1996) have shown that the steady-state levels of blood acetaldehyde (measured 30–60 minutes after ethanol administration) are not different in F_0 UChA rats (which are mostly $Aldh2^2/Aldh2^2$) from those in UChB animals (in a high proportion $Aldh2^1/Aldh2^1$). Thus, the mechanism by which the *Aldh2* genotype affected alcohol intake was not initially obvious. Starting from the concept proposed by Israel and other investigators (Israel, Khanna & Lin 1970; Seiden, Israel & Kalant 1974; Plapp 1975; Rawat 1977; Cronholm 1985; Cronholm, Jones & Skagerberg 1988) that the reoxidation of NADH limits the rate of ethanol oxidation—and thus the rate of acetaldehyde generation by alcohol dehydrogenase—we tested the hypothesis that rats with the $Aldh2^2/Aldh2^2$ genotype, with a lower capacity to oxidize acetaldehyde, would allow the accumulation of this metabolite primarily at early times after ethanol presentation, before NADH is elevated (shown by increases in the lactate/pyruvate ratio) and the velocity of acetaldehyde generation is reduced.

Data obtained (Quintanilla *et al.* 2005b) fully supported that an elevation of acetaldehyde in $Aldh2^2/Aldh2^2$ animals is short-lived. Peak blood acetaldehyde levels 5–10 minutes after ethanol administration in $Aldh2^2/Aldh2^2$ animals were three- to fivefold higher than peak levels in $Aldh2^1/Aldh2^1$ animals (Fig. 6). We also confirmed earlier observations (Tampier *et al.* 1996) that the steady-state levels of acetaldehyde were identical. We have postulated that such an 'acetaldehyde burst', occurring virtually at the same time that ethanol is ingested, leads to an association between the dysphoric effects of acetaldehyde and ethanol consumption (Quintanilla *et al.* 2005b). These studies also suggest that animals that have a higher alcohol dehydrogenase activity may similarly display higher peak acetaldehyde levels and a lower voluntary ethanol consumption (unpublished data by the authors is in line with this view). In humans, alcohol dehydrogenases ADH1B*2 and ADH1C*1, which have a one order of magnitude higher activity than ADH1B*1, have a strong protective effect against alcoholism with only minimal (8–13%) increases in the steady-state rate of ethanol elimination (Neumark *et al.* 2004).

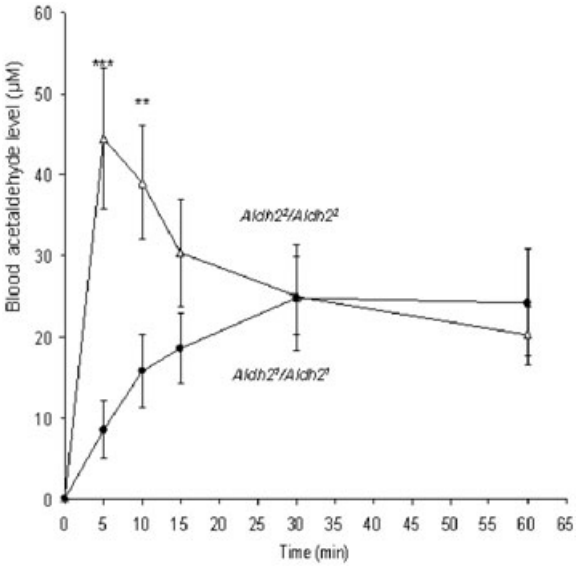

Figure 6 Blood acetaldehyde concentrations in F_2 hybrid rats genotyped as $Aldh2^2/Aldh2^2$ or $Aldh2^1/Aldh2^1$ after the administration of ethanol (1 g/kg). Significant differences in blood acetaldehyde levels: $^{***}P < 0.0025$, $^{**}P < 0.01$

Current segregation of UChA and UChB lines

As indicated earlier, in 2003 three allelic variants of ALDH2 were identified in UChA and UChB rats (Sapag *et al.* 2003). Since then selective breeding was continued by the mating of homozygous $Aldh2^2/Aldh2^2$ males and females with the lowest preference for ethanol in the UChA line, whereas in the UChB line selective breeding was divided into two sublines by mating homozygous $Aldh2^1/Aldh2^1$ males and females and also by mating homozygous $Aldh2^3/Aldh2^3$ males and females with the highest ethanol preference, such that three lines now exist with marked phenotypic differences. The UChA $Aldh2^2/Aldh2^2$ line has now been propagated to the F_{87} generation and the UChB $Aldh2^1/Aldh2^1$ and UChB $Aldh2^3/Aldh2^3$ lines have been propagated to the F_{78} generation. The current segregation of ethanol consumption between lines is shown in Fig. 3. Average ethanol intakes are: UChA $Aldh2^2/Aldh2^2 = 0.3–0.6$ g/kg/day; UChB $Aldh2^3/Aldh2^3 = 4.5–5.0$ g/kg/day; UChB $Aldh2^1/Aldh2^1 = 7.0–7.5$ g/kg/day. For both UChB groups, the consumption levels after 30 days of continuous access to 10% ethanol (and water) are 100% greater than the levels consumed over the first 1–5 days. UChA $Aldh2^2/Aldh2^2$ rats do not alter their low consumption. We suggest that the initial aversion to ethanol limits further intake.

Differences in response to acetaldehyde: conditioned taste aversion

The conditioned taste aversion paradigm is often used to test the aversive properties of drugs. This test involves pairing the consumption of a novel and detectable taste solution (0.015% v/v banana flavored solution) with the administration of acetaldehyde at a dose of 50, 100 or 150 mg/kg intraperitoneally (namely doses ranging from 1.1 to 3.4 mmoles/kg) during five conditioning trials. We wish to note that these doses would lead to supraphysiological concentrations of acetaldehyde. Aversion to acetaldehyde is indicated by a reduction of intake of the flavored solution in subsequent exposures in the absence of acetaldehyde administration.

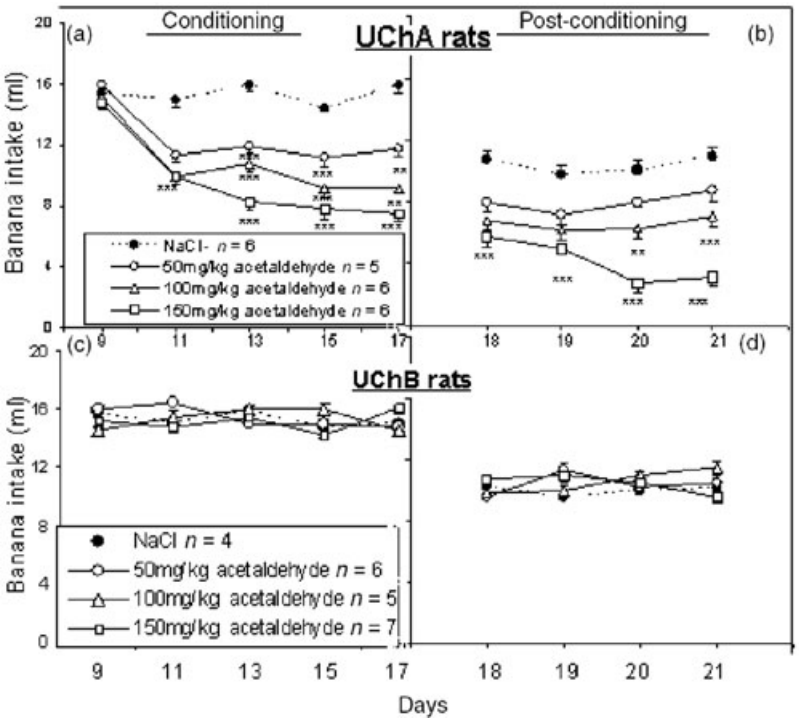

Figure 7 Left panels: Intake of banana-flavored solution in UChA (a) and UChB (c) rats receiving an intraperitoneal injection of saline (NaCl) or acetaldehyde at a dose of 50, 100 or 150 mg/kg paired with banana solution during conditioning. Right panels: Intake of banana-flavored solution in UChA (b) and UChB (d) rats when banana-flavored solution was presented concurrently with water during post-conditioning in the absence of drug treatment. Asterisks indicate significant differences between intake of banana-flavored solution by rats receiving acetaldehyde and rats of the same line receiving saline. ***$P < 0.001$; **$P < 0.005$

Figure 7 shows that a strong dose-dependent aversion to acetaldehyde was found in UChA rats, whereas UChB rats did not show aversion to any dose of acetaldehyde (Quintanilla *et al*. 2002). At equal doses of acetaldehyde, cerebral venous blood acetaldehyde levels in UChA rats were higher than in UChB rats, a finding that may reflect the previously observed difference in the activity of ALDH2 between these rat lines. However, this observation is unlikely to explain fully the differences observed because aversion to acetaldehyde was developed in the UChA rats at cerebral venous blood levels of acetaldehyde that did not produce any aversion in the UChB rats. Thus, at 5 minutes after injection, for example, significantly higher acetaldehyde levels were found in UChA rats after administration of a dose of 50 mg/kg [1.37 ± 0.04 (UChA) versus 0.68 ± 0.08 mg/dl (UChB), $P < 0.001$], 100 mg/kg [3.38 ± 0.03 (UChA) versus 1.32 ± 0.27 mg/dl (UChB), $P < 0.001$] and 150 mg/kg [5.41 ± 0.49 (UChA) versus 3.83 ± 0.05 mg/dl (UChB), $P < 0.01$]. These studies were conducted on UChB and UChA rats before the separation according to genotype described above.

The failure to demonstrate the aversive properties of acetaldehyde in UChB rats may be related to the paradoxical hedonic value of acetaldehyde. Indeed, it was postulated (Quertemont & Tambour 2004) that the overall hedonic value of acetaldehyde accumulation is a balance between its central reinforcing and peripheral aversive effects. Interestingly, when tested in place conditioning, UChB rats displayed an acetaldehyde-induced conditioned place preference, whereas at the same doses UChA rats showed an acetaldehyde-induced conditioned place aversion (Quintanilla & Tampier 2003). It should be noted that the doses of acetaldehyde used and the blood levels attained allow this molecule to cross the blood–brain barrier. Whether the acetaldehyde aversion of UChA rats is limited to central or peripheral effects is not known. In the Lewis rat, also derived from the Wistar strain, an aversion to acetaldehyde starts at low levels of acetaldehyde in the periphery, as suggested by our studies in which administration of an anti-*Aldh2* antisense oligonucleotide, which does not cross the blood brain barrier, elevated fourfold the blood acetaldehyde levels (from 2.2 to 8.3 μM), which led to a 60% inhibition of ethanol intake (Garver *et al*. 2001).

Brain ethanol and acetaldehyde metabolism and voluntary ethanol intake

A clear difference between UChA and UChB lines relates to the behavioral differences observed following the administration of acetaldehyde. At doses of 50 mg and 100 mg/kg acetaldehyde, UChA rats lose their righting reflex for 2.4 and 7.2 minutes, respectively. Conversely, UChB rats do not lose their righting reflex at these concentrations of acetaldehyde (Tampier & Quintanilla 2002b). While this might result from the fact that UChB rats metabolize systemic acetaldehyde at a faster rate than UChA rats, this does not provide a full explanation for the differences in the depressant effects of acetaldehyde as UChA rats lose their righting reflex at a blood

level of 600 μM acetaldehyde, while at 1100 μM acetaldehyde UChB rats do not lose such a reflex.

Clearly, acetaldehyde is a CNS depressant *per se* and, based on its higher lipid/water partition coefficient, according to the classical Overton–Meyer rule (Meyer 1901), it is expected to be a more potent CNS depressant than ethanol. One can therefore suggest that an anesthetic effect is the reason by which acetaldehyde elicits a greater sleep time in the UChA rats than in UChB animals. One clear possibility is the slower brain metabolism of acetaldehyde in UChA animals, such that any brain acetaldehyde originating in the periphery could achieve a higher steady-state concentration in the CNS. It should be kept in mind that ALDH2 exists in virtually all tissues, and that this metabolic difference may reside at the neuronal or glial level as well as in the capillaries that constitute the blood–brain barrier. Brain homogenates of UChA animals metabolize acetaldehyde at a significantly slower rate than that of UChB animals (Tampier, Quintanilla & Mardones 1994), but we have not pursued the identification of the cells responsible for this difference.

It should be noted that, in the above studies, high concentrations of peripheral acetaldehyde were administered, which could thus readily enter the brain. It is generally accepted that little systemic acetaldehyde crosses the blood brain barrier at concentrations below 50 μM, concentration that is only achieved under pharmacological inhibition of ALDH (Eriksson & Sippel 1977; Wescott *et al.* 1980; Eriksson & Fukunaga 1993). However, acetaldehyde generation in the CNS itself is also possible. A number of authors have presented data that strongly suggest that ethanol is metabolized in the brain into acetaldehyde by the catalase system (Tampier & Mardones 1979; Aragon, Rogan & Amit 1992; Gill *et al.* 1992; Hamby-Mason *et al.* 1997; Zimatkin, Liopo & Deitrich 1998). Evidence for a functional role of ethanol oxidation by the brain catalase system in the mediation of the acetaldehyde-related actions of ethanol is given by the fact that pre-treatment of rats with the catalase inhibitor 3-amino-1,2,4-triazole (AT) entails the consequent loss of brain catalase activity in rodents and reduces many ethanol-induced behaviors. On the other hand, recent studies (Vasiliou *et al.* 2006) show that acatalasemic mice display a prolonged sleep time versus that of control animals.

It has been shown that AT reduces the loss of righting reflex induced by ethanol in rats (Tampier *et al.* 1988; Aragon, Spivak & Amit 1991), blocks the taste aversion induced by ethanol (Aragon, Spivak & Amit 1985), reduces the voluntary ethanol consumption in rats (Aragon & Amit 1992; Tampier, Quintanilla & Mardones 1995) and mice (Koechling & Amit 1994) and avoids development of acute tolerance to the motor impairment induced by a dose of ethanol in UChB rats (Tampier & Quintanilla 2003). These AT effects cannot be ascribed to changes in the rate of ethanol disposal because no significant difference in the levels of blood ethanol was observed between animals pretreated with AT or saline and injected with ethanol (Tampier & Mardones 1986). UChA and UChB rats do not differ in their catalase activity (Tampier *et al.* 1995). However, it could be argued that UChA animals, with a less active ALDH2, would accumulate a greater concentration of brain acetaldehyde derived from equal levels of acetaldehyde formed by catalase. Indeed, the administration of AT was more effective in reducing sleep time in UChA rats than in UChB rats (Tampier, Quintanilla & Mardones 1981). Nevertheless, studies with AT should be interpreted with caution as it has been reported to inhibit several enzymes in addition to catalase, including a variety of peroxidases (Doerge & Niemezura 1989) and nitric oxide synthase (Buchmuller-Rouiller *et al.* 1992), the latter being known to mediate the action of some neurotransmitter systems and learning, which is an important component of tolerance to ethanol and, thus, of ethanol intake.

Our genotyping studies showing that there is a deficient aldehyde dehydrogenase (in addition to a slower mitochondrial reoxidation of NADH) in non-drinker UChA rats and not in drinker UChB rats are in line with studies by Amir (1977) and Socaransky, Aragon & Amit (1985) who showed a direct association between alcohol intake and ALDH activity in rat brain tissues. It is not unlikely, however, that liver and brain ALDH activities may be related in the same animal, as we have shown in our studies in the UChA and UChB lines. Thus, concluding that the effects are mainly central should await clarification.

CONCLUSIONS

Credible animal models of heavy alcohol use should display some of the characteristics seen in alcoholism in humans. However, their real value rests in the investigator's ability to ask in these animal models questions that could not be readily addressed in the clinical situation.

The rat models developed at the University of Chile showed: (1) an association between acute tolerance and heavy alcohol use (in the UChB line); and (2) an association between low ethanol consumption and the existence of a less efficient mitochondrial aldehyde dehydrogenase (in the UChA line), thus sharing, respectively, two characteristics of alcoholism and alcohol use in humans. Indeed, studies in children have shown that individuals who are more tolerant to the intoxicating effects of ethanol are more likely to become alcoholics in their older years (Schuckit 1994). In humans, as in UChB rats, this is an inherited trait. A parallel between rats and humans is also observed in East Asian adults, who are protected

against alcohol abuse and alcoholism when carrying a low-activity mitochondrial aldehyde dehydrogenase (Harada *et al.* 1982).

In relation to tolerance, data from UChB animals showing a direct relationship between the development of acute tolerance and high alcohol consumption led to the demonstration that drugs that block acute tolerance reduce ethanol intake, thus indicating the correlation is not fortuitous. This knowledge, added to the clinical correlation between tolerance and alcoholism, may contribute to the development of prevention strategies in individuals at risk.

In relation to mitochondrial aldehyde dehydrogenase, studies in UChA showed that maternal genetic influence expressed in mitochondria either allow or block a low ethanol consumption phenotype, a previously unknown biological interaction between ALDH2 and the mitochondrial respiratory chain. Future studies could determine whether some *ALDH2*2/ALDH2*1* heterozygous humans (and even subjects with a normal ALDH2) who drink heavily have mitochondria with a high capacity of reoxidizing NADH, thus not allowing acetaldehyde accumulation. More active mitochondria would also increase the generation of free radicals. Recent studies have shown important individual differences in the ability of mitochondria to burn substrates by transforming the energy of oxidation into heat rather than into adenosine triphosphate (Antonicka *et al.* 2003).

A distinct finding rested on the fact that a low-activity ALDH22 in UChA rats, which predicts a low ethanol consumption phenotype, did not lead to differences in the steady-state levels of acetaldehyde, but rather was associated to an 'acetaldehyde burst'. We postulated that this burst results from the combination of an inefficient mitochondrial ALDH2 being overwhelmed by the high amount of acetaldehyde generated immediately after ethanol ingestion when the hepatic NADH availability is low (sustained by the initial low lactate/pyruvate ratio, being transferred via blood into the hepatocyte). The release of NADH from the NADH–alcohol dehydrogenase enzyme complex (ADH–NADH), and thus of the ADH-mediated rate of acetaldehyde generation, is an important rate-limiting factor in the catalytic ability of alcohol dehydrogenase (Plapp 1975; Cronholm 1985; Cronholm *et al.* 1988), as is the amount of enzyme. Our data predict that human subjects who are protected against alcoholism by the presence of the more active alcohol dehydrogenases, e.g. ADH1B*2 (ADH2*2), would also show the 'acetaldehyde burst'.

Finally, we wish to emphasize that animals selectively bred for many generations as high alcohol drinkers will accumulate many permissive genes and will exclude protective genes and, vice versa, rats bred as low ethanol consumers accumulate protective genes and exclude permissive genes. Genes present in the two divergent lines will only depend on the gene pool existent in the original stock. Thus, while it is unlikely that all drinker and non-drinker animal lines or strains developed by different groups will share the same gene combinations, it is even more remote that either all alcoholics or all subjects who avoid alcohol will share equal gene combinations. From data in the literature, there appears to be a smaller repertoire of protective genes than of permissive genes. We suggest that studies on the genetics of alcohol aversion be pursued with as much vigor as the genetics of alcoholism. Both will strongly contribute to illuminate us in years to come.

Acknowledgements

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References

Agabio R, Cortis G, Fadda F, Gessa GL, Lobina C, Reali R, Colombo G (1996) Circadian drinking pattern of Sardinian alcohol preferring rats. Alcohol Alcohol 31:385–388.

Amir S (1977) Brain and liver aldehyde dehydrogenase: relations to ethanol consumption in Wistar rats. Neuropharmacology 16:781–784.

Antonicka H, Ogilvic I, Taivassalo T, Anitori RP, Haller RG, Vissing J, Kennaway NG, Shoubridge EA (2003) Identification and characterization of a common set of complex I assembly intermediates in mitochondria from patients with complex I deficiency. J Biol Chem 278:43081–43088.

Aragon CM, Amit Z (1992) The effect of 3-amino-1,2,4-triazole on voluntary ethanol consumption: evidence for brain catalase involvement in the mechanism of action. Neuropharmacology 31:712.

Aragon CMG, Rogan F, Amit Z (1992) Ethanol metabolism in rat brain homogenates by catalase H_2O_2 system. Biochem Pharmacol 44:93–98.

Aragon CM, Spivak K, Amit Z (1985) Blockade of ethanol induced conditioned taste aversion by 3-amino-1,2,4-triazole: evidence for catalase mediated synthesis of acetaldehyde in rat brain. Life Sci 37:2077–2084.

Aragon CMG, Spivak K, Amit Z (1991) Effects of 3-amino-1,2,4-triazole on ethanol-induced narcosis, lethality and hypothermia in rats. Pharmacol Biochem Behav 39:55–68.

Blednov YA, Jung S, Alva H, Wallace D, Rosahl T, Whiting PJ, Harris RA (2003) Deletion of the alpha 1 or beta 2 subunit of GABA A receptors reduces actions of alcohol and other drugs. J Pharmacol Exp Ther 304:30–36.

Buchmuller-Rouiller Y, Schneider P, Betz-Corradin S, Smith J, Mauel J (1992) 3-Amino-1,2,4-triazole inhibits macrophage NO synthase. Biochem Biophys Res Commun 183:150–155.

Chambers GK, Marshall SJ, Robinson GM, Maguire S, Newton-Howes J, Chong NL (2002) The genetic of alcoholism in

Polynesians: alcohol and aldehyde dehydrogenase genotypes in young men. Alcohol Clin Exp Res 26:949–955.

Chen CC, Lu RB, Chen YC, Wang MF, Chang YC, Li TK, Yin SJ (1999) Interaction between the functional polymorphisms of the alcohol-metabolism genes in protection against alcoholism. Am J Hum Genet 65:795–807.

Crabbe JC, Rigter H, Uijlen J, Strijbos C (1979) Rapid development of tolerance to the hypothermic effect of ethanol in mice. J Pharmacol Exp Ther 208:128–133.

Cronholm T (1985) Hydrogen transfer between ethanol molecules during oxidoreduction in vivo. Biochem J 15:315–322.

Cronholm T, Jones AW, Skagerberg S (1988) Mechanism and regulation of ethanol elimination in humans: intermolecular hydrogen transfer and oxidoreduction in vivo. Alcohol Clin Exp Res 12:683–686.

Doerge DR, Niemezura WP (1989) Suicide inactivation of lactoperoxidase by 3-amino-1,2,4-triazole. Chem Res Toxicol 2:100–103.

El-Ghundi M, George SR, Drago J, Fletcher PJ, Fan T, Nguyen T, Lin C, Sibley DR, Westphal H, O'Dowd BF (1998) Disruption of dopamine D_1 receptor gene expression attenuates alcohol-seeking behavior. Eur J Pharmacol 353:149–158.

Engel SR, Lyons CR, Allan AM (1998) 5-HT3 receptor overexpression decreases ethanol self administration in transgenic mice. Psychopharmacology (Berl) 140:243–248.

Eriksson CJP (2001) The role of acetaldehyde in the actions of alcohol (update 2000). Alcohol Clin Exp Res 25:15S–32S.

Eriksson CJP, Fukunaga T (1993) Human blood acetaldehyde Update 1992. Alcohol Alcohol Suppl 2:9–25.

Eriksson CJP, Marselos M, Koivula T (1975) Role of cytosolic rat liver aldehyde dehydrogenase in the oxidation of acetaldehyde during ethanol metabolism in vivo. Biochem J 152:709–712.

Eriksson CJP, Sippel HW (1977) The distribution and metabolism of acetaldehyde in rats during ethanol oxidation. I. The distribution of acetaldehyde in liver, brain, blood and breath. Biochem Pharmacol 26:241–247.

Erwin VG, McClearn GE, Kuse AR (1980) Interrelationships of alcohol consumption, actions of alcohol, and biochemical traits. Pharmacol Biochem Behav 13:297–302.

Garver E, Tu G, Cao Q, Ain M, Zhou F, Israel Y (2001) Eliciting the low-activity aldehyde dehydrogenase Asian phenotype by an antisense mechanism results in an aversion to ethanol. J Exp Med 194:571–580.

Gill K, Mendez JF, Lucas D, Deitrich RA (1992) Enzymatic production of acetaldehyde from ethanol in rat brain tissue. Alcohol Clin Exp Res 16:910–915.

Hall FS, Sora I, Uhl GR (2001) Ethanol consumption and reward are decreased in mu-opiate receptor knockout mice. Psychopharmacology (Berl) 154:43–49.

Hamby-Mason R, Chen JJ, Schenker S, Perez A, Henderson GY (1997) Catalase mediates acetaldehyde formation from ethanol in fetal and neonatal brain. Alcohol Clin Exp Res 21: 1063–1072.

Harada S, Agarwal DP, Goedde HW, Tagaki S, Ishikawa B (1982) Possible protective role against alcoholism for aldehyde dehydrogenase isozyme deficiency in Japan. Lancet 2: 827.

Higuchi S (1994) Polymorphisms of ethanol metabolizing enzyme genes and alcoholism. Alcohol Alcohol Suppl 2:29–34.

Horton AA, Barret MC (1975) The subcellular localization of aldehyde dehydrogenase in rat liver. Arch Biochem Biophys 167:426–436.

Israel Y, Khanna JM, Lin R (1970) Effect of 2,4-dinitrophenol on the rate of ethanol elimination in the rat in vivo. Biochem J 120:447–448.

Khanna JM, Chau A, Shah G (1996) Characterization of the phenomenon of rapid tolerance to ethanol. Alcohol 13:621–628.

Koechling UM, Amit Z (1994) Effects of 3-amino-1,2,4-triazole on brain catalase in the mediation of ethanol consumption in mice. Alcohol 11:235–241.

Koivula T, Koivusalo M (1975) Different forms of rat liver aldehyde dehydrogenase and their subcellular distribution. Biochim Biophys Acta 397:9–23.

Koob GF, Sanna PP, Bloom FE (1998) Neuroscience of addiction. Neuron 21:467–476.

Kurtz DL, Stewart RB, Zweifel M, Li TK, Froehlich JC (1996) Genetic differences in tolerance and sensitization to the sedative/hypnotic effects of alcohol. Pharmacol Biochem Behav 53:585–591.

Marchner H, Tottmar O (1976) Inhibition of the acetaldehyde dehydrogenase in rat liver by a cyanamide derivative present in a commercial standard diet for small animals. Acta Pharmacol Tox 39:331–343.

Mardones J, Quintanilla ME (1996) Effects of bromocriptine on the voluntary consumption of ethanol, water, and solid food by UChA and UChB rats. Alcohol 13:355–357.

Mardones J, Segovia-Riquelme N (1983) Thirty-two years of selection of rats by ethanol preference: UChA and UChB strains. Neurobehav Toxicol Teratol 5:171–178.

Mardones J, Segovia-Riquelme N (1984) Effects of different diets on voluntary consumption of ethanol in UChA and UChB rats. Acta Physiol Pharmacol Latinoam 34:31–36.

Mardones J, Segovia-Riquelme N, Hederra A (1953) Heredity of experimental alcohol preference in rats. II. Coefficient of heredity. Q J Stud Alcohol 14:1–2.

Marjanen L (1972) Intracellular localization of aldehyde dehydrogenase in rat liver. Biochem J 127:633–639.

Mellamby E (1919) Alcohol: its absorption into and disappearance from the blood under different conditions. MRC Spec Rep Ser (Lond) 31:1–48.

Meyer HH (1901) Zur theorie der alkoholnarkose. Der einfluss wechsel on der temperature auf wirkungsstärke und teilungkoefficient der narkotika. Arch Exp Pathol Pharmacol 46:338.

Neumark YD, Friedlander Y, Durst R, Leitersdorf E, Jaffe D, Ramchandani VA, O'Connor S, Carr LG, Li TK (2004) Alcohol dehydrogenase polymorphisms influence alcohol-elimination rates in a male Jewish population. Alcohol Clin Exp Res 28:10–14.

Parrilla R, Okawa K, Lindros KO, Zimmerman UJP, Kobayashi K, Williamson JR (1974) Functional compartmentation of acetaldehyde oxidation in rat liver. J Biol Chem 249:4926–4933.

Plapp BV (1975) Rate-limiting steps in ethanol metabolism and approaches to changing these rates biochemically. Adv Exp Med Biol 56:77–109.

Quertemont E, Tambour S (2004) Is ethanol a pro-drug? Role of acetaldehyde in the central effects of ethanol. Trends Pharmacol Sci 25:130–134.

Quintanilla ME, Callejas O, Tampier L (2001) Differences in sensitivity to the aversive effects of ethanol in low-alcohol-drinking (UChA) and high-alcohol-drinking (UChB) rats. Alcohol 23:177–182.

Quintanilla ME, Callejas O, Tampier L (2002) Aversion to acetaldehyde: differences in low-alcohol-drinking (UChA) and high-alcohol-drinking (UChB) rats. Alcohol 26:69–74.

Quintanilla ME, Sepúlveda S, Tampier L (1993) Effect of diet and disulfiram on acetaldehyde blood levels after ethanol in UChA and UChB rats. Alcohol 10:381–385.

Quintanilla ME, Tampier L (1995) Acetaldehyde metabolism by brain mitochondria from UChA and UChB rats. Alcohol 12:519–524.

Quintanilla ME, Tampier L (2000) Effect of naltrexone on acute tolerance to ethanol in UChB rats. Alcohol 21:245–249.

Quintanilla ME, Tampier L (2003) Acetaldehyde-reinforcing effects: differences in low-alcohol-drinking (UChA) and high-alcohol-drinking (UChB) rats. Alcohol 31:63–69.

Quintanilla ME, Tampier L, Sapag A, Israel Y (2005b) Polymorphisms in the mitochondrial aldehyde dehydrogenase gene (*Aldh2)* determine peak blood acetaldehyde levels and voluntary ethanol consumption in rats. Pharmacogenet Genomics 15:427–431.

Quintanilla ME, Tampier L, Valle-Prieto A, Sapag A, Israel Y (2005a) Complex I regulates mutant mitochondrial aldehyde dehydrogenase activity and voluntary ethanol consumption in rats. FASEB J 19:36–42.

Radlow R (1994) A quantitative theory of acute tolerance to alcohol. Psychopharmacology 114:1–8.

Rawat AK (1977) Effects of fructose and other substances on ethanol and acetaldehyde metabolism in man. Res Commun Chem Pathol Pharmacol 16:281–290.

Sapag A, Tampier L, Valle-Prieto A, Quintanilla ME, Moncada C, Israel Y (2003) Mutations in mitochondrial aldehyde dehydrogenase (ALDH2) change cofactor affinity and segregate with voluntary alcohol consumption in rats. Pharmacogenetics 13:509–515.

Schuckit MA (1994) Low level of response to alcohol as a predictor of future alcoholism. Am J Psychiatry 151:184–189.

Segovia-Riquelme N, Hederra A, Anex M, Barnier O, Figuerola-Camps I, Campos-Hope I, Jara N, Mardones J (1970) Nutritional and genetic factor in the appetite for alcohol. In: Popham RE, ed. Alcohol and Alcoholism, pp. 56–63. Toronto: University of Toronto Press.

Segovia-Riquelme N, Vitale JJ, Hegsted DM, Mardones J (1956) Alcohol metabolism in 'drinking' and 'non-drinking' rats. J Biol Chem 233:399–403.

Seiden H, Israel Y, Kalant H (1974) Activation of ethanol metabolism by 2,4-dinitrophenol in the isolated perfused rat liver. Biochem Pharmacol 23:2234–2237.

Socaransky SM, Aragon CMG, Amit Z (1985) Brain ALDH as a possible modulator of voluntary ethanol intake. Alcohol 2:361–365.

Strachan T, Read AP (1999) Human Molecular Genetics, 2nd edn. New York: Wiley-Liss.

Tabakoff B, Ritzmann RF (1979) Acute tolerance in inbred and selected lines of mice. Drug Alcohol Depend 4:87–90.

Tampier L, Mardones J (1979) Catalase mediated oxidation of ethanol by rat brain homogenates. IRCS Med Sci-Biochem 7:389.

Tampier L, Mardones J (1986) Effect of 3 amino-1,2,4-triazole on ethanol blood levels after intraperitoneal administration. Alcohol 3:181–183.

Tampier L, Mardones J (1999) Differences in ethanol sensitivity and acute tolerance between UChA and UChB rats. J Stud Alcohol 60:68–171.

Tampier L, Prado C, Quintanilla ME, Mardones J (1999b) Effect of bromocriptine on acute ethanol tolerance in UChB rats. Addict Biol 4:317–321.

Tampier L, Quintanilla ME (2002a) Effect of a dose of ethanol on acute tolerance and ethanol consumption in alcohol drinker (UChB) and non-drinker (UChA) rats. Addict Biol 7:279–284.

Tampier L, Quintanilla ME (2002b) Effect of acetaldehyde on acute tolerance and ethanol consumption in drinker and non-drinker rats. J Stud Alcohol 63:257–262.

Tampier L, Quintanilla ME (2003) Involvement of brain ethanol metabolism on acute tolerance development and on ethanol consumption in alcohol-drinker (UChB) and non-drinker (UChA) rats. Addict Biol 8:279–286.

Tampier L, Quintanilla ME, Contreras S, Segovia-Riquelme N, Mardones J (1984) Biological similarities and differences between rats genetically different in alcohol preference. Alcohol Alcohol 19:203–209.

Tampier L, Quintanilla ME, Letelier C, Mardones J (1988) Effect of 3-amino-1,2,4-triazole on narcosis time and lethality of ethanol in UChA rats. Alcohol 5:5–8.

Tampier L, Quintanilla ME, Mardones J (1981) Effects of 3-amino-1,2,4-triazole pretreatment on ethanol induced narcosis in UChA and UChB rats with different previous ethanol intake. IRCS Med Sci-Biochem 9:188–189.

Tampier L, Quintanilla ME, Mardones J (1985) Effects of diets decreasing ethanol consumption on acetaldehyde metabolism in UChA and UChB rats. Alcohol Alcohol 20:411–416.

Tampier L, Quintanilla ME, Mardones J (1994) Acetaldehyde metabolism: differences between UChA and UChB rats. Alcohol Alcohol 29:751–755.

Tampier L, Quintanilla ME, Mardones J (1995) Effects of aminotriazole on ethanol, water and food intake and on brain catalase in UChA and UChB rats. Alcohol 4:341–344.

Tampier L, Quintanilla ME, Mardones J (1999a) Effect of nicotinamide administration on ethanol consumption and on liver and brain acetaldehyde oxidation rate, by UChB rats. Addict Biol 4:191–195.

Tampier L, Quintanilla ME, Mardones J (2000) Acute tolerance, alcohol sensitivity and drinking pattern in the F_2 generation of UChA and UChB rats. J Stud Alcohol 61:647–651.

Tampier L, Sánchez E, Quintanilla ME (1996) Acetaldehyde metabolism by liver mitochondrial ALDH from UChA and UChB rats: effect of inhibitors. Addict Biol 1:379–384.

Thanos PK, Volkow ND, Freimuth P, Umegaki H, Ikari H, Roth G, Ingram DK, Hitzemann R (2001) Over expression of dopamine D_2 receptors reduces alcohol self-administration. J Neurochem 78:1094–1103.

Thiele TE, Marsch DJ, Ste Marie L, Bernstein IL, Palmiter RD (1998) Ethanol consumption and resistance are inversely related to neuropeptide Y levels. Nature 396:366–369.

Thomasson HR, Edenberg HJ, Crabb DW, Mai XL, Jerome RE, Li TK, Wang SP, Lin YT, Lu RB, Yin SJ (1991) Alcohol and aldehyde dehydrogenase genotypes and alcoholism in Chinese men. Am J Hum Genet 48:677–681.

Tottmar SO, Pettersson H, Kiessling KH (1973) The subcellular distribution and properties of aldehyde dehydrogenases in rat liver. Biochem J 135:577–586.

Tu GC, Israel Y (1995) Alcohol consumption by Orientals in North America is predicted largely by a single gene. Behav Genet 25:59–65.

Vasiliou V, Ziegler TL, Bludeau P, Petersen DR, Gonzalez FL, Deitrich RA (2006) CyP2E1 and catalase influence ethanol sensitivity in the central nervous system. Pharmacogenet Genomics 16:51–58.

Waller MB, McBride WJ, Lumeng L, Li TK (1983) Initial sensitivity and acute tolerance to ethanol in the P and NP lines of rats. Pharmacol Biochem Behav 19:683–686.

Wescott JY, Weiner H, Schultz J, Myers RD (1980) In vivo acetaldehyde in the brain of the rat treated with ethanol. Biochem Pharmacol 29:411–417.

Zimatkin SM, Liopo AV, Deitrich RA (1998) Distribution and kinetics of ethanol metabolism in rat brain. Alcohol Clin Exp Res 22:1623–1627.