Novartis Foundation Symposium 285

ACETALDEHYDE-RELATED PATHOLOGY: BRIDGING THE TRANS-DISCIPLINARY DIVIDE

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ACETALDEHYDE-RELATED PATHOLOGY: BRIDGING THE TRANS-DISCIPLINARY DIVIDE

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Chair's introduction

Peter Emery

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Although there are three proposers listed for this symposium, the idea for this meeting came largely from Victor Preedy, and his enthusiasm caught up Mikko Salaspuro and myself. I have been working with Victor for a few years on a number of projects, one of which has involved looking at aspects of acetaldehyde protein adducts. As we were working on this project, the idea came to us that although many people are interested in acetaldehyde because of its role as a metabolite of alcohol, there are in fact many other aspects of acetaldehyde that are equally important. The idea here was to bring together people who are approaching acetaldehyde from a number of different perspectives to try to understand more about what these different approaches can bring.

Acetaldehyde is an appealingly simple small molecule that is pretty reactive *in vitro*, and has some fairly serious effects *in vivo* in a variety of physiological systems. I would like to outline some of the questions that we might want to explore during this meeting.

As a nutritionist I tend to start from a simple point of view, asking questions such as 'where does it come from?' Many people will think of it mainly as the first metabolite of alcohol. Perhaps the key to understanding many of the damaging effects of acetaldehyde is the distribution of the alcohol dehydrogenase (ADH) enzymes in tissues. We know that there is a fair amount of acetaldehyde produced quite separately from the ingestion of ethanol. In particular, the bacteria in the oral cavity and throughout the gut can produce it. They may be contributing significant amounts of acetaldehyde: does this have systemic effects or is it a local phenomenon?

We also know that acetaldehyde is a product of cigarette smoke. We will hear about the effects of acetaldehyde from this source and its interaction with alcohol intake. There are also increasing amounts of acetaldehyde in the air: it is a volatile molecule produced by combustion of hydrocarbons. As we live in an increasingly polluted world, more of our exposure to acetaldehyde may be coming from the air.

There is also acetaldehyde in some foodstuffs, particularly fermented foods. We don't know whether this is a significant source of acetaldehyde intake as well.

Once it is in the body, how is it metabolized? It is metabolized by various dehydrogenase and oxidase enzymes. There are background levels of these activities, which may increase considerably in response to exposure. We will hear a lot about polymorphisms of these different enzymes, which give us useful biological models for studying exposure to acetaldehyde. People produce very different amounts of acetaldehyde in response to the same amount of alcohol intake.

There are other aldehydes present, and some of the systems we have for metabolizing acetaldehyde will also metabolize other aldehydes. The interaction with other aldehydes could be key to understanding some of the physiological actions of acetaldehyde. This leads us to consider the whole question of the variety of antioxidant defences that may be induced and up-regulated when we are exposed to acetaldehyde.

It comes in, it is metabolized, but what we really need to measure is how much acetaldehyde is present in various tissues and for how long. This is difficult with a molecule like this that is short-lived and moves around. Instead of tissue concentrations we may have to look at proxies such as blood levels. Saliva may be useful: of course, this will reflect what is produced in the mouth, but it could also reflect systemic production and exposure to an extent. Acetaldehyde is a volatile molecule so we may be able to measure it in the breath. We may be able to measure products of acetaldehyde metabolism in the urine; this could be a long-term integrative measure of exposure, rather than reflecting acute changes.

A key question from a biochemical viewpoint is the mechanism by which damage is caused. I'm sure we'll hear a lot about this fairly vague term 'oxidative stress'. Hopefully, we can be more precise in our discussion to clarify what is meant by this term. As an electrophilic molecule, acetaldehyde will attack many nucleophilic centres in a variety of important molecules, particularly forming adducts with DNA and protein, and indeed the lipid components. The question then becomes, what is the subsequent damage caused by production of these adducts? Which sorts of proteins may be affected? Proteins in signalling pathways may be amplifying the signal and the damage that is caused. There could be effects within the nucleus through transcription factors or DNA repair enzymes, or epigenetic effects on histone decoration.

We will consider the tissues that are affected. Victor Preedy always teaches his students about the effects of alcohol on different tissues. When we look at where acetaldehyde might be having its damaging effects, it concerns a great variety of tissues, and not just the liver. There is the gut, and many cancers through the gastrointestinal tract may relate to acetaldehyde damage. There could be effects on the brain, which could lead not only to behavioural effects but also degenerative diseases. There are effects on the heart and cardiovascular system, and effects on skeletal muscle. The lung will be exposed to acetaldehyde from the atmosphere, and asthma, bronchitis and emphysema have all been linked with acetaldehyde.

Breast cancer development seems to be sensitive to differences in acetaldehyde production.

Finally, the question we would like to move towards: what can we do about it? We are developing the idea that acetaldehyde is a damaging molecule, so are there ways of avoiding or minimizing this damage? Are there agents that could be used to bind acetaldehyde and reduce the amount that is present? Can we affect the amounts that are produced by reducing bacterial populations or manipulating them to change their characteristics? What can we do with the enzymes that are involved in producing or removing acetaldehyde to minimize the amount of acetaldehyde we are exposed to?

The following list highlights some of the questions about acetaldehyde that we might want to explore over the next few days:

- How does it get into the body?
- How is it metabolized?
- How much is there?
- How does it cause damage?
- Which tissues are affected?
- What can we do about it?

So let's start the story where it needs to begin, by looking at alcohol dehydrogenase and other enzymes involved in acetaldehyde production.

Acetaldehyde generating enzyme systems: roles of alcohol dehydrogenase, CYP2E1 and catalase, and speculations on the role of other enzymes and processes

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Abstract. Most acetaldehyde is generated in the liver by alcohol dehydrogenase (ADH) during ethanol metabolism. Polymorphic variants of these genes encode enzymes with altered kinetic properties, and pathophysiological effects of these variants may be mediated by accumulation of acetaldehyde. Two additional pathways of acetaldehyde generation are by the cytochrome P450 2E1 (CYP2E1) and catalase. While the amount of ethanol oxidized by these enzymes comprises a small fraction of total body ethanol clearance, the local formation of acetaldehyde by these enzymes may have important effects. Additional sources of acetaldehyde include other minor enzymes (nitric oxide synthase, other cytochrome P450s, P450 reductase, xanthine oxidoreductase) as well as non-enzymatic pathways (formation of hydroxyethyl radicals from the reaction of ethanol with hydroxyl radical, and its subsequent decomposition to acetaldehyde). Acetaldehyde may have effects locally (in the cells generating it), or when delivered to other cells by the blood stream or saliva, or by diffusion from the lumen of the gastrointestinal tract. The ultimate determinants of acetaldehyde toxicity include rates of its formation, rates of oxidation, and the capacity of cellular systems to prevent or repair chemical effects of acetaldehyde (e.g. formation of protein adducts or modification of nucleic acid bases).

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Chronic consumption of large amounts of ethanol has well known effects on the heart, liver, brain, muscles, fetus and pancreas, and is involved in the pathogenesis of a number of neoplasms. The susceptibility of individuals to the ill effects of alcohol consumption is due to complex interactions of genes and the environment. Many of the effects of ethanol are mediated by acetaldehyde, which is mainly

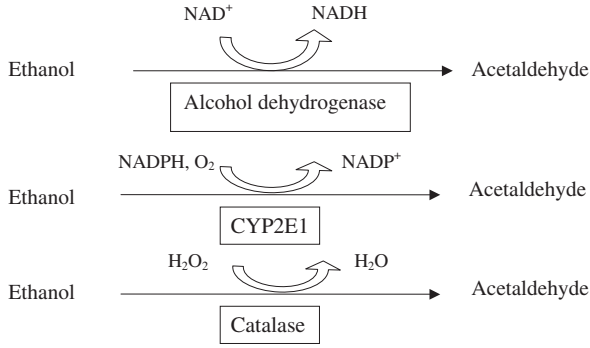

FIG. 1. Major enzymatic pathways for acetaldehyde formation. The major pathways of acetaldehyde formation, alcohol dehydrogenase, cytochrome P450 2E1 (CYP2E1), and catalase are shown with their cofactors, substrates and products.

generated by alcohol dehydrogenase (ADH), cytochrome P450 2E1 (CYP2E1) and catalase (Fig. 1). Some of the enzymes involved in acetaldehyde formation are genetically polymorphic; when the polymorphism alters the enzymatic properties of the enzyme or the level of its expression, different individuals may generate varying amounts of acetaldehyde in a given tissue. Furthermore, the enzyme activities are in some cases regulated by transcriptional or translational mechanisms, controls, concentrations of substrates and products, and by post-translational modification (Table 1). These enzymes, the regulation of their activity, and tissue distribution, as well as some minor enzymatic processes that form acetaldehyde, are the subject of this overview.

Enzymology of acetaldehyde formation

Alcohol dehydrogenases

General description. The enzymes responsible for the bulk of alcohol oxidation are the ADHs. All are dimeric enzymes with subunit molecular weight of about 40 kDa; subunits are identified by Greek letters. These enzymes are grouped into classes based upon enzymatic properties and the degree of sequence similarities. Enzyme subunits belonging to the same class can heterodimerize. The general properties of these enzymes are summarized in Table 2. Class I contains α, β, and γ isozymes. These enzymes have a low K_m for ethanol and are highly sensitive to inhibition by pyrazole derivatives. They are very abundant in liver, and play a major role in alcohol metabolism. Class II ADH (πADH) is also abundant in liver, has a higher K_m for ethanol, and is less sensitive to pyrazole inhibition than class I enzymes (Ehrig

TABLE 1 Properties of alcohol dehydrogenases (ADHs) in humans

Gene locus	*New nomenclature*	*Subunit type*	*K_m (ethanol)*	*V_{max}*	*Tissue distribution*
Class I					
ADH1	*ADH1A*	α	4	54	Liver
ADH2	*ADH1B*	β	0.05–34**	—	Liver, lung
ADH3	*ADH1C*	γ	0.6–1**	—	Liver, stomach
Class II					
ADH4	*ADH4*	π	34	40	Liver, cornea
Class III					
ADH5	*ADH5*	χ	1000	—	Most tissues
Class IV*					
ADH7	*ADH7*	σ, μ	20	1510	Stomach, oesophagus, other mucosae
Class V*					
ADH6	*ADH6*	–	30	?	Liver, stomach

*Tentative assignments based upon sequence homologies. K_m values are given in mM and V_{max} values are given in terms of turnover number (min^{-1}). Tissue distributions indicate tissues with relatively high expression; see Fig. 3 for more detailed distribution information.
**Kinetic constants vary with isozyme, see Table 2.

TABLE 2 Properties of polymorphic forms of human alcohol dehydrogenase (ADH)

Gene locus	*Subunit type*	*K_m (ethanol)*	*V_{max}*	*Population*
*ADH2*1 (ADH1B*1)*	β1	0.05	9	Caucasians, African-Americans
*ADH2*2 (ADH1B*2)*	β2	0.9	400	Asians
*ADH2*3 (ADH1B*3)*	β3	34	300	African-Americans
*ADH3*1 (ADH1C*1)*	γ1	1.0	87	All groups
*ADH3*2 (ADH1C*2)*	γ2	0.63	35	Caucasians

The kinetic constants are noted for the homodimers of the subunits listed (Ehrig et al 1990). Heterodimers behave as if the active sites were independent. The K_m values are in mM and the V_{max} values are given in terms of turnover numbers (min^{-1}), as in Table 1. The column labelled population indicates which populations have high allele frequencies for these variants. The alleles are not limited to those populations.

et al 1990). Class III ADH (χADH) is present in nearly all tissues, is virtually inactive with ethanol, but can metabolize longer chain alcohols, ω-hydroxy-fatty acids, and formaldehyde. A recent paper suggested that class III ADH might be more active towards ethanol in a hydrophobic environment, and argues that liver cytosol may be such an environment (Haseba et al 2006).

The class IV enzyme was purified from stomach and oesophagus (Pares et al 1994). σADH has the highest V_{max} of the known ADHs and is very active towards

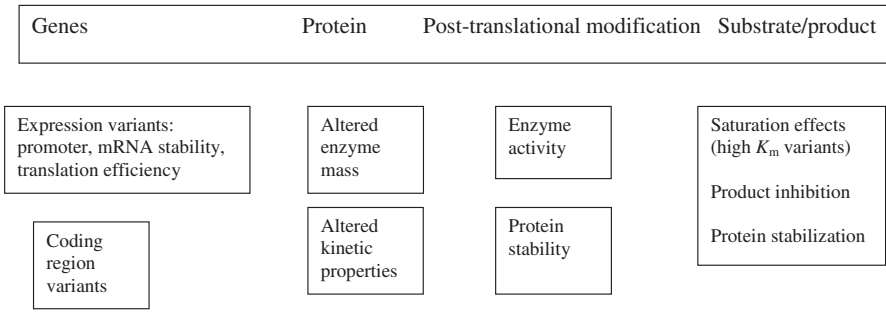

FIG. 2. Factors which control the rate of enzymatic generation of acetaldehyde. Genetic variation can influence the expression of the gene (transcriptional effects) or the stability or translational efficiency of the mRNA, as well as alter the coding sequence. Transcriptional or mRNA effects will result in varying amounts of active enzyme, and thus determine the maximum flux through the pathway. Coding region variants for ADH have widely varying kinetic properties. Post-translational modifications can influence the activity of an enzyme or its susceptibility to degradation, as can the degree of substrate binding to the enzyme, as in the case of CYP2E1 stabilization by substrate. The enzyme activity is ultimately determined by the concentrations of substrate and product, the kinetic constants for each isozyme, and the total activity of the enzyme, as defined by the kinetic rate equation for the enzyme (Crabb et al 1983).

retinol. This may be relevant to its expression in numerous epithelia which are dependent on retinol for their integrity. Class V ADH, encoded by the *ADH6* gene, is expressed in liver and in stomach, but the enzyme itself has not been purified. *In vitro* expressed enzyme had a high K_m for ethanol (about 30 mM), and moderate sensitivity to pyrazole inhibition (Cheng & Yoshida 1991). Class VI ADH was reported in deer, mouse and rat liver; class VII ADH was cloned from chicken, but the human homologues have not been found.

Genetic variants. The nomenclature for *ADH* genes was recently revised. The *ADH1, 2* and *3* genes are now designated *ADH1A*, *ADH1B*, and *ADH1C* genes, respectively. Two of the seven human *ADH* gene loci are polymorphic, and the prevalence of the alleles depends on continental origin. The kinetic properties and population distributions of these allelic enzymes are shown in Fig. 3. The isozymes encoded by the three *ADH1B* alleles, differing at single amino acids, vary markedly in K_m for ethanol and V_{max}. β1 is most common in Caucasians, has a low V_{max} and a very low K_m for ethanol. β2 is found in Asians and Ashkenazi Jews. It has a substantially higher V_{max} and somewhat higher K_m compared with β1. The β3 isozyme was first detected in samples from African-Americans, and has also been found in Southwest Native Americans. It has a high K_m for ethanol and high V_{max}. Smaller differences in enzymatic properties are observed between the products of the *ADH1C* alleles. The γ1 isozyme has about twice the V_{max} of the γ2 isozyme,

Tissue	ADH1C	ADH4	ADH6	ADH7	CYP2E1	CAT
blood	0	17	0	0	53	367
bone	13	0	0	0	13	55
bone marrow	0	0	0	0	0	634
brain	27	0	1	0	19	47
connective tissue	74	0	0	0	0	65
adipose tissue	4251	0	0	0	0	144
liver	1930	729	252	0	843	319
pancreas	36	4	4	0	0	95
adrenal gland	611	0	0	0	0	32
thyroid	0	0	0	0	18	163
placenta	16	0	0	0	0	121
eye	9	0	0	19	0	67
cervix	62	0	20	0	0	41
ovary	0	0	9	0	28	0
uterus	217	0	8	0	4	62
prostate	32	0	0	0	6	51
testis	28	0	11	0	8	48
bladder	132	0	0	33	0	99
kidney	56	0	84	0	0	79
tongue	30	0	15	90	0	30
larynx	32	0	0	32	0	98
pharynx	0	0	0	0	0	0
salivary gland	0	0	48	0	0	146
heart	602	0	55	0	0	100
lymph node	10	0	0	0	0	146
spleen	416	0	0	0	0	37
thymus	135	0	0	0	13	0
mammary gland	450	29	23	0	29	58
muscle	122	0	8	17	8	69
lung	169	0	0	40	28	69
trachea	1444	0	0	288	0	20
skin	21	0	0	0	0	85
vascular	118	0	0	0	0	157
small intestine	1558	22	90	0	0	22
colon	153	0	14	0	0	84
stomach	254	0	48	9	0	19
esophagus	472	0	52	996	0	0
nerve tissue	550	0	0	0	39	118

FIG. 3. Tissue distribution of ADH, CYP2E1, and catalase transcripts reflected by the abundance of expressed sequence tags (ESTs). Tissue distribution of ESTs for the noted genes were obtained from the NCBI Unigene Database using the EST Profile Viewer (e.g. *http://www.ncbi.nlm.nih.gov/UniGene/ESTProfileViewer.cgi?uglist=Hs.78989* for ADH5). The number in each cell is the number of transcripts per million, a measure of the abundance of the transcripts. The spot intensity is based on the transcripts per million.

while the K_ms for ethanol are similar. γ1 ADH is found at high frequency in Asians and African-Americans; Caucasians have about equal frequency of γ1 and γ2 ADH alleles (Burnell & Bosron 1989). The other *ADH* loci have not been found to be polymorphic to date.

Individuals expressing *ADH1B*2* and *ADH1B*3* would be predicted to metabolize ethanol more rapidly and generate more acetaldehyde; however, effects of the polymorphism on ethanol elimination rates are small. Different *ADH1B*2* genotypes are correlated with only a small fraction of the between-individual differences in alcohol elimination rates. The *ADH1B*3* polymorphism confers a 10% increase in the rate of ethanol metabolism; both it and *ADH1B*2* are protective against alcoholism (Edenberg et al 2006). The *ADH1C* polymorphism did not affect alcohol elimination rate, but recent data link the *ADH1C*1* allele with head and neck, oesophageal, breast and hepatocellular carcinomas (Homann et al 2006), which could reflect increased rates of acetaldehyde formation.

An additional *ADH* genetic variant is a *Pvu* II restriction fragment length polymorphism (RFLP) in an intron of the *ADH1B* gene. It is not known if the variant alters expression of the gene or is linked to another susceptibility locus; the B allele was found at higher frequency in alcoholics and in patients with alcoholic cirrhosis (Sherman et al 1993). Single nucleotide polymorphisms (SNPs) presumed to influence expression of the *ADH4* gene have been linked to risk of alcoholism (Edenberg et al 2006); one polymorphism in the promoter affects gene expression (Edenberg et al 1999). Similarly, sequence variants in the promoter of *ADH1C* may affect its expression (Chen et al 2005).

Control of expression of ADHs. The *ADH1* promoters are all active in liver. They interact with ubiquitous transcription factors (e.g. TATAA binding factors, upstream stimulatory factor [USF], CTF/NF-I and Sp1-like factors), as well as tissue-specific factors (e.g. hepatocyte nuclear factor 1 [HNF-1], D-box binding protein [DBP] and CCAAT-enhancer binding proteins [C/EBPα and β]). An HNF-1 site was recently reported to serve as a master control for all three of the class I genes (Su et al 2006). The *ADH5* and *ADH7* promoters lack TATAA boxes. The *ADH5* promoter is G+C rich, a characteristic of housekeeping genes and consistent with its ubiquitous expression. Binding sites for thyroid hormone, retinoic acid and glucocorticoid receptors have been identified in the upstream regions of *ADH1* genes. In *in vitro* experiments, retinoic acid and glucocorticoids activated the promoters and thyroid hormone antagonized the effect of retinoic acid; these hormones had less dramatic effects *in vivo*. Growth hormone increased ADH activity in rats and cultured hepatocytes, while androgens and thyroid hormones decreased it.

Chronic ethanol consumption can affect the expression of ADH. Ethanol increased hepatic ADH activity in male rats by reducing testosterone levels. The amount of ethanol consumed from conventional liquid diets did not alter liver ADH activity, whereas higher doses achieved by intragastric ethanol infusion induced liver

ADH activity. This resulted from induction of the transcription factor C/EBPβ and suppression of C/EBPγ and a truncated, inhibitory form of C/EBPβ called LIP (He et al 2002). In addition, chronic intragastric infusion of ethanol increases portal vein endotoxin, which can induce ADH mRNA via increased binding of USF (Potter et al 2003).

In humans, the amount of ADH in the liver was not induced by chronic drinking; however, with fasting, protein malnutrition and liver disease, ADH activity and the ethanol elimination rate were decreased. Orchiectomy increased alcohol elimination rates in humans. Little is known about expression of extrahepatic ADH, with the exception of gastric ADH, which is reduced with age, in women, and with heavy drinking (Seitz et al 1993).

Post-translational modifications. No post-translational modifications of the ADH enzyme are recognized. However, peroxynitrite can oxidize the active site, causing disulfide formation and release of zinc, inactivating the enzyme (Daiber et al 2002); whether this is physiologically relevant remains to be seen.

Role of substrate and product concentrations. The ADH isozymes with high K_m for ethanol, e.g. β3, π, and σ will be more active when blood ethanol concentrations are high or in tissues of the upper gastrointestinal (GI) tract that are directly exposed to beverage ethanol. Modelling of alcohol oxidation in rat liver indicated that ADH activity was controlled by the total activity of the enzyme as well as product inhibition by NADH and acetaldehyde (Crabb et al 1983); thus ADH operates below its V_{max} at steady state. Our laboratory determined the rate of ethanol oxidation by cells expressing *ADH1B*1*, *ADH1B*2* and *ADH1B*3*. The inhibition constants for β1, β2 and β3 ADH were 1.5 ± 0.1, 22 ± 14 and 210 ± 5 μM, respectively (Matsumoto et al, unpublished data), indicating that activity of β1 and β2 ADH could be limited by the accumulation of acetaldehyde.

Tissue distribution. ADHs are expressed in a variety of tissues. High levels of class I ADH mRNA were found in kidney, stomach, duodenum, colon and uterus of rats, with lower levels in many organs including the lung, small intestine and hepatic Ito cells, and much lower levels were found in brain, thymus, muscle or heart (Estonius et al 1996). Cytosolic ADH has been found in parotid gland, and chronic alcohol use was associated with parotid steatosis (Maier et al 1986). Class I ADH is found in blood vessels, which may be relevant to alcohol-induced flushing and cardiovascular effects of ethanol consumption. Class II ADH was detected in liver and duodenum (Estonius et al 1996). Gastric mucosa contains several ADHs (γ-, χ-, and σADH). σADH is absent in the stomach biopsies of about 30% of Asians, and those lacking this enzyme had lower first pass metabolism of ethanol (Dohmen et al 1996), suggesting that σADH is important in gastric oxidation of ethanol.

Relative expression of various mRNAs can be estimated from the frequency of expressed sequence tags (ESTs) detected in cDNA libraries. Figure 3 shows the relative expression of *ADH1C, ADH4, ADH6* and *ADH7* transcripts in a number of tissues. Microorganisms express numerous forms of alcohol dehydrogenase, which can contribute to the formation of acetaldehyde in the lower GI tract, or wherever microbial overgrowth occurs.

Cytochrome P450 2E1

General description. Ethanol can be metabolized by microsomal ethanol oxidizing systems, predominantly via cytochrome P450 2E1 (CYP2E1). Other cytochromes, CYP1A2 and CYP3A4, also contribute to a lesser extent (Lieber 2004). CYP2E1 is associated with NADPH-cytochrome P450 reductase in the endoplasmic reticulum, and reduces molecular oxygen to water as ethanol is oxidized to acetaldehyde. It is responsible for perhaps 10% of ethanol elimination. CYP2E1 is inducible by chronic drinking especially in the perivenular zone, and it may contribute to the increased rates of ethanol elimination in heavy drinkers. CYP2E1 is induced in fasting, diabetes and by a diet high in fat, which may relate to its ability to oxidize the ketone body acetone. Its K_m for ethanol is about 10 mM; thus CYP2E1 may assume a greater role in ethanol metabolism at high blood alcohol levels. CYP2E1 is unusually 'leaky' and generates reactive oxygen species (ROS) including hydroxyl radical ($OH^{\bullet}$), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyethyl radical ($HER^{\bullet}$). Thus, CYP2E1 is a major source of oxidative stress. CYP2E1 knockout animals had longer sleep times than normal counterparts, suggesting a role for CYP2E1 in brain sensitivity to ethanol (Vasiliou et al 2006).

Genetic variants. An Rsa I (−1053C > T) polymorphism (the Rsa I^+ allele is also named the c1 allele) is located in the 5′-flanking region of the CYP2E1 gene (Hayashi et al 1991) in a region interacting with HNF-1. The Rsa I^- allele (c2) was more active in *in vitro* transcriptional assays, although a corresponding increase in CYP2E1 activity *in vivo* has not been unequivocally confirmed using the clearance of chlorzoxazone as a probe. The frequency of this polymorphism depends on continental origin: the c2 variant is found in 2–8% of Caucasians and in 25–36% of East Asians. Another polymorphism, detectable with the Dra I restriction enzyme, is located in intron 6. The distribution of the variant genotype (lacking the Dra I site) also depends on continental origin: 40–50% of East Asians carry this genotype, while only 10% of Caucasians lack the Dra I site. A more recently described polymorphism is the −71G > T polymorphism in exon 1, which has been associated with enhanced transcriptional activity of promoter constructs in HepG2 cells. Heterozygosity for this allele occurs in about 10% of Caucasians. The effects of the various genotypes on alcohol pharmacokinetics or risk of alcoholic

complications have been inconsistent, and there is no direct evidence of differences in rates of acetaldehyde formation.

Control of expression of CYP2E1. The human *CYP2E1* gene spans 11 kb, contains 9 exons, and contains a typical TATAA box. HNF-1 is critical for its expression. Expression is also controlled both at the level of mRNA (high concentrations of ethanol can induce transcription of the *CYP2E1* gene [Takahashi et al 1993]) and by the stabilization of the protein, as observed for ethanol, acetone and pyrazole derivatives, which reduce the rate of proteasomal degradation (Lieber 2004). Recent data suggest that additional signals may affect its expression. For instance, CYP2E1 can be induced by interleukin (IL) 4 in liver (Lagadic-Gossmann et al 2000) and by phorbol ester and other cellular stresses in astrocytes (Tindberg 2003). Insulin post-transcriptionally reduced the expression of CYP2E1 by destabilizing its mRNA.

Role of substrate and product concentrations. Since CYP2E1 has a high K_m for ethanol, it will generate more acetaldehyde when ethanol concentrations are elevated. There is no evidence that acetaldehyde is a product inhibitor of CYP2E1; in fact, CYP2E1 can oxidize acetaldehyde to acetate, although probably not in the presence of ethanol.

Post-translational modification. CYP2E1 is reported to be a substrate for cAMP-dependent protein kinase A (PKA). Phosphorylation of a serine residue inactivates the enzyme (Oesch-Bartlomowicz et al 1998). Whether this plays a physiological role in controlling activity of this enzyme is not clear, although in several conditions in which CYP2E1 activity is low (fasting, diabetes), hepatic PKA activity is high.

Tissue distribution. CYP2E1 is expressed at highest levels in the liver, as well as numerous other tissues, as demonstrated by western blotting, mRNA, or EST analyses (Fig. 3). These include kidney, lung, oesophagus, biliary epithelium, pancreas, uterus, leukocytes, breast, brain, colon, urinary bladder, nasal mucosa and pancreatic beta cells. Western blots and activity assays have confirmed expression of CYP2E1 in oesophagus, pancreas and lung, among others. In brain, CYP2E1 was reported to be expressed in neurons and inducible by ethanol administration (Tindberg & Ingelman-Sundberg 1996).

Catalase

General description. The peroxisomal catalase is a tetrameric, haem-containing enzyme. In addition to converting hydrogen peroxide (H_2O_2) to water and oxygen, it can oxidize ethanol to acetaldehyde in an H_2O_2-dependent fashion. This pathway is not

thought to be a major elimination pathway under most physiological conditions, but it may be important in certain tissues such as brain; in fact, acatalasaemic mice had longer sleep times than their normal counterparts (Vasiliou et al 2006).

Genetic variation. The absence of active catalase (acatalasaemia) is encountered in Asian populations. A number of SNPs in the 5′ untranslated region and introns are reported, but there are no known effects of these variants on expression or activity of the enzyme, nor on responses to ethanol.

Control of expression. Little is known regarding transcriptional control of catalase expression in mammalian cells. The rat catalase gene is a single-copy gene spanning 33 kb. The promoter region lacks a TATAA box and an initiator consensus sequence, contains multiple CCAAT boxes and GC boxes, and contains multiple transcription initiation sites, consistent with its housekeeping function. Chronic ethanol feeding was reported to increase catalase activity (Orellano et al 1998). The rat catalase promoter contains a peroxisome proliferator responsive element (PPRE [Girnun et al 2002]) and can be induced by peroxisome proliferators.

Post-translational modification. In cells exposed to H_2O_2, Abl and Arg (non-receptor protein tyrosine kinases) associate with catalase and can activate it by phosphorylating two tyrosine residues. However, at higher concentrations of H_2O_2, phosphorylation of these residues can stimulate ubiquitination and proteasomal degradation of the enzyme (Cao et al 2003).

Control by substrate and product levels. The activity of catalase depends upon the availability of H_2O_2. This was observed with perfused rat liver: when fatty acids were added to the perfusate, peroxisomal β oxidation generated H_2O_2 and stimulated ethanol oxidation. This raises the possibility that under conditions of oxidant stress (and H_2O_2 production) catalase-mediated ethanol oxidation may be increased.

Tissue distribution. Catalase is expressed in nearly all tissues (Fig. 3). Catalase is also expressed by colonic micro-organisms and contributes to the formation of acetaldehyde from ethanol in the lower GI tract (Tillonen et al 1998).

Other pathways of acetaldehyde generation

A number of minor pathways of acetaldehyde generation have been suggested. Nitric oxide synthases 1 and 2 were reported to generate 1-hydroxyethyl radical from ethanol in the presence of NADPH and arginine. This is perhaps not

surprising given the presence of a CYP motif within the structure of the enzymes. 1-Hydroxyethyl radical can break down to form acetaldehyde (Porasuphatana et al 2006). Castro et al (2001) reported that cytosolic xanthine oxidoreductase is capable of oxidizing ethanol to acetaldehyde. CYP reductase (in the absence of CYP proteins such as CYP2E1) was reported to oxidize ethanol to 1-hydroxyethyl radical and acetaldehyde, possibly via the semiquinone form of FAD (Diaz-Gomez et al 2000). Other investigators report formation of acetaldehyde from ethanol in tissue extracts for which the responsible enzymes have not been identified in studies using different cofactors and inhibitors. It is possible that other oxidant species (hydroxyl radical) formed non-enzymatically might be able to oxidize ethanol to acetaldehyde. In addition, acetaldehyde can be formed during the degradation of threonine, putatively by threonine aldolase.

Summary

Three major enzymes and several minor enzymes can generate acetaldehyde when ethanol is present. These enzymes are present in virtually all cells; thus, the ability of acetaldehyde to alter cellular function or to modify DNA or proteins, will depend on the rate of acetaldehyde formation (related to ethanol concentration, activity of the enzyme, and the presence or absence of inhibitors of the enzymes), and of its further oxidation by aldehyde dehydrogenases.

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DISCUSSION

Deitrich: I was wondering about the protection against alcoholism given by the ADH variants. Yedi Israel has pointed out that we have been measuring steady state acetaldehyde an hour or two after alcohol has been given, but in UChA rats which have a defective ALDH2 enzyme, the major effect on acetaldehyde levels is seen in 30 min or less (Quintanilla et al 2005, Israel et al 2006). He proposes that it is the burst of acetaldehyde rather than the steady-state level which is preventing people from going ahead and drinking, and not the steady state levels. When were these acetaldehyde levels measured? It could be the burst rather than the steady state level that is important.

Crabb: This thought occurred to us as we were looking at the data from the cells that have ALDH2 and ADH. It seems possible that during the first pass of alcohol through the liver, there would not be that restraining effect of acetaldehyde or alcohol oxidation, and a pre-steady-state burst of acetaldehyde might come out in the hepatic veins. I think we need someone to do the hepatic vein catheterizations as were done in Finland many years ago, to catch that early time point.

M Salaspuro: The question as to why some ADH isoforms may protect from alcoholism is very interesting. We are used to working with hepatocytes, and know

very well how acetaldehyde formation and ethanol oxidation is regulated in the liver. But we don't know much about how they are regulated in the mouth, especially in different populations. And we don't know how either ADH and ALDH are expressed in various cell lines of the mouth mucosa. If acetaldehyde is released in the mouth some of its effects may be much more potent in the brain. Acetaldehyde for example may release histamine from the mast cells or mucosal cells and in this case it escapes the liver. Acetaldehyde may get to the CNS via the arterial tree.

Crabb: I think you can say the same about the oesophagus. Acetaldehyde made there won't pass through the liver, either. If the EST data are correct, acetaldehyde might even be formed beyond where we have been measuring it (i.e. in the hepatic veins) and closer to the brain—even in the arterial tree.

Eriksson: On the other hand, there is work showing that when 4-methylpyrazole is used in normal conditions, there isn't any effect on salivary acetaldehyde. This suggests that no measurable ADH-dependent levels are formed during normal conditions. If acetaldehyde is elevated, e.g. by deficient ALDH activity, then there is an effect of 4-methylpyrazole. I will speak more about this aspect in my paper.

Apte: With regard to the local production of acetaldehyde, the pancreas is a bit of a forgotten organ in terms of its ability to produce acetaldehyde locally. It has been shown that the pancreas can metabolize alcohol. It has ADH. Interestingly, the kinetics of ADH in the acinar cells of the pancreas seems to match most closely to ADH5. It has a very high K_m. In the cells I am interested in, the stellate cells which produce fibrosis, we think we have found ADH1. The problem I have with alcohol and acetaldehyde experiments is that when I read the literature I can't work out whether people are using the concentrations of either ethanol or acetaldehyde that the cells may actually be exposed to *in vivo*. People use concentrations big enough to get an effect. This has always been a worry of mine: I'm concerned that in our own work we are using concentrations as high as 200 μM acetaldehyde, and we justify this by saying that local production during a burst of acetaldehyde might reach as high as that, and in the 30 min it persists for it has enough time to produce these toxic effects. Should we be looking more at steady-state levels? I also have a point regarding your table about ESTs. We have found CYP2E1 protein expression in the pancreas. Not only is it present, but it is also inducible in alcohol-fed rats.

Crabb: I work with neurochemists, and they do interesting things such as *in vitro* microdialysis to get a sense of concentrations present at the pericellular level. I don't know whether this has been done with the liver or other solid organs. If it has been, I don't know whether our analytical methods are sensitive enough to detect acetaldehyde in those dialysates. We could do all sorts of things if we could get real time acetaldehyde concentrations. We need engineers and physicists to give us this kind of instrumentation.