Liver Alcohol Dehydrogenase in Japanese: High Population Frequency of Atypical Form and Its Possible Role in Alcohol Sensitivity

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Liver alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) are two of the enzymes believed to be responsible for ethanol oxidation in man: alcohol dehydrogenase oxidizes alcohol to acetaldehyde, which is then converted to acetic acid by acetaldehyde dehydrogenase. Although several molecular and kinetic investigations of the two enzymes have been performed, their role in the development of the biologically and socially important phenomena of alcohol tolerance and dependence have not yet been resolved.

Human alcohol dehydrogenase is a dimer formed with the random association of three types of subunits (α, β, and γ) encoded at three structural loci designated ADH1, ADH2, and ADH3, respectively [1–4]. Locus ADH1 is probably monomorphic, while polymorphism exists at the ADH2 and ADH3 loci. An “atypical” alcohol dehydrogenase which differs from the “usual” one in total activity, pH optima, and several kinetic parameters has been described [5, 6]. Smith et al. [1, 2] have shown that the atypical enzyme contains a variant β subunit which is encoded at the ADH2 locus. The atypical ADH is differentiated readily from the usual enzyme by the determination of the ratio of ADH activities at pH 8.8 and pH 11.0. Using such measurements, Von Wartburg and co-workers [5, 6] found that 5% of the English and 20% of the Swiss population had the atypical form of alcohol dehydrogenase. A frequency of 10% of the atypical ADH was determined among 166 English subjects by Smith et al. [1]. Fukui and Wakasugi [7] found the situation quite different in Japanese: quantitative determinations of alcohol dehydrogenase in liver samples showed that 90% of the samples tested had activities consistent with presence of the atypical ADH, while 10% had ADH activities characteristic of the usual enzyme.

In this paper data confirming the high frequency of ADH polymorphism in Japanese are presented. Our evidence is based on quantitative data as well as electrophoretic findings. The possible importance of genetically determined dif-

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ferences in alcohol dehydrogenase in the development of the phenomena of alcohol intoxication as well as of tolerance and dependence is discussed. In contrast to the findings for alcohol dehydrogenase, the second enzyme involved in alcohol oxidation, acetaldehyde dehydrogenase, was not found to be polymorphic in the Japanese population.

MATERIALS AND METHODS

A total of 40 liver specimens from Japanese individuals were obtained from autopsies performed in Osaka, Japan. Of these, 28 specimens were from males and 12 from females; one of the individuals was a newborn, while the others ranged in age from 1 to 60 years. Approximately 10 g of liver was packed in a sealed plastic bag, frozen in dry ice, and flown to Seattle. The specimens were received in frozen condition and stored at −70°C before use. Liver homogenates were prepared by grinding the sliced tissue in 10 vol of distilled water in a tissue grinder with a teflon pestle. The homogenates were then frozen and thawed three times and centrifuged twice at 27,000 g for 30 min. The clear supernatants were used for enzyme assays.

Assays of alcohol dehydrogenase were carried out according to the method of Von Wartburg et al. [5] using ethanol as the substrate at pH 8.8 and pH 11.0. Acetaldehyde dehydrogenase was assayed according to the method of Deitrich and Erwin [8] using 0.3 mM acetaldehyde as the substrate at pH 9.5 and 0.1 mM pyrazole to inhibit alcohol dehydrogenase activity. For both assays, the rate of NADH appearance at 25°C was recorded at 340 nm with a Gilford 2400 S recording spectrophotometer. One unit of enzyme activity was defined as the conversion of 1 μmol of substrate per minute. The protein content of the liver extract was determined by the method of Lowry et al. [9], and the specific activity of the enzymes was expressed as units per gram of soluble protein.

Starch gel electrophoresis of homogenates adjusted to similar activities was carried out vertically using a slight modification of the technique of Smith et al. [2]. Both gel and tray contained Tris-PO₄ buffer (0.005 M and 0.1 M, respectively) at pH 7.7. To the gel and the cathodal buffers 0.1 mM of NAD was added. Gels were stained for alcohol dehydrogenase by the method of Smith et al. [2]. For acetaldehyde dehydrogenase, the staining method of Feinstein and Cameron [10] was used.

RESULTS

Alcohol Dehydrogenase

Electrophoretic examination of the liver extracts revealed the complex patterns of liver alcohol dehydrogenase described by Smith et al. [1–4]. However, the samples studied clearly fell into two distinct groups, one with faster migrating cathodal bands and a second with slower migrating bands. The electrophoretic migration of the former phenotype was consistent with that observed for the atypical ADH, while the latter corresponded to the usual form. Representative patterns are shown in figure 1. Since the products of ADH₃¹ and ADH₃² alleles are electrophoretically distinguishable, an electrophoretic resolution between samples from homozygotes and heterozygotes for the atypical enzymes would be expected a priori. However, in our electrophoretic system, atypical ADH₃² homozygotes and ADH₃²/ADH₃² heterozygotes were indistinguishable. This is probably due to the 10-fold difference in relative activity and the minor differences in electrophoretic mobilities of β¹ and β² subunits; these properties make resolution
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Fig. 1.—Starch gel electrophoretic patterns of "usual" (U) and "atypical" (A) forms of alcohol dehydrogenase in three Japanese liver extracts. Samples were adjusted to similar ADH activities by dilution of specimens with atypical enzyme. Note faster cathodal electrophoretic migration of atypical alcohol dehydrogenase.

among several of the expected electrophoretic zones impossible. However, the distinction of the atypical phenotypes from the usual ones is quite obvious (fig. 1). Of the 40 Japanese specimens studied, 34 were atypical while six had the usual ADH phenotype.

The results of assays of ADH activities in the two electrophoretically distinguished groups are shown in table 1 and figure 2. The enzyme assays confirmed the electrophoretic observations that the atypical phenotype has much higher activity.

TABLE 1

ACTIVITIES OF ALCOHOL (ADH) AND ACETALDEHYDE (ALDH) DEHYDROGENASE

<table>
<thead>
<tr>
<th>ADH Type</th>
<th>No. Cases</th>
<th>pH 8.8</th>
<th>pH 11.0</th>
<th>Ratio*</th>
<th>ALDH Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atypical ......</td>
<td>34</td>
<td>120.5 ± 47.7</td>
<td>40.5 ± 15.1</td>
<td>0.36 ± 0.11</td>
<td>13.8 ± 7.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(32-215.0)</td>
<td>(13.4-60.5)</td>
<td>(.15-.59)</td>
<td>(2.1-35.8)</td>
</tr>
<tr>
<td>Usual ..........</td>
<td>6</td>
<td>20.3</td>
<td>45.4</td>
<td>2.4</td>
<td>20.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(11.9-25.9)</td>
<td>(28-72.5)</td>
<td>(1.8-3.7)</td>
<td>(8.9-24.7)</td>
</tr>
</tbody>
</table>

* Mean ratio of activity at pH 11.0 to activity at pH 8.8.

Note.—Activities in IU/g protein. Range of measurements shown in parentheses.
than the usual one. Thus, in the group of atypical specimens, mean ADH activity at pH 8.8 was 120.5 U compared to 20.3 U in the group with usual electrophoretic migration. This sixfold difference agrees well with observations of others [1, 5]. In spite of the broad range of activities, there was no overlap between the measurements for specimens characterized as atypical or usual by electrophoretic examination (fig. 2). Our observations also confirm the findings [1, 5] that the atypical and usual enzymes do not differ in activities at pH 11.0; obviously the ratio of activity at pH 11.0 to that at pH 8.8 is a useful discriminating factor between the two ADH phenotypes.

**Acetaldehyde Dehydrogenase**

Electrophoretic examination of this enzyme failed to reveal the presence of variant forms, at least under the conditions employed. Only two anodal bands were present in all the specimens studied (fig. 3).

Assays of this enzyme revealed a broad range of activities (2.1–35.8 U/g protein), but there was no evidence of bimodality. The activities of acetaldehyde dehydrogenase in specimens having the atypical form of alcohol dehydrogenase were similar to those in specimens with the usual form.

**DISCUSSION**

Our data suggest that 85% of the Japanese specimens examined were either homozygous or heterozygous for the atypical $ADH_2^2$ allele, while 15% of the liver
specimens were from individuals homozygous for the usual $ADH_2^1$ allele. These findings agree well with the data of Fukui and Wakasugi [7] based on assays of liver extracts. From our data, the frequencies of $ADH_2^1$ and $ADH_2^2$ alleles in the Japanese population are calculated to be .39 and .61, respectively. This is in sharp contrast to the estimated frequency of .95 for $ADH_2^1$ and .05 for $ADH_2^2$ in the English population [1].

The biological significance of the polymorphism of alcohol dehydrogenase remains uncertain. Since at physiological pH the atypical alcohol dehydrogenase is many times more active than the usual enzyme, it is possible that persons with the atypical $ADH_2^2$ allele metabolize alcohol differently than the $ADH_2^1$ homozygotes. However, the limited pharmacokinetic data testing this assumption are not conclusive. Edwards and Evans [11] studied the disappearance of alcohol from blood in three persons who were found after assay of liver biopsy specimens to have the atypical enzyme; in only one of these was the disappearance of ethanol faster than normal. Von Wartburg and Schurch [6], on the other hand, have observed significantly more rapid alcohol elimination in a person with the atypical liver ADH. However, measurements of the rates of alcohol elimination over long periods of time may not be the most appropriate tool for testing the biological importance of $ADH_2$ polymorphism. Orme-Johnson and Ziegler [12] have presented evidence that a hepatic ethanol-inducible microsomal enzyme system is involved in the oxidation of ethanol to acetaldehyde, and this has been supported by observations of others [13, 14]. If two enzyme systems (alcohol dehydrogenase
and microsomal oxidases) are involved in the oxidation of ethanol, measurements of rates of alcohol elimination in different ADH phenotypes over a long period of time might not produce clear patterns, since initial ADH-dependent differences in rates of ethanol oxidation might be quickly compensated by the involvement of the microsomal oxidation system.

Several of the pharmacological effects of alcohol drinking have been attributed to acetaldehyde rather than to ethanol (reviewed in [15]). This metabolite has been shown to increase after alcohol administration, reach higher levels in alcoholic than nonalcoholic individuals [16, 17], and has been assumed to play a role in the pathogenesis of both alcohol intoxication [15, 18] and alcohol dependence [19]. Several interesting pharmacological actions have been attributed to acetaldehyde: inhibition of pyruvate-stimulated oxidative carboxylation in brain mitochondria [20]; stimulation of epinephrine release from neural storage depots [21]; and participation in the formation of compounds with structures similar to those of plant alkaloids with high additive potency [19]. The signs of acute alcohol intoxication produced in alcoholics treated with disulfiram after alcohol administration are probably due to the disulfiram-mediated suppression of acetaldehyde dehydrogenase and the consequent acetaldehyde accumulation [22]. In fact, alcohol intoxication phenomena have been produced by administration of acetaldehyde to normal volunteers [23]. The high activity (at physiological pH) of the alcohol dehydrogenase in persons with the \( ADH_2^a \) homozygote is thought to result in rapid oxidation of ethanol to acetaldehyde. Even if the microsomal oxidases play a substantial role in the handling of ethanol, the person with atypical alcohol dehydrogenase might differ from the individual with the usual type of enzyme in the initial rate of acetaldehyde formation and the amount of acetaldehyde released in the circulation soon after alcohol drinking; persons with different ADH genotypes might, therefore, differ in the rapidity of development and in the intensity of signs of alcohol intoxication.

Data on initial acetaldehyde levels after alcohol consumption in persons with various ADH phenotypes are not available, but some indirect evidence for the above assumptions comes from studies on the ethnic differences in alcohol sensitivity. Many Mongoloids respond with rapid, intense flushing of the face and with symptoms of alcohol intoxication after administration of alcohol in amounts which have no apparent effect in Caucasoids. In a study of alcohol sensitivity conducted on Caucasian and Mongoloid infants by Wolff [24], only 5% of the Caucasian infants responded abnormally after alcohol administration, while 83% of the Japanese infants showed visible flushing and increase in red discoloration of the skin on direct spectroscopic examination. This latter frequency is identical with the combined frequencies of heterozygotes and homozygotes for atypical alcohol dehydrogenase observed in our study. Although Wolff [25] prefers to attribute these phenomena to a direct effect of ethanol, we postulate that they are a consequence of the initially high amount of acetaldehyde produced by the highly active atypical alcohol dehydrogenase. The present data and those of Wolff [24] point to a difference between the various ADH genotypes in at least one biologi-
cally important parameter, that is, the level of alcohol administration necessary to produce symptoms of intoxication. Higher sensitivity to alcohol intoxication due to the higher frequency of the atypical \( ADH_2^2 \) gene might thus be the genetic factor underlying the lower frequency of alcoholism in Mongoloids.

Contrasted to the above assumptions are Wolff's studies [25] which show a high frequency of abnormal flushing response among American Indians; alcoholism, however, is a known social problem in the American Indian population. These observations can be reconciled by postulating that the high acetaldehyde production in a person with \( ADH_2^2 \) genotype might have a dual effect. It might lead to increased risk of intoxication, thus deterring the individual from further exposure to alcohol and decreasing the probability of alcoholism. On the other hand, in \( ADH_2^2 \) carriers who persist in drinking alcohol, high acetaldehyde production could lead to an enhancement of endogenous formation of additive compounds [19] and thus be responsible for a faster appearance of the phenomenon of dependence. The interaction of \( ADH_2^2 \) polymorphism with social barriers and peculiarities in social structure could be responsible for the paucity of alcoholism in certain populations and, at the same time, for the high frequency of alcoholism in others.

**SUMMARY**

Electrophoretic and quantitative studies reveal that 85% of Japanese carry an atypical liver alcohol dehydrogenase (ADH). The frequency of ADH polymorphism is identical with the reported frequency of alcohol sensitivity in the Japanese population. This identity in population frequencies points to a causative relationship between the two phenomena and suggests that alcohol sensitivity might be due to the increased acetaldehyde formation in individuals carrying the atypical ADH gene.

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