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EXTRAHEPATIC, DIFFERENTIAL EXPRESSION OF FOUR CLASSES OF HUMAN ALCOHOL DEHYDROGENASE

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Summary. The amounts of mRNA expressed for different alcohol dehydrogenase (ADH) classes were determined in human tissues by Northern hybridization. ADH classes I, II, and III were expressed in all tissues. The mRNAs were highest for class I ADHs, with particularly strong signals in liver, lung, ileum, colon, and uterus. For class II ADH, such a wide tissue distribution had not been recognized previously. Expression of class III ADH was highest in testis, followed by uterus, colon, and ileum. The amounts of class I and III ADH mRNAs varied significantly, indicating that tissue-specific factors modulate the expression of these enzymes above a basal level. Class V ADH (ADH6) was not detected in any of the tissues, including stomach. This suggests that class V ADH is not identical with human stomach σ-ADH (class IV). The results support the general proposition that ADHs are not restricted to liver and have functions other than those in ethanol oxidation.

Most mammalian alcohol dehydrogenases (ADH, EC 1.1.1.1) were isolated from liver. In humans, at least six distinct ADHs have been recognized and categorized in five classes based on their structural homology. Class I ADHs comprise three highly homologous polypeptide chains (α-, β- and γ-subunits) that combine randomly to form homo- and heterodimers. Class II ADH (π) and class III ADH (χ) exist as homodimers only (1). Recently, an additional ADH has been isolated from human stomach and designated σ-ADH (2), while another, ADH6, was identified by sequencing a genomic clone (3). We will employ the assignment of σ-ADH as class IV (4) and ADH6 as class V ADH.

Despite extensive structural and kinetic data on human ADH isozymes and those from other species, distinct physiological functions have not been assigned to most of these. Scattered data on substrate specificities and on inhibitor and activator binding indicate a wide spectrum of potential functions for human ADHs. Primary and secondary alcohols (5) and alcohols in the metabolic pathways of monoamines and serotonin (6-8) are almost equally good substrates for class I ADHs. Testosterone inhibits only the γ-subunit, which oxidizes 3β-hydroxy-5β-steroids

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(9) and also exhibits a strong preference for alcohols in the mevalonate metabolic pathway (10). Class II ADH acts on primary aliphatic alcohols and aromatic aldehydes (11,12). Class III ADH can be activated by fatty acids (13), acts on ω-hydroxy fatty acids (14), and is probably identical with 20-hydroxy-leukotriene B_4 dehydrogenase (15). Furthermore, this enzyme has a glutathione-dependent formaldehyde dehydrogenase activity (16,17). The substrate specificity of class IV ADH is reminiscent of that of class II ADH (2). Thus, there is a wide range of physiological and non-physiological ADH substrates, and it is apparent that *in vitro* kinetic data are insufficient to assign *in vivo* functions.

A thorough study of the extrahepatic tissue distribution of human ADHs has not been conducted much as it could yield important clues on possible physiological roles of these enzymes. With the exception of class III ADH, which is expressed constitutively in virtually all tissues examined, ADHs seem to be associated with particular tissues and expressed to a different extent (18). These past studies of extrahepatic ADHs, however, had been limited mainly by methodology. For example, measurement of enzyme activities is neither sensitive enough nor capable of discriminating between the different ADH isozymes because of overlapping substrate specificities and the lack of specific inhibitors. Similarly, immunological techniques were not applied, because antibodies that could discriminate between all ADH classes have not been available. Therefore, we have used DNA probes to detect the expression of the individual ADH genes, an approach that had been limited so far to class I and III ADH and a few human tissues (19-21).

MATERIALS AND METHODS

Human Tissue Samples. Surgical specimens of human tissues were procured from the National Disease Research Interchange (NDRI), Philadelphia, PA. The samples were snap-frozen, stored at liquid nitrogen temperatures, and shipped on dry ice.

Preparation of Total RNA. Extraction of RNA was performed according to the method of Chomczynski and Sacchi (22). Frozen human tissue samples were ground under liquid nitrogen in a mortar, followed by homogenization in denaturing solution with a Polytron homogenizer (Brinkmann Instruments). For human hepatoma cells (Hep G2, HB8065, American Type Culture Collection), denaturing solution was poured directly into the tissue culture flasks. A liver RNA sample was obtained from Clontech Laboratories. The concentration of RNA was estimated by optical absorbance measurements at 260 nm.

Preparation of Membranes. Samples were prepared in gel-loading buffer (23) containing 40 μg/mL ethidium bromide. 30 μg total RNA of each tissue was fractionated in 1.1% (w/v) agarose gels (Seakem GTG, FMC BioProducts) containing 2.2 M formaldehyde (23). Also applied to the gels were RNA molecular weight markers from Bethesda Research Laboratories. After rinsing several times with water, the gel was kept in 50 mM sodium hydroxide for 20 min, rinsed with 20x SSC (23) and blotted on a Hybond N nylon membrane (Amersham) by vacuum transfer (VacuGene Blotting System from LKB). The membrane was then briefly rinsed with 6x SSC, air-dried, and baked *in vacuo* for 2 h at 80 °C.

Northern Hybridization. Human alcohol dehydrogenase cDNAs were labeled with [α-^{32}P]dCTP (6000 Ci/mmol, DuPont NEN) with a multiprime DNA labeling system (Amersham). The cDNA probes used were class I β-ADH (24), prepared from clone pADH2 by KpnI digestion,

class II ADH (25), prepared as described (26), class III ADH (27), excised from clone 30L by EcoRI digestion, and ADH6 (class V), digested with EcoRI and covering the region from bp 318 to 814 (J.-O. Höög, personal communication). Membranes were prehybridized in 50% (v/v) formamide, 5x SSPE (23), 5x Denhardt's solution, 0.005% (w/v) sodium pyrophosphate, 0.2 mg/mL salmon sperm DNA (Sigma), 80 mM ammonium acetate, and 0.1% SDS (sodium dodecylsulfate) for 3 h at 42 °C (23). Labeled DNA probes were desalted on NAP-5 columns (Pharmacia), heat-denatured, added to the prehybridization solution, and the hybridization continued for 40 h at 42 °C. Blots were washed twice with 1x SSC/0.1% SDS at 22 °C for 15 min, twice with 0.2x SSC/0.1% SDS for 15 min at the same temperature, and once with 0.1x SSC/0.1% SDS for 20 min at the hybridization temperature. X-ray films (X-Omat AR, Kodak) were exposed at -70 °C with two intensifying screens. Exposure times were 4 d, 10 d, 20 h, and 6 d for class I, II, III, and V ADH, respectively. Between hybridizations, the membranes were stored at -70 °C after removal of the DNA probes (28).

RESULTS AND DISCUSSION

Northern hybridization analysis has served to determine the amount of expression of four ADH classes in human tissues and a human liver cell line. Human tissues were chosen for this study owing to the unique isozyme pattern and importance of this enzyme in human biology. Though fresh human tissue samples are generally difficult to obtain and the quality of isolated RNA rarely meets the highest standards of quality, relatively undegraded total RNA was obtained from the snap-frozen tissues (Figure 1).

To establish the sensitivity and specificity of the four cDNA probes, control membranes with different amounts of the four ADH cDNAs were included in every hybridization. The sensitivity of the method was better than 10 pg with a detection limit of approximately 1 pg. Since cross-hybridization between cDNAs was not observed (data not shown), the probes were specific for each of the four ADH classes.

CLASS I ADHS. Class I ADH mRNAs are expressed in all tissues examined (Figure 2A and Table1). The β-ADH cDNA probe detects mRNAs of all three class I ADHs, since sequence differences between the α-, β-, and γ-subunits are only minor. Expression levels in most tissues are higher than those of class II or III ADH (Table1). The amount of mRNA is particularly high in liver, but significant amounts are also found in lung, ileum, colon, and uterus. Three different mRNA sizes of approximately 1.8, 2.8, and 4.2 kb can be distinguished. In lung, ileum, and uterus, a particularly strong hybridization signal of the 2.8 kb mRNA is found.

Although our results indicate a tissue-specific expression of the individual class I ADHs, we do not assign the different message sizes to the three individual class I ADHs, since previous assignments are equivocal. For example, mRNA sizes of 1.6, 1.9, 2.2, 2.6, and 4.3 kb have been detected when two different cDNA probes for β-ADH were used to study class I ADH expression (19,29). Thus, the 1.6 kb mRNA was assigned to α-ADH and the 2.6 kb mRNA to β- and γ-ADH. In liver, size heterogeneity of the transcripts for β-ADH was described (20) and found in different cDNA clones of β-ADH (24). However, the use of specific oligonucleotide probes selected for individual ADH genes has yielded conflicting results. In liver, a 1.6 kb

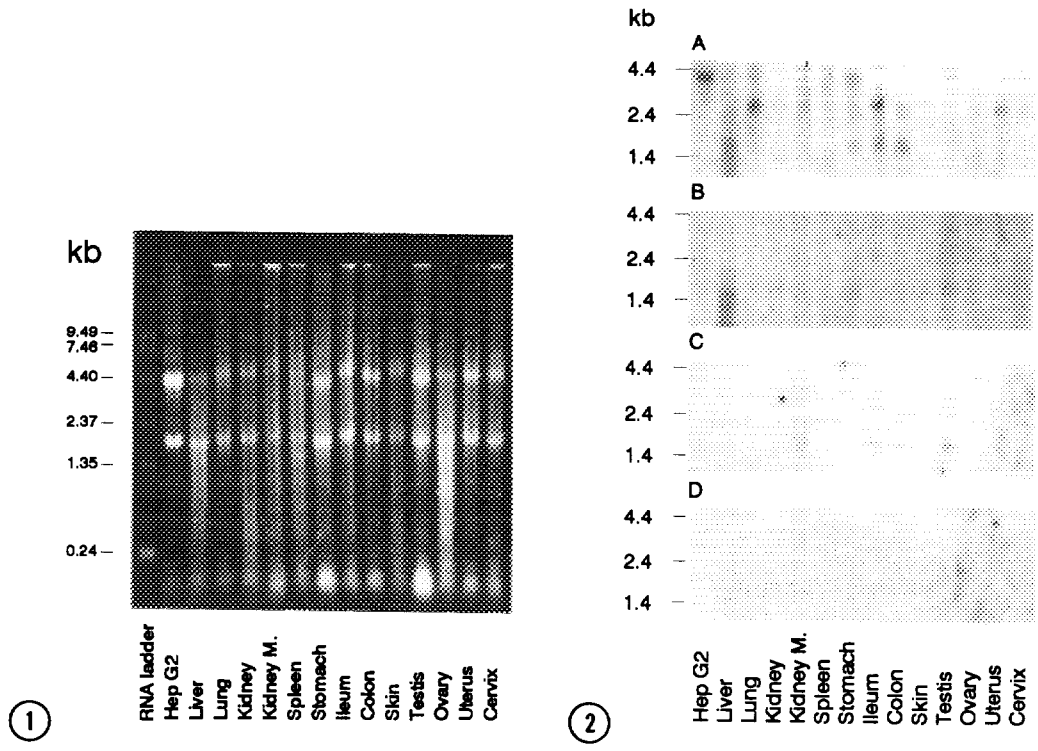

Figure 1. **Agarose Gel Stained with Ethidium Bromide for Human Total RNA.** Each lane contains 30 μg of RNA. The preparation of the samples and the agarose gel are described in "Materials and Methods".

Figure 2. **Northern Blot Analysis of the Expression of Human Alcohol Dehydrogenases (ADHs).** A) class I ADHs; B) class II ADH; C) class III ADH; D) class V ADH; hybridization conditions are described in "Materials and Methods".

mRNA with a minor component at 4.3 kb was attributed to α-ADH, and the mRNA of γ-ADH was thought to show only one band of 1.6 kb (instead of 2.6 kb) (20).

CLASS II ADH. Class II ADH mRNA is also expressed in all tissues, but generally in much lower amounts than class I ADHs (Figure 2B and Table1). It is difficult to assess the signals in the commercial liver RNA sample quantitatively, owing to the poor quality of this particular sample. The signals in liver, kidney medulla, stomach, and testis seem to be slightly higher compared to those of the other tissues. The major signal is seen at an mRNA size of 1.8 kb, accompanied by minor bands at about 3.0 and 4.0 kb. Class II ADH has, thus far, been detected primarily in the liver (30). The present data establish the ubiquity of class II ADH mRNA, in agreement with an earlier indication that this enzyme is expressed in extrahepatic tissues (18).

CLASS III ADH. Class III ADH mRNA is found in all tissues with the highest amount of expression observed in testis, followed by uterus, colon, and ileum (Figure 2C and Table1). The major mRNA sizes, i.e., 1.8 and 2.8 kb, are detected with about equal intensity, a result that

Table 1 **mRNA Levels of Alcohol Dehydrogenases in Human Tissues[a]**

	Alcohol Dehydrogenase mRNA			
Tissue/Cell Line	Class I	Class II	Class III	Class V
Hep G2	+ + + +	+ +	+	-
Liver	+ + + + +	+ +	+ +	-
Lung	+ + + +	+	+ +	-
Kidney	+ +	+	+	-
Kidney Medulla	+ + +	+ +	+ +	-
Spleen	+ +	+	+	-
Stomach	+ + + +	+ +	+	-
Ileum	+ + + + +	+	+ + +	-
Colon	+ + + +	+	+ + +	-
Skin	+ +	+	+	-
Testis	+ + +	+ +	+ + + + +	-
Ovary	+ + +	+	+	-
Uterus	+ + + +	+	+ + +	-
Cervix	+ + +	+	+	-

[a] "+" indicates the ADH expression level estimated by visual inspection within one ADH class as well as in relation to the other classes, taking into account the different times of autoradiography. "-" indicates the absence of a hybridization signal.

differs from a report of multiple mRNAs ranging from 1.7 to 5.6 kb in human liver, spleen, and several unidentified cell lines (21). The strong signal observed in the testis sample is in accord with the fact that testes are a rich source for the isolation of class III ADH (31). The varying amounts of mRNA for this enzyme suggest that there is tissue-specific regulation of χ-ADH in addition to a relatively high basal expression. This is supported by the finding that different regulatory elements are present in the promoter region of the class III ADH gene (32).

CLASS V ADH (ADH6). The mRNA of this enzyme could not be detected in any of the tissues (Figure 2D and Table1). A positive control hybridization established the same sensitivity and selectivity for class V ADH as for the other probes.

The lack of a signal in the stomach sample is in contrast with a report that human stomach contains ADH6 mRNA (3). This latter observation may likely be artifactual, since the method used was based on the polymerase chain reaction. It is well known that after reverse-transcription, this method can over-amplify basal levels of mRNA that are present in almost any cell. The ADH6 gene product has been thought (33) to be identical to human stomach σ-ADH (class IV) (2), an enzyme of possible relevance for human alcohol metabolism. The oxidation of ethanol by gastric mucosa has been reported to be gender-specific with the ADH activity being lower in women (34). Our finding that ADH6 is not expressed in stomach is especially telling

since the RNA sample originated from the stomach of a Caucasian male which would be expected to exhibit a high ethanol-oxidizing activity (34). This result is based on a single sample, however, and should be verified before definitive conclusions can be reached. If confirmed it would demonstrate that ADH6 is not identical with stomach σ-ADH. The role of class V ADH remains elusive since significant expression of this ADH has yet to be demonstrated in any tissue.

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