Alcohol dehydrogenase of human and rat blood vessels

Role in ethanol metabolism

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Abstract Alcohol dehydrogenase (ADH) activity has been detected in all arteries and veins examined from humans and rat. In distinct human autopsy vessels, activity values range from 0.9 ± 0.2 to 9.9 ± 7.7 mU/mg. Distribution of the activity in human aorta was: intima (23.5%), media (74%) and adventia (2.5%). In most of the samples the β1β1 isozyme of class I ADH was the only form responsible for the ADH activity. Class IV ADH (σσ-ADH) was present in three of the 28 individuals examined. The rat blood vessels showed class IV, but not class I, ADH localized in endothelium and media. The physiological role of vascular ADH is probably related to retinoid metabolism and elimination of lipid peroxidation aldehydes. A contribution to human ethanol metabolism is supported by the significant amount of low-K_m activity and the extension of the vascular system.

Key words: Alcohol dehydrogenase; Alcohol metabolism; Blood vessel; Histochemical analysis; Lipid peroxidation; Vascular disease

1. Introduction

Ethanol exerts different effects on the cardiovascular system. Depending upon the concentration of ethanol employed and the type of vessel investigated, ethanol can produce vasodilatation or vasoconstriction [1]. It has also been suggested that both acute and chronic ethanol ingestion contribute to the etiology of hypertension, coronary heart disease, and stroke, and that acetaldehyde plays a role in the cardiovascular effects of ethanol [2–4]. In contrast, several studies have shown that moderate drinking protects against coronary disease (for reviews see [2,5]). One of the possible origins of both the negative and positive effects of ethanol on the cardiovascular system is the metabolism of ethanol by the same system, with the concomitant in situ production of acetaldehyde and NADH. The presence of a class I alcohol dehydrogenase (ADH) isozyme in human aorta has been reported [6] but the level of ADH activity and, therefore, the importance of ethanol metabolism in the blood vessels was not known. In the present work we recognize the ADH forms present in vascular tissues, report the ADH activity in different types of blood vessels, and estimate the contribution of the vascular system to human ethanol metabolism. In addition we have investigated the presence of ADH in the blood vessels of the rat, the most frequently used species in toxicological studies of ethanol.

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Abbreviations: ADH, alcohol dehydrogenase; AMPPD, 3-(2′-spiroadamantane)-4-methoxy-4-(3′′-phosphoryloxy)phenyl1,2-dioxanetane

2. Materials and methods

2.1. Human tissues

Different veins and arteries were obtained at autopsy, 12–24 h postmortem, from individuals (25–75 years old) without circulatory system disease. Blood vessel biopsies were also obtained at surgery of subjects with vascular diseases (varicose veins and atherosclerosis) or trauma. All samples were carefully cleaned from remaining blood by rinsing thoroughly in distilled water, and stored at −80°C. Prior to analysis the specimens were thawed, cut into small fractions and homogenized. Crude homogenates were centrifuged (24 000 × *g*, 1 h) and supernatants were used for activity and electrophoretic analyses.

2.2. Rat tissues

Sprague-Dawley rats (250–350 g) were used. After being killed distinct blood vessel types from six animals were dissected, samples of each type were pooled and kept frozen at −80°C until analysis. Tissues were processed under the same conditions as those for the human samples.

2.3. Histochemical staining

Sections 20 μm thick were cut in a cryostat (Leica) at −20°C, mounted on poly-L-lysine-coated slides and kept at −80°C until use. Prior to staining, sections were dried at room temperature for 30 min, then incubated for 1 h at 42°C in the dark in a staining solution containing 50 mM sodium phosphate, pH 7.6, 5 mM NAD, 2 mM 4-methylpyrazole, 11 mM pyruvic acid, 3.4 mM nitroblue tetrazolium, and 500 mM ethanol as a substrate. After staining, the slides were washed with phosphate-buffered saline, fixed with 4% paraformaldehyde neutral solution for 10 min, dehydrated in ethanol/water, cleared in xylene, and moistened in DPX.

2.4. RNA analysis

Total RNA was isolated by the guanidine isothiocyanate method [7]. PolyA$^+$-RNA was purified by using the PolyAtract System 1000 (Promega). First-strand cDNA synthesis was performed with a *Not*I primer-adaptor (Promega) as a primer. A 1 μl aliquot from the cDNA reaction was subsequently used as a template in a PCR mixture to amplify a 449-bp fragment of class IV cDNA, as previously described [8]. PCR products were separated on a 1% agarose gel, transferred to a Nylon membrane (BioRad) and subjected to hybridization for 24 h with a dUTP-digoxigenin-labeled class IV-specific probe. Detection was performed by incubation at 37°C for 15 min with the chemiluminescent substrate AMPPD, and exposure to an X-ray film for 15 s.

2.5. Enzymology and protein analysis

Alcohol dehydrogenase activity was determined by measurement in a Cary 219 spectrophotometer, the increase of absorbance at 340 nm, due to NADH formation. The enzymatic assay was performed at 25°C in 0.1 M Gly/NaOH, pH 10.0, 2.4 mM NAD, and 33 mM or 1 M ethanol for the human and rat tissues, respectively. Activities are expressed in mU (nmols NADH produced per min)/mg protein, and

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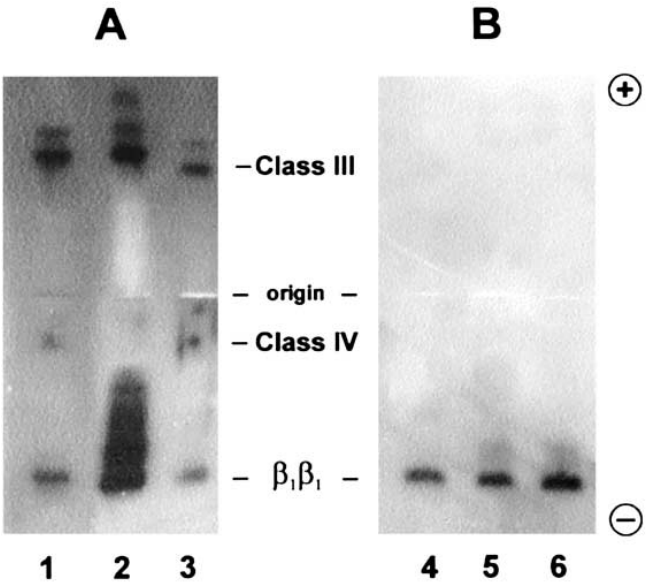

Fig. 1. Starch gel electrophoresis of homogenates from human samples. A: Activity staining with crotyl alcohol. Lane 1: aorta; lane 2: liver; lane 3: esophagus. Samples are from different individuals. B: Activity staining with ethanol. Lanes 4–6: superior vena cava, portal vein and aorta, respectively, from the same individual. All samples were obtained at autopsy.

indicated as the mean ± SD. Protein was determined using the Bio-Rad protein assay. Starch gel electrophoresis was performed as previously reported [9], using 0.1 M crotyl alcohol or 0.2 M ethanol for the activity staining.

The study was approved by the Ethical Committees of the Universitat Autònoma de Barcelona and Hospital de Tarragona Joan XXIII. Informed written consent was obtained from each patient before surgery.

3. Results

3.1. Alcohol dehydrogenase in human blood vessels

Blood vessel samples were obtained from eight individuals at autopsy and from surgical biopsy of 20 individuals. The homogenates of biopsy samples exhibited little ethanol dehydrogenase activity and, in general, faint bands of activity were observed on starch gel electrophoresis. In contrast, the samples obtained at autopsy were much more active, and ADH bands clearly distinguished by electrophoresis (Fig. 1). Apparently the amount of active enzyme was much higher in post-mortem but healthy tissue than in biopsies of blood vessels with pathology. The class III ADH bands were observed in all samples stained with crotyl alcohol, including those from biopsies. In all samples with measurable ethanol dehydrogenase activity, the β1β1-ADH isozyme could be always detected by electrophoresis. All blood vessels analyzed (Table 1 and Fig. 1) exhibited the isozyme. In samples from three individuals (two from autopsy and one from biopsy) σσ-ADH, the class IV ADH, was also clearly detected (Fig. 1).

All specimens obtained at autopsy exhibited measurable ADH activity (Table 1). The wide range of the activity values found for each vessel type results from the interindividual variability. Some subjects exhibited high activity levels for all vessels analyzed, while others showed low activities for all vascular tissues. At present the source of this variability is not clear. It is not related to the age of the patient, neither to the post-mortem time.

Activity of the different layers dissected from a human aorta was measured in the most active specimen, exhibiting 19 mU/mg protein and 94 mU/g fresh tissue. Activity was present in all layers although the contribution of the media was the highest, about 74%; the intima showed 23.5% of the activity, while the adventia only 2.5%.

3.2. Alcohol dehydrogenase in rat blood vessels

The typical alcohol dehydrogenase of the rat vessels is class IV, which is the ADH form characteristic of the stomach (Fig. 2). Class I was not detected while class III ADH was barely observed in the rat vascular tissues. The activity was similar for each vessel studied (Table 1). A histochemical staining of a rat artery, under conditions for detection of class IV activity (in the presence of 2 mM 4-methylpyrazole to inhibit any class I activity), showed the highest activity in the media, lower activity in the intima and no activity in the adventia (Fig. 3). The endothelial cells appeared clearly stained. Class IV cDNA was also detected in rat vessels by RT-PCR and subsequent hybridization with a chemiluminescent class IV-specific probe (Fig. 4), demonstrating the presence of class IV mRNA.

Table 1
Alcohol dehydrogenase activity (mU/mg) in human and rat blood vessels

Vessel	Human (*n*)	Rat
Aorta	9.9 ± 7.7 (4)	n.d.
Carotid	n.d.	6.1 ± 0.7
Portal vein	3.4 ± 3.0 (5)	6.5 ± 0.6
Inferior vena cava	0.9 ± 0.2 (4)	3.8 ± 0.6
Superior vena cava	8.3 ± 7.3 (4)	5.3 ± 1.0

Human samples were obtained at autopsy. Rat samples from six animals were pooled, homogenized and analyzed in triplicate.
n.d., not determined.

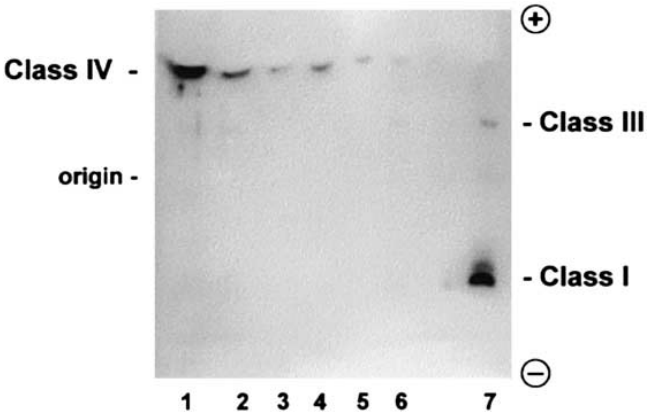

Fig. 2. Starch gel electrophoresis of rat tissue homogenates. Lane 1: stomach; lanes 2–6: blood vessels: portal vein (2), superior vena cava (3), jugular vein (4), carotid artery (5), inferior vena cava (6); lane 7, liver. Gel was stained for activity with crotyl alcohol.

4. Discussion

4.1. Characteristics of alcohol dehydrogenase in blood vessels

Human blood vessels exhibit significant alcohol dehydrogenase activity. The β1β1 class I isozyme is responsible for most of this activity and is present in all vessels studied. Activity is detected in all layers of the aorta, with a higher contribution of the media. Contribution of the intima is significant, while the adventia shows a small activity which may result from contamination of media cells. This distribution is consistent with the histochemical analysis of ADH in rat artery. All human vessels also contain the class III ADH form, i.e. the glutathione-dependent formaldehyde dehydrogenase, whose contribution to ethanol metabolism is negligible. In some individuals the class IV ADH is also detected. We do not know, with the present data, whether the presence of class IV ADH in a few individuals (3 out of 28) results from genetic polymorphism or from a lability of the enzyme, making it undetectable in most of our human samples.

The rat blood vessels show alcohol dehydrogenase characteristics different from those of the human vascular enzyme. We have demonstrated the presence of class IV, at both protein and mRNA levels, in the rat circulatory system. Class I was not detected. Histochemical analyses reveal that class IV is present in the arterial endothelium and mostly in the media, a layer formed by smooth muscle and connective tissue. This demonstrates that class IV, which was initially thought to be essentially an ADH of the epithelia [10,11], is also present in non-epithelial tissues.

The physiological function of ADH in blood vessels is probably similar in both the human and rat species. Both class I and class IV are highly active in the transformation of alcohols and aldehydes of importance for the maintenance of the epithelia. Both enzymes can oxidize retinol to retinal, an important reaction for the production of retinoic acid, an essential modulator of epithelial development [11–13]. Recent evidence suggests that retinoids might also be involved in the differentiation and ageing of vascular smooth muscle cells [14]. Moreover, classes I and IV may play a relevant role in the elimination of aldehydes resulting from lipid peroxidation [12,15]. Class III ADH is also present in blood vessels, and is the main enzyme responsible for formaldehyde elimination, also a major product of lipid peroxidation. It is therefore reasonable that the set of ADH classes in blood vessels constitutes a defence mechanism against cytotoxic aldehydes generated by lipid peroxidation in a highly oxygenated tissue. Glutathione-S-transferase, another enzyme involved in the elimination of these cytotoxic aldehydes, is also present in blood vessels [16]. Both enzymatic systems can, therefore, play a role in the protection against atherosclerosis since lipid peroxidation is believed to be a factor that stimulates the formation of the atherosclerotic plaque [17] and adducts of

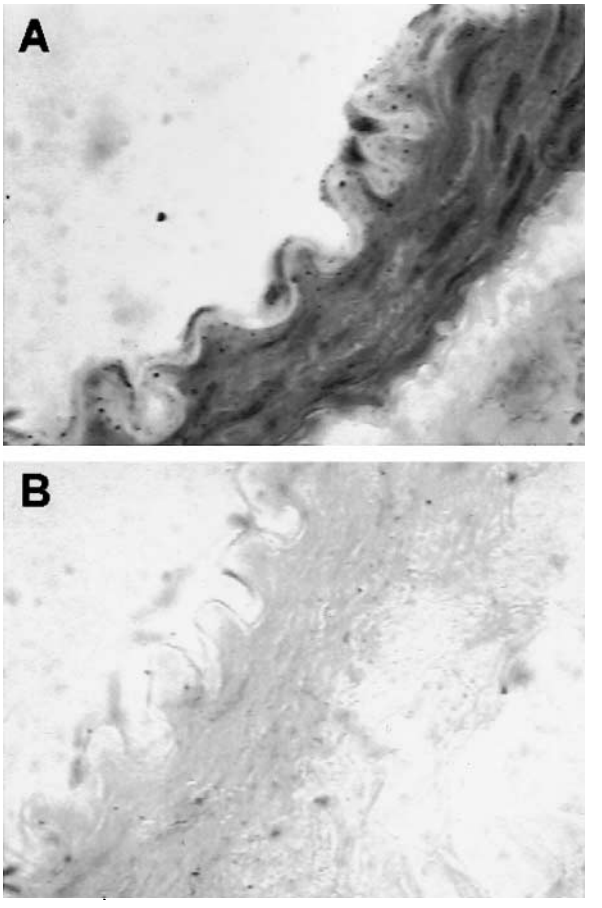

Fig. 3. Histochemical staining for ADH activity of rat arterial tissue sections. Original magnification: ×100. From lumen to the outer part of the vessel: intima, media, and adventia layers. A: 500 mM ethanol and 2 mM 4-methylpyrazole. B: No substrate was added.

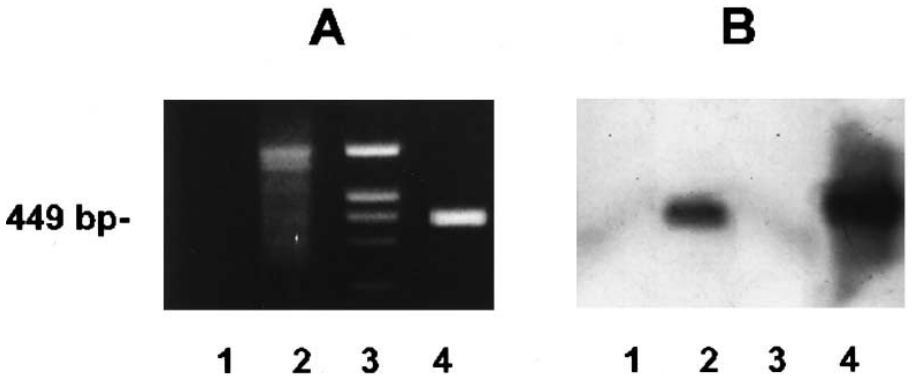

Fig. 4. Detection of mRNA of ADH class IV from rat blood vessels by RT-PCR. Lane 1: Control sample without added DNA template; land 2: PCR products from blood vessels; lane 3: pBR328 DNA digested with *BglI* and *HinfI* (molecular weight marker VI, Boehringer Mannheim); lane 4: PCR products from stomach. A: Ethidium-bromide staining after 1% agarose gel electrophoresis. B: Chemiluminescent bands after hybridization with a class IV-specific probe. The 449-bp band corresponds to a fragment of class IV cDNA.

4-hydroxynonenal and lipoprotein have been localized in human atheroma [18].

4.2. *Role of blood vessel alcohol dehydrogenase in ethanol metabolism*

The isozymes of the *ADH2* gene are characteristically expressed in human vascular tissue. The β1β1 form shows a low K_m for ethanol ($K_m = 0.05$ mM, $k_{cat} = 9$ min^{-1}) [19] which makes it suitable for metabolizing the blood ethanol at the low concentration found after social drinking. Individuals expressing the *ADH2*2* allele will exhibit the highly active β2β2 form ($k_{cat} = 400$ min^{-1}), and therefore a much higher ADH activity in blood vessels. A contribution of class IV ADH could be also possible, at least in some individuals, in spite of the higher K_m (37 mM), because of an extremely high k_{cat} (1510 min^{-1}) [8]. The importance of vascular ADH in ethanol metabolism may differ between individuals. Thus, values ranging from 2.9 to 19 mU/mg have been found in human aorta samples from different subjects.

Human blood vessels also contain a significant amount of ADH of the low-K_m type. Analysis by starch gel electrophoresis demonstrates that both cytosolic (class 1) and mitochondrial (class 2) forms are present in all vessels studied (results not shown), while measurements using 66 μM propanol as substrate, at pH 8.5, show an activity of about 1 mU/mg protein. This activity is lower than the ADH activity for most of the blood vessels, suggesting a possible local accumulation of acetaldehyde in the vascular tissue due to alcohol oxidation by endogenous ADH.

Rat class IV oxidizes ethanol poorly. Its K_m at physiological pH is extremely high (3 M), which makes the enzyme practically inactive at the pharmacological concentrations of ethanol [10]. Thus, with 33 mM ethanol, at pH 7.5 and 37°C, the activity is about 100-fold lower than the values reported in Table 1. The situation is quite different for the human species. Under physiological conditions the activity is similar to that reported in Table 1, with values close to those found in gastric mucosa [9,20]. Since the low-K_m ββ form is responsible for most of the activity, the enzyme will be readily saturated at usual blood ethanol concentrations, and therefore with maximal velocity. All blood vessels examined contained the isozyme, which has been localized in the endothelium and lamina media (present work and [6]). The presence of ADH in endothelium has been also demonstrated in rat arteries (Fig. 3) and veins (not shown) by histochemical analysis, suggesting a wide distribution of the enzyme in the whole vascular system. The vascular endothelium corresponds to an enormous surface when all vessels of the human body are considered (estimates of 500–700 m^2, [21]). It is therefore likely that blood vessels contribute significantly to extrahepatic ethanol metabolism. ADH of blood vessels may also have a role in the first-pass metabolism of ethanol. The origin of this first pass is controversial. Physiological experiments support a role of stomach ADH in the first pass [22], but the amount of enzyme is low in gastric tissue [9,20]. The enzyme of the blood vessels can oxidize ethanol during the absorption from the stomach and intestine, and during the first passage through the veins to the lungs, before it reaches the general circulation. The contribution of vascular ADH to the overall and first-pass metabolism of ethanol may be significant in humans but of less importance in the rat, where vascular ADH has low activity with ethanol.

The presence of some level of ethanol metabolism in blood vessels has been interpreted as a positive mechanism of preventing cardiovascular diseases through maintenance of a reduced environment in the vessel and minimization of lipoprotein oxidation [6]. However, several reports support the possibility that some of the detrimental effects of ethanol are mediated via increased oxidative stress which would be, at least in part, induced by ethanol metabolism, that in addition may lead to lipid accumulation [2,23,24]. Moreover, ethanol metabolism by vascular ADH could interfere with the proposed function of this enzyme in the elimination of lipid peroxidation products, therefore contributing to vascular damage.

Some of the direct effects of ethanol on blood vessels [1] can be a consequence of its metabolism, particularly through the action of the in situ generated acetaldehyde. Concentration of blood acetaldehyde is low even at intoxicating ethanol doses; however, the acetaldehyde concentration in the blood vessel tissues could be higher because of local ethanol metabolism, suggesting a stronger cardiovascular effect of this toxic compound than previously thought [1,25], and a contribution of blood vessel acetaldehyde to the flushing response of Orientals. Finally, it has been suggested that some acetaldehyde effects, such as the stimulation of vascular prostacyclin release, may take part in the protective effect of moderate consumption against some cardiovascular complications [26]. In

conclusion, the metabolism of ethanol in blood vessels by alcohol dehydrogenase represents a new mechanism to explain the effects of ethanol on the cardiovascular system.

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