Gen. Pharmac. Vol. 21, No. 5, pp. 697–702, 1990
Printed in Great Britain.

0306-3623/90 $3.00 + 0.00

DIFFERENCES IN KINETIC CHARACTERISTICS AND IN SENSITIVITY TO INHIBITORS BETWEEN HUMAN AND RAT LIVER ALCOHOL DEHYDROGENASE AND ALDEHYDE DEHYDROGENASE

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(*Received* 6 *November* 1989)

Abstract—1. On the basis of kinetic properties and sensitivity to pyrazole inhibition, it is shown that liver alcohol dehydrogenase present in human mainly corresponded to class I and in rat to class ADH-3 which differed in a number of parameters.

2. Two different aldehyde dehydrogenase (ALDH) isoenzymes were detected in both human and rat liver. The human isoenzymes corresponded to the ALDH-I and ALDH-II type.

3. In the rat, one isoenzyme had low K_m and showed similar activity than in human liver but differed in their sensitivity to both disulfiran and nitrofazole inhibition whereas the other presented high K_m and showed greater activity than the human one.

4. Caution must be therefore paid when extrapolating to human subjects the data on ethanol metabolism obtained with rats.

INTRODUCTION

Ethanol is mainly metabolized in the liver by the sequential activities of alcohol dehydrogenase (EC 1.1.1.1) and aldehyde dehydrogenase (EC 1.2.1.3) although other alternative enzymatic systems have been described (Lieber and De Carli, 1968, 1970). Mammalian alcohol dehydrogenase (ADH) is a polymorphic dimeric zinc metalloenzyme composed of 40,000 Da subunits. The relative electrophoretic mobility of liver ADH molecules differs in most of the animal species studied. Human ADH subunits can be divided into three classes: I, II and III (Strydom and Vallee, 1982). Class I subunits derive from at least three genetic loci and constitute the α-subunit, different β-subunits, and two γ-subunits (Von Wartburg *et al.*, 1965; Smith *et al.*, 1971; Wagner *et al.*, 1983; Jornvall *et al.*, 1984). The genetic organization and gene structures of π-subunits of the class II type and the χ-subunits of the class III type have only recently been reported (Duester *et al.*, 1986). Three different alcohol dehydrogenase isoenzymes have been reported in rat tissues (Julià *et al.*, 1987).

In mammals, the greatest aldehyde dehydrogenase (ALDH) concentrations are found in the liver. The enzyme, a homotetramer, exists as isoenzymes which are specific to different subcellular compartments: cytoplasm, mitochondria and microsomes (Deitrich, 1966; Marjanen, 1972; Tottmar *et al.*, 1973; Koivula, 1975; Koivula and Koivusalo, 1975). ALDH isoenzymes have been divided into two broad classes: isoenzymes with micromolar K_m values or isoenzymes with millimolar K_m values for short-chain aliphatic aldehydes. Low and high K_m isoenzymes can be found within the same subcellular compartment, and both have been partially purified from rat liver mitochondria (Siew *et al.*, 1976) and cytoplasm (Truesdale-Mahoney *et al.*, 1981). When human liver homogenates are electrophoresed on starch gel, four bands of aldehyde dehydrogenase activity are visualized after staining (Harada *et al.*, 1978, 1980). The two major isoenzymes, called ALDH-I (or E_2) and ALDH-II (or E_1), have been purified to homogeneity (Greenfield and Pietruszko, 1977; Hempel *et al.*, 1982). ALDH-I and ALDH-II are both low K_m aldehyde dehydrogenases. Two additional slow migrating bands, ALDH-III and ALDH-IV, represent high K_m aldehyde dehydrogenase isoenzymes (Harada *et al.*, 1978, 1980).

Much attention has been devoted in the past to study on the effects of acute and chronic ethanol consumption. Many of these studies have been performed using animals particularly the rat, but we have recently reported the existence of major differences in the *in vivo* pattern of ethanol elimination between humans and monkeys or rats (Zorzano and Herrera, 1990a). Due to these differences we decided to perform a comparative study on the activities of the two major ethanol metabolizing enzymes, alcohol dehydrogenase and aldehyde dehydrogenase, using fresh human and rat liver biopsies. A partial and preliminary report has been previously published (Herrera *et al.*, 1983).

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Table 1. Alcohol dehydrogenase activity in human and rat liver

	ADH activity (Units/g tissue)		Kinetic parameters at pH 10.5	
	pH 7.4	pH 10.5	K_m (mmol/l)	V_{max} (Units/g tissue)
Human liver	1.54 ± 0.17	6.24 ± 0.56	2.10 ± 0.36	7.70 ± 0.70
Rat liver	1.00 ± 0.15*	2.72 ± 0.21*	1.02 ± 0.25*	2.96 ± 0.43*

Results are means ± SE of 4–8 observations per group. ADH activity was assayed in tissue extracts at pH 7.4 or 10.5, 25°C and different ethanol concentrations (ranging from 0.02 to 17 mmol/l). *Significant difference between human and rat liver groups, at $P < 0.05$.

MATERIALS AND METHODS

Fresh human liver biopsies taken for diagnosis after overnight fasting during abdominal surgery (cholecystectomy) in 9 patients who were later proven to be histopathologically healthy were used together with material from female Wistar rats from our own colony weighing about 200 g. The animals were kept in a light cycle and temperature controlled room and fed *ad libitum* with Purina chow pellets. Rats were sacrificed with a guillotine after overnight fast.

Determination of alcohol dehydrogenase and aldehyde dehydrogenase activities

Immediately after excising human and rat liver, they were placed in ice-cold 50 mM sodium phosphate buffer, at pH 7.4. Samples were homogenized in 50 mM phosphate buffer (ratio 1:1, weight:volume) in a glass Potter vessel with Teflon pestle in an ice-bath and then sonicated in a MSE sonifier (set at 12 μ for 1 min) to disrupt the integrity of subcellular organelles. Final homogenates were spun at 56,000 *g* for 20 min, at 4°C. The supernatants were then centrifuged at 143,000 *g* for 60 min, and final supernatants containing the overall non-membrane bound ethanol-metabolizing enzymes, were immediately used for enzymatic assays. ADH and ALDH activities were assayed on the same day following the spectrophotometric methods described by Von Wartburg *et al.* (1965) and by Blair and Bodley (1969) with a few modifications (Zorzano *et al.*, 1989).

ADH activity was assayed at 25°C in cuvettes containing 67 mM sodium phosphate (pH 7.4) or 100 mM glycine-NaOH buffers (pH 8.8, 9.6, 10.5 or 12.0), 1.3 mM NAD^+ and 0.8–1.0 mg of protein (20 μl of sample) in 3 ml. The reaction was initiated by the addition of ethanol to reach a final concentration of 17 mM for measurement of total activity, and 0.02, 0.05, 0.1, 1.0 and 17 mM for V_{max} and K_m measurements.

ALDH assay was performed at 37°C in cuvettes containing 100 mmol/l glycine-NaOH, 130 mmol/l KCl, 1 mmol/l pyrazole and 1 mmol/l NAD^+, pH 7.4 or 8.8, and 0.8–1.0 mg of protein in 1 ml. The reaction was started by adding 0.05, 0.3 or 20 mmol/l acetaldehyde (final concentration). For K_m and V_{max} measurements of different ALDH isoenzymes, acetaldehyde was added at final concentrations ranging from 0.005 to 40 mmol/l. In some experiments, the ALDH inhibitors disulfiram and nitrefazole were dissolved in dimethylsulfoxide and added to the incubation media. In these experiments, identical volumes of dimethylsulfoxide were added to the control (no inhibitor) cuvettes. Measurements were made with a Beckman DU-8B (Kinetics II) spectrophotometer at 340 nm, and one unit of enzymatic activity was considered to correspond to 1 μmol of substrate transformed per min. Proteins were determined by the Lowry method (Lowry *et al.*, 1951). Results are expressed as means ± SE and statistical comparisons among groups was done by Student's *t*-test.

RESULTS

Alcohol dehydrogenase activities in human and rat liver

Alcohol dehydrogenase activity (ADH) in human and rat liver was initially investigated. Human liver biopsies displaying the typical phenotype of ADH activity under the basis of kinetic characteristics were only included for comparison with rat liver biopsies, whereas those showing atypical ADH characteristics (Von Wartburg *et al.*, 1965) were discarded. As shown in Table 1, ADH activity was significantly increased in human liver at both pH 7.4 and 10.5 as compared to the activity found in rat liver. At pH 10.5, both V_{max} and K_m values were greater in human as compared to rat liver extracts (Table 1). On the basis of kinetic properties, human hepatic ADH resembles class I ADH isoenzyme, whereas rat liver ADH is similar to the previously reported ADH-3 (Julià *et al.*, 1987). The effect of pH on ADH activity was next investigated (Fig. 1). ADH

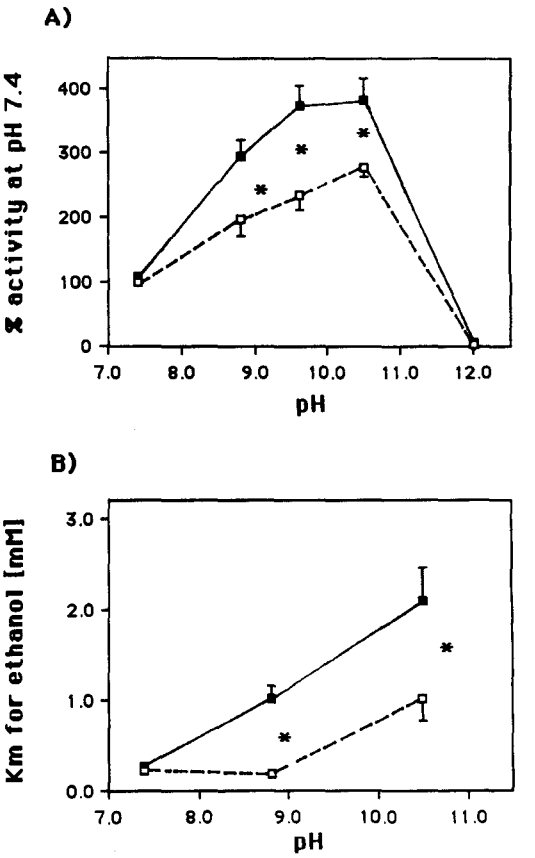

Fig. 1. Effect of pH on ADH activity in human rat liver. Results are means ± SE of 4–8 observations per group. Alcohol dehydrogenase activity was assayed in human (■——■) and rat (□-----□) liver extracts at different pH, 25°C and different ethanol concentrations (ranging from 0.02 to 17 mmol/l). In (A) ADH activity assayed at 17 mmol/l ethanol is shown. K_m values obtained at different pH are presented in (B). *Significant difference between human and rat liver groups, at $P < 0.05$.

activity increased with pH both in human and rat liver so at pH 10.5 ADH activity was maximal. However, when data were expressed as percentage of ADH activity observed at pH 7.4, a clear dissociation between human and rat liver ADH was detected (Fig. 1A). Thus, increasing the pH caused a greater activation of ADH in human liver than in rat liver. Since these differences are a reflection of changes in V_{max}, we also investigated possible changes in K_m for ethanol (Fig. 1B). At physiological pH of 7.4, K_m values for ethanol were low and undistinguishable both in human and in rat liver ADH. When pH was raised to 8.8, the K_m values substantially increased in human liver but not in rat liver, which remained constant. At pH 10.5, K_m values for human liver were even higher than at pH 8.8, and under these conditions, K_m values also increased in rat liver preparations. Thus, alkaline pH caused a raise in K_m values, and human liver ADH was more sensitive to these changes than the rat ADH isoenzyme.

The effect of pyrazole, a competitive ADH inhibitor (Theorell and Yonetani, 1963), was next investigated (Fig. 2). At pH 8.8 and 17 mM ethanol, pyrazole inhibited ADH activity similarly in human and rat liver preparations. Half maximal inhibitions were found at about 50 μM pyrazole (Fig. 2A). However, at pH 10.5 pyrazole inhibited rat liver ADH activity to a much greater extent than at pH 8.8; under these conditions 50 μM pyrazole caused a 95% inhibition of rat liver ADH activity (Fig. 2B). The ADH sensitivity to pyrazole was not modified in human liver at pH 10.5 and therefore large differences in the pattern of pyrazole inhibition were detected between human and rat liver preparations (Fig. 2B).

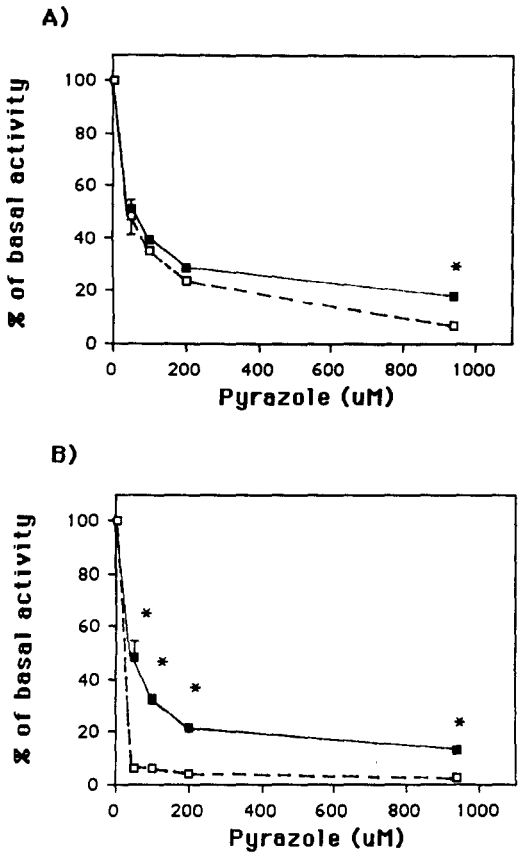

Fig. 2. Effect of pyrazole on ADH activity in human and rat liver. Results are means ± SE of 4–5 observations per group. Alcohol dehydrogenase activity was assayed in human (■——■) and rat (□-----□) liver extracts at pH 8.8 (A) or 10.5 (B), 25°C and 17 mmol/l ethanol in the presence of different concentrations of pyrazole (ranging from 0 to 940 μM). *Significant difference between human and rat liver groups, at $P < 0.05$.

Aldehyde dehydrogenase activities in human and rat liver

In the present study, aldehyde dehydrogenase (ALDH) activities in human and rat liver extracts were also investigated. We have already reported that in soluble (microsome-free) extracts from human liver only the isoenzymes ALDH-I (or E_2) and ALDH-II (or E_1) are detected (Zorzano and Herrera, 1989; Zorzano *et al.*, 1989). The ALDH-I isoenzyme had a very low K_m for acetaldehyde (approximately 9 μM) (Table 2) whereas the ALDH-II isoenzyme showed a higher K_m for acetaldehyde (about 32 μM) (Table 2). The human high K_m isoenzymes of ALDH, ALDH-III and ALDH-IV, (17, 18) were not detected, suggesting that *in vivo*, they might be located in the microsomal compartment.

In rat liver extracts two different ALDH components were detected (Table 2): one had a low K_m value for acetaldehyde (approximately 10 μM); whereas the second component had a much higher K_m for acetaldehyde (1.5 mM). The activity of the low K_m ALDH isoenzyme was similar in both human and rat liver extracts (Table 2). However, the total ALDH activity was greater in rat liver than in human liver because of the increased activity of the high K_m ALDH isoenzyme in rat liver as compared to the activity present in human liver (Table 2). Surprisingly, in human liver extracts, high concentrations of acetaldehyde (above 4 mM) caused a dramatic inhibition of ALDH (Fig. 3); the inhibition was so extreme it indicated that both ALDH-I and ALDH-II isoenzymes were affected. This kind of inhibition by a substrate was not detected in rat liver extracts (data not shown).

Table 2. Aldehyde dehydrogenase activity in human and rat liver

	Low K_m isoenzyme		High K_m isoenzyme	
	K_m (μM)	V_{max} (Units/g)	K_m (μM)	V_{max} (Unit/g)
Human liver	9 ± 3	0.85 ± 0.09	32 ± 5	0.27 ± 0.10
Rat liver	10 ± 2	0.87 ± 0.10	1547 ± 223*	2.15 ± 0.30*

Results are means ± SE of 7–13 observations per group. Aldehyde dehydrogenase activity was assayed in tissue extracts at pH 8.8, 37°C and different acetaldehyde concentrations. Values of low K_m isoenzyme activities were obtained using 0.05 mM acetaldehyde in both human and rat liver extracts. In human liver extracts, high K_m isoenzyme activities were obtained by substracting the ALDH activity found at 0.05 mM acetaldehyde from maximal ALDH activity obtained at 0.3 mM acetaldehyde. Similarly, in rat liver extracts high K_m isoenzyme activities were calculated by substracting the ALDH activity at 0.05 mM acetaldehyde from optimal ALDH activity found at 20 mM acetaldehyde. Kinetics of the ALDH isoenzymes were obtained assaying ALDH activities at acetaldehyde concentrations ranging from 0.005 to 40 mM in rat liver extracts and from 0.005 to 0.3 mM in human liver extracts. *Significant difference between human and rat liver groups, at $P < 0.05$.

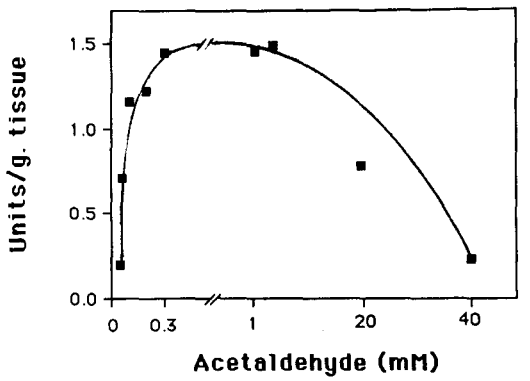

Fig. 3. Effect of acetaldehyde on aldehyde dehydrogenase activity in human liver extracts. Results are means of 4 observations per group. Aldehyde dehydrogenase activity was assayed in human liver extracts at pH 8.8, 37°C and different acetaldehyde concentrations.

The maximal ALDH activity was found at pH 8.8 both in human and rat liver extracts, and a similar extent of stimulation in ALDH activity, induced by raising the pH from 7.4 to 9.5, was also detected (data not shown).

The effect of known ALDH inhibitors, disulfiram (Neims *et al.*, 1966) and nitrefazole (McMillan, 1983), on human and rat liver ALDH was also subjected to study. The low K_m isoenzymes were inhibited to a small extent by 10 μM disulfiram in both human and rat liver extracts (Fig. 4A) whereas 100 μM disulfiram caused almost complete inhibition. No differences in the dilsulfiram inhibition pattern on the low K_m isoenzymes were detected between human and rat. Human hepatic ALDH-II isoenzyme was more sensitive to disulfiram than human ALDH-I isoenzyme (Fig. 4B). However, the rat high K_m isoenzyme was very insensitive to disulfiram and concentrations of inhibitor as high as 100 μM only caused a 22% inhibition of its activity (Fig. 4B).

Nitrefazole, another ALDH inhibitor, caused a substantial decrease in the low K_m ALDH activity in both rat liver and human liver extracts (Fig. 5A); however, the inhibitory effect of nitrefazole was greater in rat liver extracts as compared to human liver extracts (Fig. 5A). On the other hand, both human ALDH-II and rat high K_m ALDH isoenzyme were completely insensitive to inhibition by nitrefazole, even at concentrations as high as 100 μM (Fig. 5B).

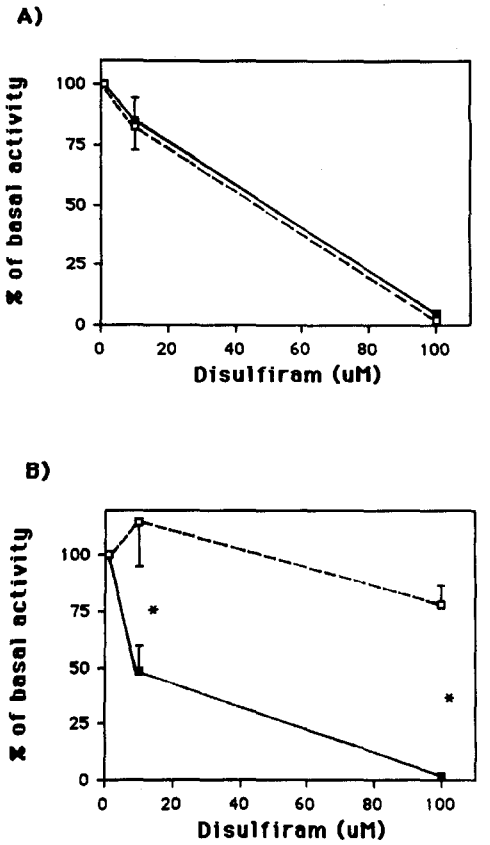

Fig. 4. Effect of disulfiram on aldehyde dehydrogenase in human and rat liver. Results are means ± SE of 5–8 observations per group. Aldehyde dehydrogenase activity was assayed in human (■——■) and rat (□-----□) liver extracts at pH 8.8, 37°C and 0.05 and 0.3 mmol/l acetaldehyde concentrations (human liver extracts) or 0.05 and 20 mmol/l in rat liver extracts in the presence of different concentrations of disulfiram. Activity of low K_m isoenzyme corresponds to activity assayed at 0.05 mM acetaldehyde. In human liver extracts, high K_m isoenzyme activities were obtained by substracting the ALDH activity found at 0.05 mM acetaldehyde from maximal ALDH activity obtained at 0.3 mM acetaldehyde. Similarly, in rat liver extracts high K_m isoenzyme activities were calculated by substracting the ALDH activity of 0.05 mM acetaldehyde from optimal ALDH activity found at 20 mM acetaldehyde. Results in panel A correspond to low K_m isoenzymes and in panel B to high K_m isoenzymes. *Significant difference between human and rat liver groups, at $P < 0.05$.

DISCUSSION

In a recent report we demonstrated the existence of major differences regarding the pattern of ethanol elimination after an oral load of ethanol in both rats and humans (Zorzano *et al.*, 1990a); thus, blood ethanol concentrations stayed very high for longer periods of time in human subjects as compared to rats. Surprisingly, hepatic alcohol dehydrogenase activity was increased in human subjects compared to rats. The results of the present study demonstrate the existence of large differences in the kinetic properties and sensitivity to inhibitors between human and rat hepatic alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). In consequence, in light of these differences between human and rat liver ADH and ALDH activities, data regarding ethanol pharmacology obtained from experimental animals might be difficult to extrapolate to human subjects.

It is known that human liver alcohol dehydrogenase mainly consists of class I (α, β, γ) ADH isoenzymes. In addition, the existence of class II (π) alcohol dehydrogenase in human liver has also been described (Bosron *et al.*, 1979; Ditlow *et al.*, 1984). Under our conditions, human liver alcohol dehydrogenase presented a low K_m for ethanol (2.1 mM) and high sensitivity to pyrazole ($K_{0.5}$ of 50 μM at pH 8.8). That clearly indicates that class II alcohol dehydrogenase (characterized by K_m values for ethanol of

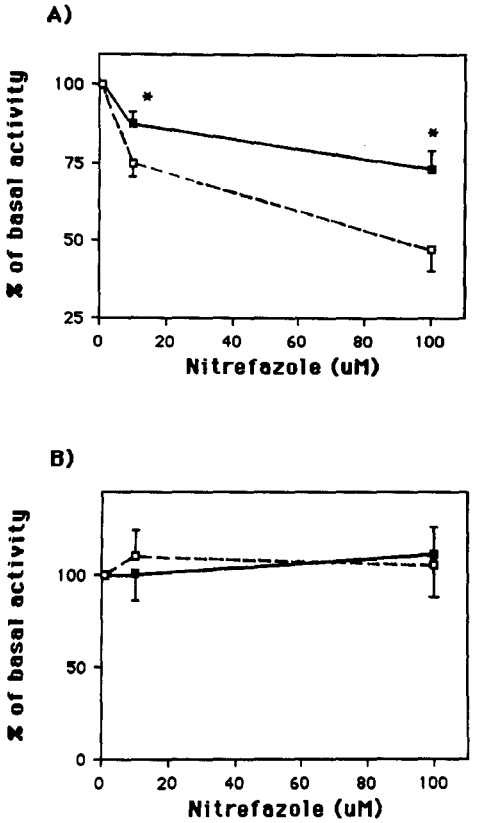

Fig. 5. Effect of nitrefazole on aldehyde dehydrogenase in human and rat liver. Results are means ± SE of 5–7 observations per group. Aldehyde dehydrogenase activity was assayed in human (■——■) and rat (□-----□) liver extracts at pH 8.8, 37°C and acetaldehyde concentrations of either 0.05 and 0.3 mmol/l in human liver extracts or 0.05 and 20 mmol/l in rat liver extracts, in the presence of different concentrations of nitrefazole. Results in panel (A) correspond to low K_m isoenzymes and in panel (B) to high K_m isoenzymes. See further details in legend to Fig. 4. *Significant difference between human and rat liver groups, at $P < 0.05$.

about 120 mM and relative insensitivity to pyrazole) was negligible in our preparation. On the other hand, it has been recently reported that rat tissues contain three different isoenzymes of alcohol dehydrogenase, named ADH-1, ADH-2 and ADH-3 (Julià *et al.*, 1987). ADH-3 is mainly present in rat liver and its reported properties are comparable to the characteristics observed by us in the present study, that is, the K_m for ethanol is 1.0 mM at pH 7.4 and it is strongly inhibited by pyrazole. Thus, although human class I ADH and rat ADH-3 seem to show similar characteristics, here we have found that they both differ in a number of properties, namely: the effect of pH on enzyme activity; K_m values for ethanol; and sensitivity to pyrazole. Thus, human class I ADH was activated by changes in pH to a greater extent than rat ADH-3; K_m values became much higher in human class I ADH than in rat ADH-3; and finally, alkaline pH caused an increase in sensitivity to pyrazole in rat ADH-3 but not in human class I ADH. The increase in sensitivity to pyrazole by raised pH concurs with data from another source describing an increase in pyrazole association rate to the enzyme–NAD^+ complex (Andersson *et al.*, 1981).

In the soluble extract (microsome-free) obtained from human liver, we have detected the two major human ALDH isoenzymes, that is, ALDH-I ($K_m \simeq 9\,\mu M$) and ALDH-II ($K_m \simeq 32\,\mu M$) (Zorzano and Herrera, 1990b; Zorzano *et al.*, 1989). It is believed that ALDH-I has a predominantly mitochondrial origin whereas ALDH-II has a cytosolic origin (Pietruszko, 1983; Goedde and Agarwal, 1987). A different pattern of ALDH isoenzymes was observed in extracts from rat liver, which were characterized by very different K_m values. According to earlier authors, the major rat low K_m ALDH isoenzymes are found in mitochondria, whereas the major high K_m ALDH isoenzymes are present in mitochondria, microsomes and cytosol (Tottmar *et al.*, 1973; Horton and Barrett, 1975; Lebsack *et al.*, 1981).

Regarding the low K_m ALDH isoenzymes, human ALDH-I showed the same K_m values for acetaldehyde as the rat low K_m ALDH isoenzyme; however, they differed in their sensitivity to nitrefazole, indicating that they are different isoenzymes. Another indication that we are dealing with different isoenzymes is that, contrary to rat low K_m ALDH isoenzyme, human ALDH-I was inhibited by acetaldehyde. As judged by kinetic studies, we did not identify the presence of any ALDH isoenzyme in rat liver extracts with acetaldehyde K_m values similar to those of human ALDH-II. Very large differences were observed between human ALDH-II and the high K_m rat ALDH isoenzyme, differences that regarded K_m values as well as sensitivity to high concentrations of acetaldehyde and disulfiram. Interestingly enough, human ALDH-I was less sensitive to disulfiram than ALDH-II; however, rat low K_m ALDH was more sensitive to disulfiram than rat high K_m ALDH isoenzyme, which in fact was extremely resistant to the inhibitor.

In conclusion, the results of the present study allow us to conclude, (a) the existence of major differences in kinetic properties and sensitivity to pyrazole between human class I ADH and rat ADH-3 isoenzymes; (b) the appearance of a different pattern of ALDH isoenzymes in liver extracts from rats and human subjects, so whereas two different low K_m ALDH isoenzymes (micromolar range) have been described in human extracts, only one low K_m isoenzyme is found in rat liver extracts; (c) human and rat liver low K_m ALDH isoenzymes differed in a number of properties, namely their sensitivity to inhibitors such as disulfiram and nitrefazole, and their sensitivity to substrate-inhibition.

Acknowledgements—The authors thank M. Morante for excellent technical work and Carol F. Warren from the ICE of Alcalá de Henares University for her editorial help. This study was supported by Grants from the "Comisión Asesora de Investigación Científica y Técnica", Ministerio de Educación, and from the "Fondo de Investigaciones Sanitarias de la Seguridad Social" of Spain.

REFERENCES

Andersson P., Kvassman J., Lindstrom A., Olden B. and Pettersson G. (1981) Effect of pH on pyrazole binding to liver alcohol dehydrogenase. *Eur. J. Biochem.* **114**, 549–554.

Blair A. H. and Bodley F. H. (1969) Human liver aldehyde dehydrogenase: partial purification and properties. *Can. J. Biochem.* **47,** 265–275.

Bosron W. F., Li T. K., Dafeldecker W. P. and Vallee B. L. (1979) Human liver π-alcohol dehydrogenase: kinetic and molecular properties. *Biochemistry* **18,** 1101–1105.

Deitrich R. A. (1966) Tissue and subcellular distribution of mammalian aldehyde-oxidizing capacity. *Biochem. Pharmac.* **15,** 1911–1922.

Ditlow C. C., Holmquist B., Morelock M. M. and Vallee B. L. (1984) Physical and enzymatic properties of class II alcohol dehydrogenase isozyme of human liver: π-ADH. *Biochemistry* **23,** 6363–6368.

Duester G., Smith M., Bilanchone V. and Hatfield G. W. (1986) Molecular analysis of the human class I alcohol dehydrogenase gene family and nucleotide sequence of the gene encoding the β subunit. *J. Biol. Chem.* **261,** 2027–2033.

Goedde H. W. and Agarwal D. P. (1987) Polymorphism of aldehyde dehydrogenase and alcohol sensitivity. *Enzyme* **37,** 29–44.

Greenfield N. J. and Pietruszko R. (1977) Two aldehyde dehydrogenases from human liver; isolation via affinity chromatography and characterization of the isozymes. *Biochim. Biophys. Acta* **483,** 35–45.

Harada S., Agarwal D. P. and Goedde H. W. (1978) Isozyme variations in acetaldehyde dehydrogenase (EC 1.2.1.3) in human tissues. *Hum. Genet.* **44,** 181–185.

Harada S., Agarwal D. P. and Goedde H. W. (1980) Electrophoretic and biochemical studies of human aldehyde dehydrogenase isozymes in various tissues. *Life Sci.* **26,** 1773–1780.

Hempel J. D. H., Reed D. M. and Pietruszko R. (1982) Human aldehyde dehydrogenase: improved purification procedure and comparison of homogeneous isoenzymes E1 and E2. *Alcoholism Clin. Exp. Res.* **6,** 417–425.

Herrera E., Fresneda V. and Zorzano A. (1983) Comparative kinetics of human and rat liver alcohol dehydrogenase. *Biochem. Soc. Trans.* **11,** 729–730.

Horton A. A. and Barrett M. (1975) The subcellular localization of aldehyde dehydrogenase in rat liver. *Arch. Biochem. Biophys.* **167,** 426–436.

Jornvall H., Hempel J., Vallee B. L., Bosron W. F. and Li T. K. (1984) Human liver alcohol dehydrogenase: amino acid substitution in the β_2 β_2 oriental isozyme explains functional properties, established an active site structure, and parallels mutational exchanges in the yeast enzyme. *Proc. Natn Acad. Sci. U.S.A.* **81,** 3024–3028.

Julià P., Farres J. and Pares X. (1987) Characterization of three isoenzymes of rat alcohol dehydrogenase. Tissue distribution and physical and enzymatic properties. *Eur. J. Biochem.* **162,** 179–189.

Koivula T. (1975) Subcellular distribution and characterization of human liver aldehyde dehydrogenase fractions. *Life Sci.* **16,** 1563–1570.

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

Lebsack M. E., Gordon E. R. and Lieber C. S. (1981) Effect of chronic ethanol consumption on aldehyde dehydrogenase activity in the baboon. *Biochem. Pharmac.* **30,** 2273–2277.

Lieber C. S. and De Carli L. M. (1968) Ethanol oxidation by hepatic microsomes: adaptive increase after ethanol feeding. *Science* **162,** 917–918.

Lieber C. S. and De Carli L. M. (1970) Hepatic microsomal ethanol oxidizing system: *in vitro* characteristics and adaptive properties *in vivo*. *J. Biol. Chem.* **245,** 2505–2512.

Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193,** 265–275.

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

McMillan D. E. (1983) Effect of EMD 15,700 and disulfiram on ethanol intake in rats under schedule-induced polydipsia. *Drug Dev. Res.* **3,** 193–198.

Neims A. H., Coffey S. and Hellerman L. (1966) A sensitive radioassay for sulfhydryl groups with tetraethylthiuram disulfide. *J. Biol. Chem.* **241,** 3036–3040.

Pietruszko R. (1983) Aldehyde dehydrogenase isoenzymes. In *Isoenzymes: Cellular Localization, Metabolism and Physiology. Current Topics in Biological and Medical Research*, Vol. 8, pp. 195–217. Alan R. Liss, New York.

Siew C., Deitrich R. A. and Erwin V. E. (1976) Localization and characteristics of rat liver mitochondrial aldehyde dehydrogenases. *Arch. Biochem. Biophys.* **176,** 638–649.

Smith M., Hopkinson D. A. and Harris H. (1971) Developmental changes and polymorphism in human alcohol dehydrogenase. *Ann. Hum. Genet.* **34,** 250–271.

Strydom D. J. and Vallee B. L. (1982) Characterization of human alcohol dehydrogenase isoenzymes by high-performance liquid chromatography peptide mapping. *Analyt. Biochem.* **123,** 422–429.

Theorell H. and Yonetani T. (1963) Liver alcohol dehydrogenase-DPN-pyrazole complex: a model of a ternary intermediate in the enzyme reaction. *Biochem. Z.* **338,** 537–553.

Tottmar S. O. C., Pettersson H. and Kiessling K. H. (1973) The subcellular distribution and properties of aldehyde dehydrogenase in rat liver. *Biochem. J.* **135,** 577–586.

Truesdale-Mahoney N., Doolittle D. R. and Weiner H. (1981) Genetic basis for the polymorphism of rat liver cytosolic aldehyde dehydrogenase. *Biochem. Genet.* **19,** 1275–1282.

Von Wartburg J. P., Papenberg J. and Aebi H. (1965) An atypical human alcohol dehydrogenase. *Can. J. Biochem.* **43,** 889–898.

Wagner F. W., Burger A. R. and Vallee B. L. (1983) Kinetic properties of human liver alcohol dehydrogenase: oxidation of alcohols by class I isoenzymes. *Biochemistry* **22,** 1857–1863.

Zorzano A. and Herrera E. (1990a) *In vivo* ethanol elimination in man, monkey and rat: a lack of relationship between the ethanol metabolism and the hepatic activities of alcohol and aldehyde dehydrogenases. *Life Sci.* **46,** 223–230.

Zorzano A. and Herrera E. (1990b) Differences in the kinetic properties and sensitivity to inhibitors of human placental, erythrocyte, and major hepatic aldehyde dehydrogenase isoenzymes. *Biochem. Pharmac.* **39,** 873–878.

Zorzano A., Ruiz Del Arbol L. and Herrera L. (1989) Effect of liver disorders on ethanol elimination and alcohol and aldehyde dehydrogenase activities in liver and erythrocytes. *Clin. Sci.* **76,** 51–57.