GASTRIC ALCOHOL DEHYDROGENASE ACTIVITY IN MAN: INFLUENCE OF GENDER, AGE, ALCOHOL CONSUMPTION AND SMOKING IN A CAUCASIAN POPULATION

ALEXANDR PARLESAK*, MICHAEL HANS-ULRICH BILLINGER1, CHRISTIANE BODE and JOHANN CHRISTIAN BODE1

Hohenheim University (140), Department of Physiology of Nutrition, Garbenstr. 28, D-70599 Stuttgart and 1Department of Gastroenterology and Hepatology, Robert-Bosch-Hospital, Stuttgart, Germany

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Abstract — Aims: The stomach is involved in first-pass metabolism of alcohol in humans. As conflicting data were published regarding the influence of age and gender on the activity of alcohol dehydrogenase (ADH) in human gastric mucosa, the present study aimed at the investigation of these and other potentially confounding factors (alcohol consumption, smoking, drug intake) on its activity in a Caucasian population. Methods: ADH activity was assessed in endoscopic gastric biopsy specimens from 111 Caucasian subjects aged 20–80 years, of whom 51 were females. Results: Highest ADH activity was measured at ethanol concentrations between 150 and 500 mM. Mean ADH activity was higher in antral specimens than in those from the gastric corpus of the same subjects. ADH activity decreased with increasing age in males, while the values in females aged 41–60 years were higher than those in women aged 20–40 or 61–80 years. In men aged 20–40 years, consumption of larger quantities of alcohol (>0.8 g/kg body weight/day) was associated with reduced ADH activity. H2-Receptor antagonist treatment also decreased gastric ADH activity. Conclusions: The results indicate that ADH activity in human gastric mucosa is negatively associated with consumption of larger quantities of alcohol. The question of whether ADH activity is higher in males or females can only be answered with respect to age. The gastric ADH activity in young men is distinctly higher compared to young women, but the opposite holds true in middle-aged subjects.

INTRODUCTION

The presence of several isoenzymes of alcohol dehydrogenase (ADH, EC 1.1.1.1) in different parts of the gastrointestinal tract, especially the stomach, of various animal species and in man was first described more than three decades ago (Moser et al., 1968) and confirmed soon thereafter (Murray and Motulsky, 1971). In subsequent studies, several isoenzymes belonging to class I and III ADH have been found in the stomach and, in addition, a new class IV ADH has been detected in the stomach, but not the liver (Pestalozzi et al., 1983; Yin et al., 1990; Moreno and Pares, 1991).

Determination of total ADH activity, comprised of class I, III and IV isoenzymes in the human stomach has attracted new interest following the description of gastric first-pass metabolism of alcohol in rats (Julkunen et al., 1985) and, shortly thereafter, in man (DiPadova et al., 1987; Caballeria et al., 1989a,b). Gastric ADH activity has been reported to be affected by several factors, including ethnicity (Baraona et al., 1991; Moreno et al., 1994), age (Harada and Okubo, 1993; Seitz et al., 1993; Moreno et al., 1994), gender (Frezza et al., 1990; Seitz et al., 1993; Moreno et al., 1994; Yin et al., 1997; Seitz and Oneta, 1998) and certain drugs which inhibit ADH activity, such as cimetidine and ranitidine (Caballeria et al., 1989a; Hernandez-Munos et al., 1990; DiPadova et al., 1992; Stone et al., 1995). For some of these factors, such as ethnicity and H2-receptor antagonists, the results published by different groups are in agreement. For other factors, including age (Seitz et al., 1993; Harada and Okubo, 1993; Moreno et al., 1994; Seitz and Oneta, 1998) and gender (Frezza et al., 1990; Seitz et al., 1993; Moreno et al., 1994; Yin et al., 1997; Seitz and Oneta, 1998), the published data are not equivocal.

The aim of the present study was to determine the influence of age, gender, alcohol consumption, smoking and drug intake and the interaction of these factors on gastric ADH activity in a Caucasian population. Since it has been claimed that freezing and thawing of gastric biopsies prior to measurement may lead to loss of ADH activity (Seitz and Oneta, 1998) and that test conditions (substrate concentration, pH of the incubation medium) might markedly influence the results, a careful evaluation of these methodological factors preceded this investigation.

PATIENTS AND METHODS

Patients

Caucasian patients in whom oesophago-gastro-duodenoscopy was performed were eligible for the study. In all cases, the examination was carried out to investigate upper abdominal complaints, such as bloating, pain, or heartburn. Exclusion criteria were any of the following: (1) acute illness of any type or operation during the preceding 2 weeks; (2) all clinically relevant chronic diseases, such as chronic liver disease, renal insufficiency (serum creatinine >2 mg/dl) or malignancies; (3) gastro-duodenal ulcer or moderate or severe gastritis (judged by endoscopy and histology) and also autoimmune type atrophic gastritis; (4) endocrine disorders; (5) severe heart insufficiency (New York Heart Association grade 3 or 4). A total of 142 patients were asked to participate. Because of ulcers or gastritis, 31 subjects had to be excluded after endoscopy. Biopsies of 111 patients were thus used to determine ADH activity.

To assess factors that might influence gastric ADH activity, all patients were interviewed using a standardized questionnaire before the endoscopy was performed. The patients were asked in detail about their frequency of alcohol consumption and serving size in terms of medium glasses or bottles of...
wine, 0.3 l cans or bottles of beer or shots of hard liquor. For calculation of mean daily alcohol consumption, the following alcohol concentrations (v/v) were assumed: beer 4.4%, wine 11%, and hard liquors 40%. In addition, all subjects were asked regarding their smoking habits. Smokers were asked the average number of cigarettes they smoked during the preceding month. Further, the present intake of drugs and drugs taken during the last 2 weeks prior to the study was recorded. In Table 1, data on age and gender distribution are given as are the numbers of smokers and of persons who consumed H₂ antagonists.

The surgical specimens for determination of pH, optimum substrate concentration and the effect of storage at –80°C on ADH activity came from five patients who underwent surgery for cancer of the stomach (three males, two females, mean age 67.8 years, range 53–74).

The study was approved by the Ethics Committee of the Robert-Bosch-Hospital. All patients gave their informed consent.

**Biopsy specimens**

The endoscopy was performed by an experienced gastroenterologist. In addition to tissue samples from the antrum, corpus and in most cases from the fundus taken for routine gastroscopy and a rapid urease test, three biopsies (fresh weight 12–21 mg) for the determination of ADH activity were obtained from the antrum 3–4 cm from the pyloric ring. To compare the ADH activity of the mucosa in different parts of the stomach, additional biopsy specimens were taken from the middle part of the body of the stomach in eight and from the fundus in seven subjects, respectively.

Biopsy specimens taken for measurement of ADH activity were immediately washed in 2 ml of ice-cold 1.15% (w/v) solution of KCl to remove mucus and blood. Following removal of the KCl solution, the remaining liquid was carefully dapped using filter paper. After weighing, the specimens were homogenized in 0.5 ml of the 1.15% KCl solution at 4°C by using a specially designed Teflon homogenizer for Eppendorf vials and then centrifuged at 100,000 g (4°C) for 15 min. The supernatant was transferred to Eppendorf vials and kept at –80°C until measurement of ADH activity (maximum storage time: 2 weeks).

Surgical specimens (size ~30×30 mm) of the stomach from the antral region were cut immediately after excision of the stomach by the pathologist in charge of the histological evaluation. The mucosa was scraped off using a glass blade. Subsequent procedures were the same as described for biopsy specimens, with the exception that mucosa samples of ~100 mg each were transferred to 3.5 ml of the 1.15% KCl solution into Teflon tubes and homogenized using a Teflon homogenizer.

**Measurement of ADH activity**

ADH activity was measured by monitoring the formation of NADH at 334 nm using a slight modification of a method described earlier (Bode et al., 1970). If not stated otherwise, 150 µl of the cytosolic fraction was mixed with sodium pyrophosphate buffer (Na₂HPO₄, 75 mM, semicarbazide 50 mM, glycine 20 mM, pH 9.6) at 25°C. The NAD⁺ concentration was 2.5 mM, the alcohol concentration 500 mM and the final volume was 1.0 ml. Using the molar absorption coefficient 6.18×10⁶ l/mM/cm for NADH at 334 nm, ADH activity was calculated in µmol/min/g of mucosal tissue. In previous studies (Bode et al., 1988) and in 40 samples of the present study, enzyme activities were correlated to both protein concentration (Lowry et al., 1951) and fresh weight of mucosal tissue. In both cases, activities correlated closely (r = 0.924; P < 0.001 for the samples of the present study). Therefore, no further protein measurements were performed and activities were calculated per g fresh tissue.

**Quality control measures**

To determine the variation in the series, samples of the five surgical specimens were measured 18-fold on the same day. The mean coefficient of variation was 4.9% (range 3.8–5.5%). To measure the variation from day to day, an ADH standard was produced, using purified commercially available enzyme (Boehringer, Mannheim, Germany). The standard samples (1100 µl) were kept at –80°C. The standards were determined on each day when samples of biopsies were measured. The mean value (± SD) of 21 measurements was 1106 ± 36.5 U/l, corresponding to a coefficient of variation of 5.7%.

**Histology**

Specimens for histology were fixed in formalin-buffered saline and embedded in paraffin wax, and 5 µm sections were prepared for light microscopy in a standard manner. Sections were stained for Helicobacter pylori with haematoxylin and eosin or cresyl violet (Thuluvath et al., 1994). In addition, H. pylori colonization was determined by a gel-based rapid urease test (CLO-Test; Astra, Wedel, Germany). Samples identified as positive for H. pylori were not included in the investigation.

**Statistics**

Data are expressed as mean values ± SD, unless indicated otherwise. For statistical evaluation, the software package

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age (years)</th>
<th>Alcohol intake (g/kg/day)</th>
<th>Consumers of H₂ antagonists</th>
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<td>20–40</td>
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<td>Females</td>
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Total no. of subjects = 111.
RESULTS

Effect of pH, alcohol concentration and storage at –80°C

In Fig. 1, the influence of increasing ethanol concentrations on ADH activity in five surgical specimens is shown. In all samples, maximal activity was reached at ethanol concentrations of ~200 mM. If the pH of the buffer was increased stepwise by 0.3 pH units in the range between pH 6.8 and 10.0, only minor effects on ADH activity were seen. A flat maximum was reached at pH 9.0 (ADH activities at pH 6.8, 9.0 and 10.0 were 0.28 ± 0.04, 0.36 ± 0.05 and 0.34 ± 0.07 μmol/min/g of tissue, respectively). After storage of the cytoplasmic fraction of gastric mucosa for 4 weeks at –80°C, average ADH activity did not differ from that measured in the fresh material (98.3 ± 9.8% of original activity).

Antrum versus body and fundus

As measured in eight subjects, the ADH activity was significantly higher (P = 0.006) in biopsies from the antrum (0.25 ± 0.10 μmol/min/g of tissue) than in those from the corpus (0.13 ± 0.01 μmol/min/g of tissue). No significant differences (P = 0.150) were observed between the values obtained from the antrum (0.27 ± 0.24 μmol/min/g of tissue) and the fundus (0.19 ± 0.11 μmol/min/g of tissue) of another seven subjects.

Age and gender

In male subjects with low alcohol consumption who did not take H₂ antagonists, activity of gastric ADH decreased with increasing age (ANOVA: P = 0.010; Fig. 2). In the group of male subjects aged 61–80 years, the activity was almost half of that in men aged 20–40 years (P = 0.003). In women aged 61–80 years, activity of gastric ADH was significantly lower than that measured in females aged 41–60 years (P = 0.029). Surprisingly, the mean value in the youngest group of females was only about one-third of that found in the group 40–60 years of age (P = 0.008, Fig. 2).

Disregarding the effect of age, average ADH activity in the stomach did not differ between females and males with an alcohol consumption of <0.4 g/kg/day (0.260 ± 0.132 vs 0.264 ± 0.154 μM/min/g, P = 0.996). Within the single groups of comparable age, significant differences became evident in subjects aged 20–40 years, where the ADH activity was higher in males than in females (n = 8/5, P = 0.013), and 41–60 years, where the opposite was true (n = 21/13, P = 0.013). No difference in ADH activity between men and women became evident in the group of the oldest subjects (n = 14/24, P = 0.683) (Fig. 2).

Alcohol consumption

In the youngest group of males not taking ranitidine or cimetidine, increasing consumption of alcoholic beverages was associated with a decreasing gastric ADH activity (ANOVA: P = 0.020, Fig. 3). In this group, especially high daily alcohol intake suppressed ADH activity in the stomach to ~19% of that in the group with the lowest intake (P < 0.001, Fig. 3). Though the average ADH activity of gastric mucosa also decreased in men aged 41–60 years (to 81 and 45%) and 61–80 years (to 86 and 33%) in the case of increasing alcohol intake (0.4–0.8 and >0.8 g/kg/day, respectively), the differences did not reach statistical significance in those groups (P = 0.160 and P = 0.529, respectively).

Within the present study, the statistically relevant number of women consuming >0.4 g/kg/day was limited to those aged...
The results of the present study demonstrate that both age and gender influence the activity of ADH in the stomach of Caucasian subjects. The finding of decreasing gastric ADH activity with increasing age in humans is in accordance with the results of some other reports (Harada and Okubo, 1993; Seitz et al., 1993; Moreno et al., 1994; Thuluvath et al., 1994; Pozzato et al., 1995). At variance with the latter publications, other authors did not find an effect of age on the activity of ADH in gastric mucosa of the antrum or corpus (Salmela et al., 1994; Yin et al., 1997; Lai et al., 2000; Kechagias et al., 2001).

Several factors might contribute to the conflicting results of gastric ADH activity in relation to age. (1) Gastric ADH activity decreases in patients with diseases of the gastric mucosa, such as chronic gastritis, predominantly caused by \textit{H. pylori}, and in atrophic gastritis (Salmela et al., 1994; Thuluvath et al., 1994; Simanowski et al., 1998). (2) Genetic polymorphism and differences in the distribution of the phenotypes of the ADH isoenzymes in the stomach may influence the enzyme activity (Baraona et al., 1991; Moreno et al., 1994; Yin et al., 1997; Lai et al., 2000). (3) Several drugs interfere with gastric ADH activity. Detailed studies have been performed on the inhibitory action of H$_2$-receptor antagonists cimetidine, ranitidine, famotidine and nizadine (Caballeria et al., 1989a; Hernandez-Munos et al., 1990; Palmer et al., 1991; DiPadova et al., 1992; Stone et al., 1995). Inhibition of gastric ADH was also observed by aspirin, salicylic acid, acetaminophen (paracetamol) and propranolol (Palmer et al., 1991; Seitz and Oneta, 1998). (4) Different substrate concentrations used in the studies may be responsible for the different values of gastric ADH activity (Yin et al., 1997; Seitz and Oneta, 1998; Lai et al., 2000). (5) A large inter-individual variation in the activity of gastric ADH in humans has been observed in nearly all studies (reviewed in Seitz and Oneta, 1998). Small sample size, therefore, is likely to produce statistical type I or II errors.

Shortcomings in earlier studies on the effect of age and gender on gastric ADH activity are small sample sizes (Frezza et al., 1990; Harada and Okubo, 1993; Moreno et al., 1994; Salmela et al., 1994; Thuluvath et al., 1994) and inclusion of subjects with gastric diseases or no information on this point (Frezza et al., 1990; Hernandez-Munos et al., 1990; Moreno et al., 1994; Salmela et al., 1994; Thuluvath et al., 1994; Kechagias et al., 2001). Further, in several studies, no information was given about other factors which might influence gastric ADH activity, such as ethnic background of the subjects (Moreno et al., 1994; Salmela et al., 1994; Thuluvath et al., 1994; Pozzato et al., 1995; Kechagias et al., 2001), alcohol consumption (Salmela et al., 1994; Thuluvath et al., 1994; Pozzato et al., 1995; Yin et al., 1997), and detailed information on drug intake (Thuluvath et al., 1994; Salmela et al., 1994; Pozzato et al., 1995).

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The purpose of the design of the present study was to overcome some of the problems mentioned above. Only subjects from the same ethnic background were included, the sample size was similar to that in the two earlier studies with the highest number of subjects included (Seitz et al., 1993; Lai et al., 2000) and possible secondary effects of gastric diseases were avoided by exclusion of all patients with endoscopic or histological evidence of moderate to severe gastritis of any type, ulcer disease or malignancies. Detailed information was collected regarding other potentially confounding factors: alcohol consumption, smoking and any type of recent or permanent medication.

In studies on the effect of factors such as age and gender on total gastric ADH activity in humans, the influence of methodological factors has to be considered. It has been claimed that ADH activity might be lost by freezing of biopsy specimens or tissue homogenate (Seitz and Oneta, 1998). From the presented results, it can be concluded that the cytosolic fraction of mucosal homogenates can be stored at –80°C for at least 4 weeks without loss of ADH activity. It has been argued that it is imperative to measure the ADH activity within the range of physiological pH 7.0–7.5 (Yin et al., 1997; Lai et al., 2000), instead of alkaline pH (9–10) which has been used in
most studies (Caballera et al., 1989; Hernandez-Munos et al., 1990; DiPadova et al., 1992; Seitz et al., 1993; Moreno et al., 1994; Salmela et al., 1994; Thuluvath et al., 1994; Pozzato et al., 1995; Stone et al., 1995; Kechagias et al., 2001). As changes of the pH in a range of 6.8 to 10.0 had only a moderate effect on total ADH activity, the measured differences in this activity between the single groups are unlikely to occur only at pH 9.0, but would also be present at physiological pH. Further, because of the presence of various ADH isoenzymes in the human gastric mucosa, the measurement of total gastric ADH activity may vary with different substrate concentrations. The kinetic properties of the ADH isoenzymes in gastric mucosa differ distinctly: the $K_m$ values of classes I ADH and IV ADH are approximately 1 and 41 mM, respectively. Class III ADH is unsaturable for alcohol (Seitz and Oneta, 1998). Since, at high alcohol concentration, substrate inhibition of class I ADH may occur and because genetic polymorphism in class I ADH and ethnic differences in the expression of class IV ADH have been described (Moreno et al., 1994; Yin et al., 1997; Seitz and Oneta, 1998), the selection of the substrate concentration needs attention. In fact, in a study on gastric ADH activity in Han Chinese, gastric antral mucosal ADH activities were slightly (8–15%), but significantly, higher at low ethanol concentration (33 mM), compared to high ethanol concentration (500 mM) (Lai et al., 2000). From the present data on the relationship between ethanol concentration in the test and ADH activity, it is evident that maximal ADH activity can be measured using ethanol concentrations of $\geq$100 mM. The ethanol concentration of 500 mM was chosen, because it has been used in most other studies (Seitz and Oneta, 1998).

The lower values (–48%) of ADH activity in biopsies from the corpus, compared to values obtained in antral biopsies, are in accordance with the data in earlier reports (Seitz et al., 1993; Oneta et al., 1998). Since in most studies on the influence of age and gender on gastric ADH, biopsies from the antrum were used (Frezza et al., 1990; Yin et al., 1997; Seitz and Oneta, 1998; Lai et al., 2000) it was decided also to include antral biopsies into this study. Because of ethical reasons biopsies were not taken from both locations.

The existence of gender differences in the bioavailability of ethanol has been generally accepted (Thomasson, 1995). These differences are in part explained by the different ratio of body water to adipose tissue in women and men (Thomasson, 1995; Kwo et al., 1998). Other authors have found a lower first-pass metabolism in females than in males and suggested that this might be the more important cause of the gender difference in alcohol metabolism. In the study by Frezza et al. (1990), gastric ADH activities correlated with the values of first-pass metabolism, and it was suggested that gastric ADH activity is responsible for gender differences in alcohol degradation.

In the present study, lower gastric ADH activities in females compared to males were only observed in the youngest age group (20–40 years). Because of the small number of females in this subgroup, this finding has to be interpreted with caution, although it is in accordance with data from another trial from Germany using the same method for ADH measurement (Seitz et al., 1993). In this study, total gastric ADH was found to be lower in young females, compared with age-matched males. No significant sex differences in gastric ADH activity were found in other studies (Moreno et al., 1994; Salmela et al., 1994; Thuluvath et al., 1994; Lai et al., 2000). In three of these studies, the sample sizes were small, thereby increasing the likelihood of a type II error. The fourth study, which was performed in Han Chinese and in which no gender differences of gastric ADH activity were observed (Lai et al., 2000), had a sample size similar to that of the present study. In the Chinese study, no differences in the distribution of the phenotypes of ADH 3 1-1/ADH 3 1-2 and $\mu$ADH (+)/$\mu$ADH (−) between males and females were found. A possible explanation for these divergent findings might be ethnic differences in the latter study and an inappropriate age grouping of females and males. As evident from the present study, the ratio of gastric ADH activity in males to that of females was found to be inverted in age groups 20–40 years and 41–60 years. The marked decrease in ADH activity in subjects with high mean daily alcohol intake compared to the group of non-drinkers or moderate drinkers confirms the results of earlier reports (Frezza et al., 1990; Seitz et al., 1993).

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