Biochemical Pharmacology, Vol. 34, No. 13, pp. 2385–2386, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

SHORT COMMUNICATIONS

Sex differences in hepatic alcohol dehydrogenase activity in animal species

(*Received* 1 *August* 1984; *accepted* 9 *November* 1984)

We have reported previously [1, 2] that hepatic alcohol dehydrogenase (ADH) activity is high in young pre-pubertal male and female spontaneously hypertensive (SH) rats. However, in sexually mature SH rats, ADH activity is markedly reduced in the males, while it remains high and virtually unchanged in the females. Castration prevents the marked reduction in ADH activity in the males, whereas chronic testosterone administration to castrated male SH rats reduces the activity of ADH to a level as low as that found in mature native males. A similar pattern was observed with respect to the rate of ethanol metabolism measured *in vivo*. We have concluded [2] that, in the male SH rat, the rate of ethanol metabolism is primarily limited by the levels of alcohol dehydrogenase which are, in turn, modulated by testosterone. Other investigators have confirmed the findings on the inhibitory effect of testosterone on hepatic ADH activity and on the rate of ethanol metabolism in Sprague–Dawley rats, which are more commonly used in the laboratory [3–6]. The repression of ADH by testosterone has also been reproduced in Lewis rats [7]. Since similar results have been obtained on the inhibitory effect of testosterone on hepatic ADH activity in three strains of rats at different laboratories, it suggests that this may be a general biological occurrence. Therefore, it was of interest to determine whether these observations can be generalized to other strains and animal species. For this purpose, in the present study, we have measured the activity of ADH in livers of adult male and female mice, rats, guinea pigs, rabbits and dogs.

Adult male and female animals of the different species were obtained from the following suppliers. C57BL/6 and DBA/2 mice, and Sprague–Dawley rats were supplied by Charles River (St. Constant, Quebec, Canada). Spontaneously hypertensive rats were purchased from Taconic Farms (Germantown, NY, U.S.A.) Albino guinea pigs and New Zealand albino rabbits were obtained from High Oak Ranch Ltd. (Goodwood, Ontario, Canada) and Riemens (St. Agatha, Ontario, Canada) respectively. The mice, rats and guinea pigs were killed by decapitation, and the rabbits by cervical dislocation. Liver samples were taken into liquid nitrogen and were kept frozen at −80° for periods not exceeding 7 days. Frozen livers of dogs, which were killed by injection of Sucostrin (succinylcholine), were supplied by Pel-Freeze (Rogers, AR, U.S.A.).

ADH activity in the liver was determined essentially as described by Crow *et al.* [8]. Liver samples were homogenized in 3 vol. of 0.05 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), pH 8.4, containing 0.33 mM dithiothreitol and centrifuged at 40,000 *g* for 45 min at 4°. Aliquots (0.02 ml) of the supernatant fractions obtained were assayed for ADH activity at 37° in 2 ml of a reaction mixture containing 0.5 M Tris buffer, pH 7.4, 2.8 mM NAD^+ and 10 mM ethanol (final concentrations). Under these conditions enzyme activity was linear with respect to both amount of supernatant fraction and time. The activity was calculated from the initial rate of NADH production at 340 nm and expressed per g liver and per kg body weight.

Hepatic alcohol dehydrogenase activities per g liver and per kg body weight are presented in Table 1. Of all the species and strains studied, only the SH rat and the mouse strains showed significant sex differences in ADH activity. No sex differences in ADH activity were observed in guinea pigs, rabbits and dogs, thus suggesting that sex-dependent modulation of ADH is not a general biological phenomenon. This does not, however, rule out a testosterone-dependent effect on ADH activity, as shown previously, but suggests that, when the effect is present, other factors override the effect.

The activity of ADH in the C57BL/6 mice, the high alcohol preference strain, was higher in the females than in the males (+19% per g liver and +13% per kg body weight). Similarly, in the DBA/2 mice, the low alcohol preference strain, ADH activity was elevated in the females relative to the males (+17% per g liver and +24% per kg body weight). While this pattern was similar to that found in the SH rat, it should be noted that the magnitude of the difference in ADH activity between the sexes was greater in the latter (+145% per g liver and +193% per kg body weight). In addition to the sex difference in ADH activity found in the mice, in the present investigation, a strain difference in ADH activity was also observed. The activity of ADH in the high alcohol preference strain (C57BL/6) was higher than in the low preference strain (DBA/2).

Similar sex differences in ADH activity to those found in our studies were observed by other investigators in adult C57BL and DBA mice [9]. Studies in sexually mature-BALB and C57BL/lbg mice have also shown that the rates of blood ethanol disappearance are elevated markedly in the females as compared with the males of these strains [10]. However, data in this study [10] suggested that these differences were not related to ADH activity. In the present study, no sex difference in ADH activity was observed in Sprague–Dawley rats, which are commonly used in the laboratory but which do not constitute an inbred strain, thus allowing for breeder differences. These results are in variance to observations by Buttner [11], who found that ADH activity in female Sprague–Dawley rats was greater than in their male counterpart. However, comparison of the values of ADH activity in male and female Sprague–Dawley rats, reported in studies by Mezey *et al.* [5, 6, 12] also shows that, in this strain, ADH activity is not sex dependent.

Another observation that emerged from the present investigation is that the activity of liver ADH in the males of the different species and strains of animals studied correlates well ($r = 0.77$, $P < 0.05$) with corresponding rates of ethanol elimination *in vivo* reported in the literature [13]. This suggests that, in the species investigated, the level of ADH plays a rate-determining factor in the metabolism of ethanol. We have, however, not measured activities in larger animals. For example, the horse is known to present high liver ADH levels but low rates of ethanol metabolism [14]. Present data are in line with the suggestion [15] that the level of ADH can become an important rate-determining step in the metabolism of ethanol in small animals. This may not be the case in larger animals with low basal metabolic rats. Indeed, a much better correlation ($r = 0.94$, $P < 0.001$) has been found between basal metabolic rate and the rate of ethanol metabolism across a wide range of species, including the horse and man [16], which supports the rate-limiting role of mitochondrial oxidation

Table 1. ADH activity in livers of adult male and female animals of different species*

Species	ADH activity (μmoles/g liver/hr)		(mmoles/kg body wt/hr)	
	Male	Female	Male	Female
Mouse				
C57BL/6	459.0 ± 7.2	547.2 ± 15.0†	24.89 ± 0.52	28.24 ± 0.78‡
DBA/2	316.2 ± 10.2	371.4 ± 4.8‡	16.35 ± 0.24	20.22 ± 0.52†
Rat				
Sprague–Dawley	292.2 ± 10.8	312.0 ± 18.6	8.46 ± 0.46	10.11 ± 0.85
SH strain	122.6 ± 3.4	300.1 ± 16.7§	4.54 ± 0.19	13.31 ± 0.64‖
Guinea pig	202.8 ±13.8	238.2 ± 13.2	8.59 ± 0.54	6.46 ± 0.61
Rabbit	577.8 ± 24.0	624.0 ± 9.0	16.57 ± 1.37	19.11 ± 0.33
Dog	117.0 ± 14.4	137.6 ± 20.4	1.59 ± 0.17	1.89 ± 0.20

* Values represent means ± S.E.M. (N = 4–5 males or females). Only statistically significant differences ($P < 0.05$) in ADH activity between males and females of each species and strain are indicated.
‡ $P < 0.01$.
† $P < 10^{-3}$.
§ $P < 10^{-4}$.
‖ $P < 10^{-5}$.

of NADH in ethanol metabolism across the zoological scale.

In conclusion, it appears that the high activity of liver ADH in adult females relative to adult males in some rodent strains does not constitute a general phenomenon, but it depends on the species and strains of animals studied. In addition, for the species studied, liver ADH levels correlated with reported rates of *in vivo* ethanol metabolism.

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Biochemical Pharmacology, Vol. 34, No. 13, pp. 2386–2389, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

Effects of dimethyl sulfoxide (DMSO) on bleomycin-induced pulmonary fibrosis

(*Received* 3 *September* 1984; *accepted* 13 *December* 1984)

Bleomycin, an anti-neoplastic agent composed of a heterogeneous mixture of at least thirteen components [1], has been demonstrated to have effective anti-tumor activity in the treatment of squamous cell carcinomas as well as some lymphomas. Since it is less immunosuppressive than most other anti-neoplastic agents, it is often used in a combination drug regimen [2]. Unfortunately, the high incidence of pulmonary toxicity in those patients treated bleomycin impedes cancer therapy and is regarded as the most significant limiting factor for an intensive and prolonged course in bleomycin cancer therapy [3].

The pulmonary toxicity is characterized by the excessive deposition of connective tissue. Collagen, a major component of connective tissue, has been the focus of many