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Modulation of Alcohol Dehydrogenase and Ethanol Metabolism by Sex Hormones in the Spontaneously Hypertensive Rat

EFFECT OF CHRONIC ETHANOL ADMINISTRATION

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In young (4-week-old) male and female spontaneously hypertensive (SH) rats, ethanol metabolic rate *in vivo* and hepatic alcohol dehydrogenase activity *in vitro* are high and not different in the two sexes. In males, ethanol metabolic rate falls markedly between 4 and 10 weeks of age, which coincides with the time of development of sexual maturity in the rat. Alcohol dehydrogenase activity is also markedly diminished in the male SH rat and correlates well with the changes in ethanol metabolism. There is virtually no influence of age on ethanol metabolic rate and alcohol dehydrogenase activity in the female SH rat. Castration of male SH rats prevents the marked decrease in ethanol metabolic rate and alcohol dehydrogenase activity, whereas ovariectomy has no effect on these parameters in female SH rats. Chronic administration of testosterone to castrated male SH rats and to female SH rats decreases ethanol metabolic rate and alcohol dehydrogenase activity to values similar to those found in mature males. Chronic administration of oestradiol-17β to male SH rats results in marked stimulation of ethanol metabolic rate and alcohol dehydrogenase activity to values similar to those found in female SH rats. Chronic administration of ethanol to male SH rats from 4 to 11 weeks of age prevents the marked age-dependent decreases in ethanol metabolic rate and alcohol dehydrogenase activity, but has virtually no effect in castrated rats. In the intoxicated chronically ethanol-fed male SH rats, serum testosterone concentrations are significantly depressed. *In vitro*, testosterone has no effect on hepatic alcohol dehydrogenase activity of young male and female SH rats. In conclusion, in the male SH rat, ethanol metabolic rate appears to be limited by alcohol dehydrogenase activity and is modulated by testosterone. Testosterone has an inhibitory effect and oestradiol has a testosterone-dependent stimulatory effect on alcohol dehydrogenase activity and ethanol metabolic rate in these animals.

There is some evidence in the literature indicating an influence of age and sex on the rate of ethanol metabolism and on the activity of the enzyme alcohol dehydrogenase, depending on the species and the strain of animals studied. Blood ethanol disappearance rates have been reported to be markedly diminished in old as compared with young male C57BL/Ibg and BALB mice, but not in females, in which they remained high and were not affected by age (Collins *et al.*, 1975, 1976). In other studies higher alcohol dehydrogenase activity has been observed in young as compared with old male BALB/c mice (Schlesinger, 1966). Young male Sprague–Dawley rats have been shown to have greater rates of ethanol elimination than have the respective old males (Hollstedt & Rydberg, 1970). Females of different Wistar-derived strains of rats have been found to eliminate ethanol from the blood faster than do males (Eriksson & Malmström, 1967; Eriksson, 1973). Also, hepatic alcohol dehydrogenase activity was found to be higher in female than in male adult mice (Eriksson & Pikkarainen, 1968;

Abbreviation used: SH rat, spontaneously hypertensive rat.

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Collins *et al.*, 1975) and Sprague–Dawley rats (Büttner, 1965). Unfortunately, in most of these studies either ethanol elimination rate or hepatic alcohol dehydrogenase activity alone was determined, so that no conclusions can be drawn with respect to the correlation between these two factors. Work done with C57BL/Ibg mice, however, has indicated that hepatic alcohol dehydrogenase activity did not correlate with the marked decrease in ethanol elimination rate in the old males (Collins *et al.*, 1975, 1976), which suggests that alcohol dehydrogenase activity does not limit ethanol metabolism in this strain. It should be noted that, in species and strains in which the rate of ethanol metabolism is primarily limited by the rate of mitochondrial reoxidation of NADH, ethanol metabolism correlates with basal metabolic rate (Videla *et al.*, 1975). The latter is higher in younger animals and may constitute the basis for the greater rate of ethanol metabolism found in them.

We have previously reported that the rate of ethanol metabolism in the male spontaneously hypertensive (SH) rat is markedly dependent on the age of the animal. Ethanol metabolism is markedly diminished as the animals age, and chronic administration of ethanol prevents this decrease. Thus ethanol appeared to keep the animals at a 'metabolically younger' age (Israel *et al.*, 1977). We have now found that, in these animals, the changes in ethanol metabolism are markedly sex-dependent, and that changes in testosterone status and alcohol dehydrogenase activity play a major role in both the metabolic tolerance that follows chronic administration of ethanol and the age-related changes in ethanol metabolism.

Materials and Methods

Animals

Spontaneously hypertensive rats (SH rats) were purchased from Taconic Farms (Germantown, NY, U.S.A.). The dates of birth of all animals were specified by the supplier. The rats were kept in our animal quarters in wire-mesh cages and received commercial laboratory chow (Wayne Lab-Blox) and water for 3–7 days before the experiments were conducted.

Chronic administration of ethanol

Unless indicated, rats were fed on laboratory chow *ad libitum*. In experiments in which ethanol was administered chronically, Lieber-type liquid diets containing 36% of the total dietary energy as ethanol, 19% as proteins, 41% as fat and the remainder as carbohydrate (Khanna *et al.*, 1967) were given to either castrated or sham-operated male SH rats from 4 to 11 weeks of age. Ethanol was replaced isocalorically by sucrose in the diets of control rats. In each group, which consisted of (*a*) one castrated ethanol-treated animal, (*b*) one sham-operated ethanol-treated animal, (*c*) one castrated sucrose-control animal and (*d*) one sham-operated sucrose-control animal, feeding was controlled by the animal with the lowest energy intake per day.

Determination of ethanol metabolic rate

Before the determination of ethanol metabolic rate, the animals that had received ethanol chronically and their pair-fed controls were given 70ml of sucrose diet overnight. Thus the animals were withdrawn from ethanol, but fed. Food and water were not offered during the measurement of ethanol metabolic rate. Ethanol was determined in tail-vein blood samples taken 2, 3, 4, 5 and 6h after the intraperitoneal administration of 2.5g of ethanol/kg as a 12.5% (w/v) solution in saline (0.9% NaCl). The samples were deproteinized and ethanol was assayed enzymically (Hawkins *et al.*, 1966). Blood ethanol concentrations were plotted as a function of time. After a distribution time of 1–2h, the disappearance of ethanol from the blood was linear with time. The intercept at the abscissa was taken as the time at which all the administered ethanol was metabolized. The value at the intercept was 4–6h for the female and young male SH rats, and 10–13h for the sexually mature male SH rats (see the Results section). Ethanol metabolic rate in terms of mg of ethanol/h per kg body wt. was obtained by dividing the dose of ethanol administered (mg/kg) by the period of time required for its complete elimination (h). The value obtained was divided by the ratio of wet liver weight to body weight (expressed as g of liver/kg body wt.), in order to express ethanol metabolic rate in terms of mg of ethanol/h per g of liver.

Alcohol dehydrogenase activity

After the determination of ethanol metabolic rate in naive or in chronically treated animals, the animals were fed on chow or sucrose liquid diet overnight respectively, and were killed by decapitation within 18–20h. Preliminary experiments had shown that freezing the liver samples, taken from the left lobe, in liquid N_2 and storing them at −80°C for a period of 1 week did not alter alcohol dehydrogenase activity. Therefore analysis of alcohol dehydrogenase activity was done within 1 week in the frozen livers. The samples were thawed at room temperature, weighed (1–2g) and homogenized in 9vol. of 1% (v/v) Triton X-100 (Räihä & Koskinen, 1964). The clear supernatants obtained after centrifugation at 46000*g* for 1h in a Sorvall RC2-B refrigerated centrifuge were used for assay of the enzyme activity.

Alcohol dehydrogenase activity was measured as described by Hawkins *et al.* (1966), with slight

modifications as follows. The reaction mixture contained 3.1ml of semicarbazide/glycine/NaOH buffer, pH9.2, 0.1ml of 0.01M-NAD^+ solution, 3.7μl of ethanol (final concentrations 0.31mM and 20mM respectively) and a 5μl sample of supernatant. A cuvette in which ethanol was not added served as a blank. The NADH formation at 22°C was followed at 340nm in a Gilford 2400 spectrophotometer for 4min. Under these conditions enzyme activity was linear with respect to both amount of supernatant and time. In order to compare alcohol dehydrogenase activity with ethanol metabolic rate, alcohol dehydrogenase activity was calculated in terms of mg of ethanol/h per kg body wt. and mg of ethanol/h per g of liver. These values were multiplied by 0.871, to correct for pH and temperature to those existent *in vivo* (Videla & Israel, 1970).

Gonadectomy and chronic administration of sex hormones

Rats were anaesthetized with ether. A single incision into the scrotum beginning near the anal end was made and each testis was ligated and removed. Ovaries were removed through paired dorsal incisions. Sham operation was performed in control animals.

Capsules filled with testosterone powder (Sigma Chemical Co.) or oestradiol-17β (Sigma Chemical Co.) were prepared from Silastic tubing (outer diameter 3.175mm, wall thickness 0.8mm) as described by Legan *et al.* (1975), and were implanted in dorsal subcutaneous pockets in the animal. A 30mm-long capsule was used for administration of testosterone. In castrated male rats, a capsule of these characteristics and length has been reported to give a steady plasma concentration of testosterone that maintains the plasma lutropin concentration at the normal range of intact animals (Damassa *et al.*, 1976). The oestradiol capsules were 5mm long, a length that was reported by Legan *et al.* (1975) to maintain normal steady serum oestradiol concentrations.

Radioimmunoassay

Animals were killed by decapitation and blood was collected from the neck. The clotted blood samples were centrifuged at 1080*g* for 15min at 4°C, and sera were stored at −80°C. The determination of free serum testosterone (testosterone plus dihydrotestosterone) concentrations was done by radioimmunoassay (Odell *et al.*, 1974).

Statistical analysis

Results are presented as means ± S.E.M. Statistical significance was determined by the Student's *t* test. Differences were considered significant when probability values of less than 0.05 were obtained.

Results

Effects of age and sex

The relationship between age and the rate of ethanol metabolism in the male and female naive chow-fed SH rats is presented in Figs. 1(*a*) and 1(*b*). A drastic decrease in ethanol metabolic rate occurs in the adult male SH rats, but not in the female rats. In the latter, the minor decrease in ethanol metabolic rate expressed per unit body weight is fully accounted for by a decrease in the liver/body weight ratio, and the rate of ethanol metabolism expressed per unit liver weight remains constant at all ages. In the males, however, a marked decrease in ethanol metabolic rate is evident when the rate of ethanol metabolism is expressed both per g of liver or per kg body wt. In these animals the rate of ethanol metabolism falls sharply, by 48% (mg/h per g of liver), between 4 and 10 weeks of age; thereafter it does not change appreciably. Liver alcohol dehydrogenase activity *in vitro* was also decreased to the same extent, by 53% (mg/h per g of liver), with increasing age in the male SH rats, at the same period. Alcohol dehydrogenase was only slightly affected by age in the females of this strain (Figs. 1*c* and 1*d*). In the males a significant positive correlation ($r = 0.909$; $P < 0.0001$) was found. Minor changes in alcohol dehydrogenase activity in the females did not correlate with ethanol metabolism *in vivo*. It should be noted that the absolute activity of alcohol dehydrogenase measured *in vitro* is lower than the rate of ethanol metabolism measured *in vivo*. This may be due to some loss of alcohol dehydrogenase activity during the preparation of the supernatant fraction and to the different conditions that might prevail *in vitro* and *in vivo*.

To determine whether the changes in the rate of ethanol metabolism and alcohol dehydrogenase activity were related to the development of hypertension in these animals, systolic blood pressures were determined (by using conventional tail cuffs connected to a calibrated pressure transducer) in male and female SH rats at 5–6 weeks and 10–11 weeks of age. In the males blood pressure increased from 99 ± 6mmHg at 5–6 weeks to 135 ± 12mmHg at 10–11 weeks. Similarly in the females blood pressure rose from 104 ± 6mmHg at 5–6 weeks to 137 ± 6mmHg at 10–11 weeks, thus indicating that hypertension as such is not related to changes in ethanol metabolism in the males. Administration of hydralazine, a drug that markedly lowered blood pressure, did not affect ethanol metabolism (results not shown).

Effects of gonadectomy and of administration of testosterone and of oestradiol

Since in the male SH rats the changes in the rate of ethanol metabolism and in alcohol dehydrogen-

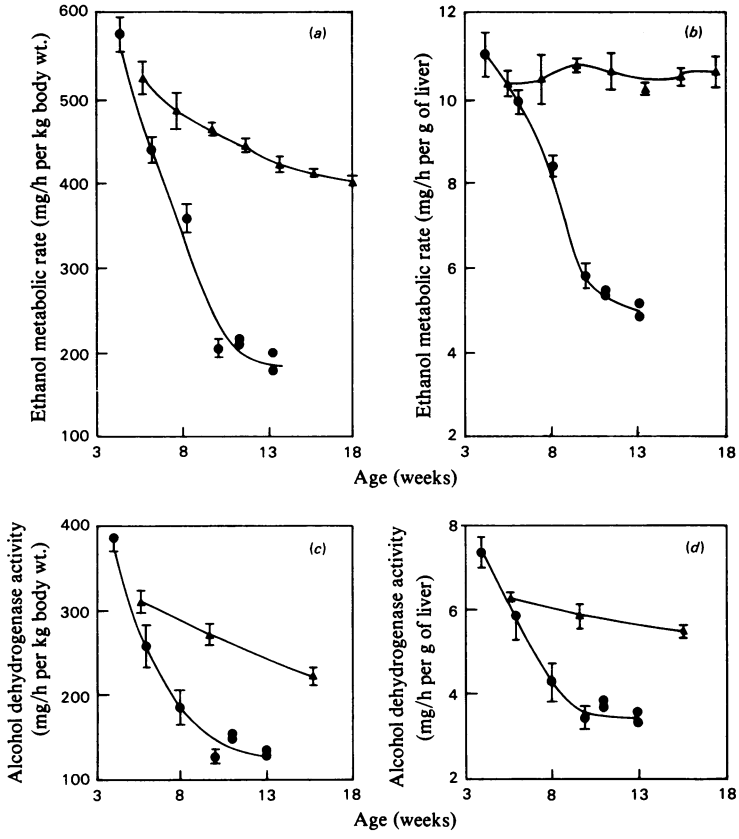

Fig. 1. *Effects of age and sex on ethanol metabolic rate and alcohol dehydrogenase activity in naive chow-fed SH rats* Experimental details are given in the text. Each point and bar represent the mean ± S.E.M. for four males (●) or females (▲) for determination of ethanol metabolic rate (*a* and *b*), and three males (●) or five females (▲) for determination of alcohol dehydrogenase activity (*c* and *d*).

ase activity coincided with the time of development of sexual maturity, we determined whether sex hormones were involved in these changes.

Fig. 2(*a*) shows that castration at pre-puberty (4–5 weeks) inhibited the marked age-dependent decrease in ethanol metabolic rate in the male SH rats. Chronic administration of testosterone completely reversed the effect of castration on ethanol metabolism, though having no significant effects on sham-operated control rats. A virtually identical pattern was observed in alcohol dehydrogenase activity. Castration inhibited the marked age-dependent fall in alcohol dehydrogenase activity, and administration of testosterone to castrated male SH rats returned their alcohol dehydrogenase activity to values as low as those found in sham-operated animals (Fig. 2*b*).

We further determined whether the ethanol-metabolizing capacity of the female SH rats, in which ethanol metabolic rate and alcohol dehydrogenase activity were maintained high and virtually constant at different ages, was also sensitive to testosterone. The results (Table 1) show that chronic administration of testosterone to female SH rats leads to marked decreases in both ethanol metabolic rate and hepatic alcohol dehydrogenase activity to values comparable with those found in the mature males of this strain. Ovariectomy did not result in significant changes in these parameters (Table 1).

Similarly chronic administration of oestradiol to intact and ovariectomized female SH rats resulted in a rate of ethanol metabolism and alcohol dehydrogenase activity comparable with those found in their respective controls (Table 1). In sharp contrast, chronic administration of oestradiol to the male SH rats leads to a striking stimulation of ethanol metabolic rate and alcohol dehydrogenase activity to values as high as those found in the female SH rats (Table 2). Similar results were observed in castrated male SH rats treated with testosterone plus oestradiol (Table 2); oestradiol prevented the inhibitory effect of testosterone on ethanol metabolism and

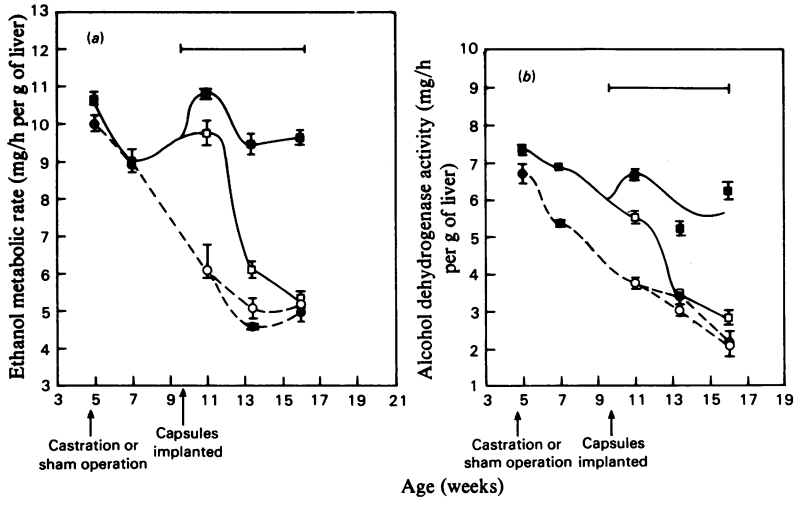

Fig. 2. *Effects of castration at pre-puberty and of chronic administration of testosterone on ethanol metabolic rate and alcohol dehydrogenase activity in the male SH rat*

Experimental details are given in the text. Key: ■, castrated; ●, sham-operated; □, castrated + testosterone; ○, sham-operated + testosterone. Each point and bar represent the mean ± S.E.M. for four or five animals.

Table 1. *Effects of ovariectomy at pre-puberty and of chronic administration of testosterone or of oestradiol on ethanol metabolic rate and alcohol dehydrogenase activity in the female SH rat*

Animals were ovariectomized at 3.9 weeks of age. Capsules for hormone administration were implanted at 5.4 weeks of age. Ethanol metabolic rate and alcohol dehydrogenase activity were determined at the age of 13.2 weeks. Experimental details are given in the text. The number of animals in each group is given in parentheses. Only statistically significant differences from respective controls are indicated.

Treatment group	Ethanol metabolic rate (mg/h per g of liver)	Alcohol dehydrogenase activity (mg/h per g of liver)
Sham-operated control	11.67 ± 0.33 (5) ($P < 10^{-6}$)	5.56 ± 0.39 (3) ($P < 0.002$)
Sham-operated + testosterone	5.75 ± 0.25 (6)	3.47 ± 0.17 (5)
Sham-operated + oestradiol	12.54 ± 0.33 (6)	5.62 ± 0.19 (4)
Ovariectomized control	11.34 ± 0.76 (4) ($P < 0.001$)	6.30 ± 0.71 (3) ($P < 0.002$)
Ovariectomized + testosterone	5.78 ± 0.08 (5)	3.20 ± 0.11 (5)
Ovariectomized + oestradiol	10.11 ± 0.88 (5)	5.07 ± 0.22 (5)

alcohol dehydrogenase activity in the male SH rats. In castrated males without testosterone implants oestradiol had no significant effect on alcohol dehydrogenase activity, but it produced a small but significant increase in the rate of ethanol metabolism. Thus in these animals a small part of the oestradiol effect on ethanol metabolism is independent of alcohol dehydrogenase activity and testosterone.

The observation that administration of testosterone *in vivo* can diminish both ethanol metabolic rate *in vivo* and hepatic alcohol dehydrogenase activity *in vitro* raised the possibility that the enzyme might be directly inhibited by testosterone. Therefore kinetic studies were conducted to investigate the effect of testosterone added *in vitro* (0.1 and 10ng/ml) on liver alcohol dehydrogenase activity from young pre-puberty male and female SH rats. No effect of testosterone was obtained *in vitro* (results not shown).

Effect of chronic treatment with ethanol

The relationship between ethanol metabolism and testosterone in naive animals suggested that the increase in the rate of ethanol metabolism that follows chronic administration of ethanol to male SH rats might be related to a decrease in testosterone concentrations. This hypothesis was supported by the observation that, in the chronically ethanol-fed male SH rats (Lieber-type liquid diet was administered from the age of 4 weeks to the age of 11 weeks), at

Table 2. *Effects of castration at pre-puberty and of chronic administration of testosterone with and without oestradiol on ethanol metabolic rate and alcohol dehydrogenase activity in the male SH rat*

Animals were castrated at 4.1 weeks of age. Capsules for hormone (testosterone or oestradiol) administration were implanted at 6 weeks of age. Ethanol metabolic rate and alcohol dehydrogenase activity were determined at the age of 13 weeks. Experimental details are given in the text. The number of animals in each group is five to seven. Ethanol metabolic rate: (*a*) versus (*b*), not significant; (*a*) versus (*c*), $P<0.001$; (*a*) versus (*d*), $P<10^{-7}$; (*e*) versus (*f*), $P<0.001$; (*e*) versus (*g*), $P<0.02$; (*e*) versus (*h*), $P<0.05$. Alcohol dehydrogenase activity: (*a*) versus (*b*), not significant; (*a*) versus (*c*), $P<10^{-7}$; (*a*) versus (*d*), $P<10^{-6}$; (*e*) versus (*f*), $P<10^{-4}$; (*e*) versus (*g*) and (*e*) versus (*h*), not significant.

Treatment group	Ethanol metabolic rate (mg/h per g of liver)	Alcohol dehydrogenase activity (mg/h per g of liver)
(*a*) Sham-operated control	5.62 ± 0.15	3.22 ± 0.13
(*b*) Sham-operated + testosterone	5.64 ± 0.30	3.30 ± 0.28
(*c*) Sham-operated + oestradiol	10.53 ± 0.70	6.23 ± 0.16
(*d*) Sham-operated + testosterone + oestradiol	9.56 ± 0.21	5.67 ± 0.15
(*e*) Castrated control	9.23 ± 0.27	5.61 ± 0.28
(*f*) Castrated + testosterone	5.52 ± 0.36	3.37 ± 0.12
(*g*) Castrated + oestradiol	10.71 ± 0.35	5.78 ± 0.19
(*h*) Castrated + testosterone + oestradiol	10.11 ± 0.20	5.79 ± 0.16

Table 3. *Effects of chronic administration of ethanol on ethanol metabolic rate and alcohol dehydrogenase activity in castrated and sham-operated male SH rats*

Animals were castrated or sham-operated at 4 weeks of age. Lieber-type liquid diets were administered from the age of 4 weeks to the age of 11 weeks, as described in the Materials and Methods section. Ethanol metabolic rate and alcohol dehydrogenase were determined at 11 weeks of age. Experimental details are given in the text. The number of animals in each group is five. N.S., Not significant.

	Ethanol metabolic rate		Alcohol dehydrogenase activity	
Treatment group	(mg/h per kg body wt.)	(mg/h per g of liver)	(mg/h per kg body wt.)	(mg/h per g of liver)
Sham-operated sucrose-fed control	301 ± 16 ($P<10^{-5}$)	6.15 ± 0.26 ($P<0.001$)	177 ± 7 ($P<10^{-3}$)	3.60 ± 0.08 ($P<10^{-3}$)
Sham-operated ethanol-fed	566 ± 21	9.83 ± 0.26	304 ± 18	5.25 ± 0.15
Castrated sucrose-fed control	423 ± 9 ($P<0.01$)	8.90 ± 0.28 (N.S.)	275 ± 12 (N.S.)	5.78 ± 0.31 (N.S.)
Castrated ethanol-fed	541 ± 18	9.96 ± 0.53	319 ± 19	5.78 ± 0.44

the peak of ethanol intoxication (232 ± 10 mg/dl) serum testosterone concentrations were indeed very low (1.76 ± 0.23 ng/ml in the chronically ethanol-fed rats versus 5.58 ± 1.27 ng/ml in the pair-fed sucrose controls; $P<0.03$, $n=4$ pairs). If ethanol exerts its effects through a decrease in testosterone concentrations, it follows that no difference should be observed in ethanol metabolism between castrated ethanol-fed and castrated sucrose-fed control male SH rats. Data in Table 3 illustrate that the development of metabolic tolerance that follows chronic treatment with ethanol is markedly diminished by castration. Results are presented both per kg body wt. and per g of liver, since ethanol is known to lead to the development of hepatomegaly (Fallon, 1975; Baraona *et al.*, 1977; Orrego *et al.*, 1979). The same pattern is obtained for liver alcohol dehydrogenase activity (Table 3), suggesting that an important part of the metabolic tolerance induced by ethanol in the male SH rat is due to an increase in alcohol dehydrogenase activity.

Discussion

The rate of ethanol metabolism and hepatic alcohol dehydrogenase activity in young pre-puberty (4–5-week-old) male and female SH rats are high, and not different between the two sexes. However, in sexually mature SH rats (10 weeks old or more) both ethanol metabolic rate and alcohol dehydrogenase activity are markedly diminished in the males, but remain virtually the same in the females. In the male SH rat, ethanol metabolism appears to be limited primarily by the activity of hepatic alcohol dehydrogenase, which is modulated by testosterone.

In the male SH rat hepatic alcohol dehydrogenase activity correlates well with ethanol metabolic rate at different ages; also, the relative changes in ethanol metabolic rate are paralleled by remarkably comparable relative changes in alcohol dehydrogenase activity under a variety of experimental conditions. Castration prevented the marked decreases in both ethanol metabolic rate and alcohol dehydrogenase activity, whereas testosterone administration to castrated male SH rats resulted in values for both ethanol metabolic rate and alcohol dehydrogenase activity as low as those in the controls. Oestradiol prevented the effect of testosterone on both ethanol metabolic rate and alcohol dehydrogenase activity. Furthermore, a large proportion of the relative increment in ethanol metabolic rate in the chronically ethanol-fed male SH rats could be explained by a relative increment in alcohol dehydrogenase activity. Taken in conjunction, these data suggest that the activity of alcohol dehydrogenase is the main limiting factor in ethanol metabolism in the male SH rat. This is further supported by the finding that an acute administration of ethanol to mature male SH rats does not alter the hepatic lactate/pyruvate concentration ratio, suggesting that the rate of production of NADH during ethanol metabolism via alcohol dehydrogenase occurs at a lower rate than that at which it can be removed by reoxidation (Wahid, 1977).

Other investigators have also indicated that, in several species and in some experimental situations, the rate of ethanol metabolism can be governed by the maximal activity of alcohol dehydrogenase (Plapp, 1975; Crow *et al.*, 1977). However, a poor correlation has often been found between the ethanol elimination rate *in vivo* and alcohol dehydrogenase activity *in vitro* in different individuals, species and stages of growth (Wallgren & Barry, 1970). Furthermore, studies indicate that mitochondrial reoxidation of NADH constitutes the primary factor in ethanol metabolism in the 'normal' strains of rats commonly used in the laboratory (Israel *et al.*, 1975; Thurman *et al.*, 1976). It seems, therefore, that some of the factors that limit the rate of ethanol oxidation are genetically determined, and care should be exercised when generalizing even within the same species.

The present studies clearly demonstrate that testosterone is responsible for the decrease in ethanol metabolic rate secondary to changes in alcohol dehydrogenase activity in the SH rat. In contrast with our findings, a testosterone-dependent regulatory mechanism for ethanol metabolism unrelated to changes in alcohol dehydrogenase activity appears to exist in mice (Collins *et al.*, 1976). Our studies have shown that testosterone added *in vitro* does not inhibit hepatic alcohol dehydrogenase activity from young male and female SH rats. This finding is in line with reports of lack of activity of hepatic alcohol dehydrogenase towards testosterone (Merritt & Tomkins, 1959; Reynier *et al.*, 1969). The mechanism for the indirect decrease in alcohol dehydrogenase activity caused by testosterone, in the SH rat, remains to be studied.

Oestradiol had no significant effect on hepatic alcohol dehydrogenase activity and ethanol metabolic rate in the female SH rat. These results are in line with observations made by other investigators, who reported that ovariectomy had no effect on hepatic alcohol dehydrogenase activity (Ohno *et al.*, 1970; Collins *et al.*, 1976) and caused only a minimal increase in ethanol elimination rate (Collins *et al.*, 1976). However, the observation that oestradiol administration prevented the inhibitory effect of testosterone on ethanol metabolic rate and alcohol dehydrogenase activity, in both the intact and castrated male SH rat, indicates that the serum testosterone/oestradiol concentration ratio influences ethanol metabolic rate and alcohol dehydrogenase activity in these animals. Oestradiol may act indirectly via mechanisms that lower the biologically active (free) concentration of testosterone in the blood (Anderson, 1974).

Chronic administration of ethanol to male SH rats starting at a young age prevented the marked age-dependent decrease in both ethanol metabolic rate and alcohol dehydrogenase activity. The marked decrease in serum testosterone concentrations observed in the chronically ethanol-fed male SH rats is in line with findings by other investigators in experimental animals (Van Thiel *et al.*, 1975; Cicero & Badger, 1977) and in man (Mendelson & Mello, 1974; Van Thiel *et al.*, 1974; Gordon *et al.*, 1975). It should be mentioned that we previously indicated (Israel *et al.*, 1977), on the basis of preliminary experiments with a small number of animals, that chronic treatment with ethanol did not change alcohol dehydrogenase activity in the male SH rat. In that study, alcohol dehydrogenase activity was measured only at one time and in older animals. The results presented in the present paper are based on many studies comprising a very large number of animals, and therefore are more representative of the general biological phenomenon in the SH rat.

In conclusion, in the male SH rat, ethanol metabolic rate appears to be limited primarily by alcohol dehydrogenase activity and is modulated by testosterone. Testosterone has an inhibitory effect, and oestradiol has a testosterone-dependent stimulatory effect on ethanol metabolic rate and alcohol dehydrogenase activity in these animals. In the female SH rats, alcohol dehydrogenase does not appear to limit ethanol metabolic rate. Administration of testosterone to females, however, produces decreases in ethanol metabolic rate and alcohol dehydrogenase activity comparable with those found in mature male

SH rats. Since the standard laboratory rat is not rigidly inbred, many substrains exist. It should be noted that SH rats were originally derived from the Wistar strain (Okamoto & Aoki, 1963). The existence, extent and individual variation of these regulatory mechanisms in other strains and species, including man, require further investigation.

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