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THE DISTRIBUTION AND METABOLISM OF ACETALDEHYDE IN RATS DURING ETHANOL OXIDATION—II.

REGULATION OF THE HEPATIC ACETALDEHYDE LEVEL

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Abstract—The regulation of the hepatic acetaldehyde (AcH) level during ethanol oxidation was investigated *in vivo* in fed male and female Sprague–Dawley rats. Various doses of ethanol were administered orally, the livers were freeze-clamped during pentobarbital anaesthesia, and ethanol, AcH, lactate, pyruvate, acetoacetate, 3-hydroxybutyrate and the aldehyde dehydrogenase activity were measured. A positive correlation was found between the ethanol and AcH concentration, when the ethanol concentrations were between 5–30 μmole/g wet wt liver. A negative correlation was found within this ethanol range between the hepatic mitochondrial free NADH/free NAD^+ ratio and the AcH concentration. A negative correlation was also obtained between the hepatic aldehyde dehydrogenase activity and the AcH concentration. A positive correlation was demonstrated between the hepatic mitochondrial free NADH/free NAD^+ ratio and the AcH concentration at ethanol concentrations above 30 μmole/g. The results are discussed in relation to the regulation of the hepatic AcH level by the metabolism of ethanol and AcH within the liver.

In the preceding paper [1] it was demonstrated that the liver is the primary site for the metabolism of acetaldehyde (AcH) during ethanol oxidation in rat. The difference between AcH formation and elimination in the liver determines the amount of AcH which leaves this organ, and which can cause eventual pharmacological effects. Furthermore this difference, determining the hepatic AcH concentration, was found to represent only a very small fraction of quantity of AcH formed or eliminated in the liver. Consequently, the relative levels of these two functions must be assumed to exert a sensitive control on the amount of AcH escaping into the rest of the body.

The hepatic AcH formation (ethanol oxidation) rate is generally believed to be determined by the cytosolic free NADH/free NAD^+ redox ratio, which in turn is regulated by the re-oxidation of NADH in the mitochondrial respiratory chain [2–5]. The regulation of hepatic AcH oxidation is, however, more obscure. The enhancement of the mitochondrial re-oxidation of NADH by uncoupling has recently been found to increase AcH oxidation during ethanol metabolism in perfused rat livers[5]. In addition, an increase of the hepatic NAD^+ concentration has been shown to decrease the *in vivo* AcH level in the liver during ethanol oxidation [6]. However, in a study of rat strains with different ethanol preferences, the higher mitochondrial free NADH/free NAD^+ ratio was found in the strain with the lower hepatic AcH concentration, suggesting that this redox ratio was a reflection of the hepatic AcH oxidation rather than a regulator of this reaction [7]. Aldehyde dehydrogenase activity was proposed in this study to be the main regulator of the hepatic AcH metabolism during the ethanol oxidation. Supporting findings have also been reported with studies in which changes in the aldehyde dehydrogenase activity and corresponding changes in the hepatic AcH levels were produced by dietary manipulations[8, 9].

The preceding study investigated the distribution of AcH during normal ethanol oxidation, i.e. when only the ethanol dose and ethanol oxidation time, but not the aldehyde dehydrogenase activity or the free NADH/free NAD^+ ratio were varied. The present work represents a continuation of this research in which the aim was to analyse the regulation of the hepatic AcH level during such normal ethanol oxidation.

MATERIALS AND METHOD

Three doses of ethanol, 0.75, 1.5 and 3.0 g/kg body wt, were administered orally to fed male and female Sprague–Dawley rats and a part of each rat's liver was freeze-clamped during pentobarbital anaesthesia at either 0.25, 0.5, 1.0, 2.0 or 4.0 hr after the ethanol administration, as described in the preceding paper [1]. The liver proteins were precipitated and ethanol and AcH were measured gas chromatographically from the supernatants containing 45 mM thiourea as previously described [1, 10].

Lactate and pyruvate were assayed enzymatically from neutralized supernatants, containing no thiourea, by the method of Hohorst *et al.* [11]. Acetoacetate and 3-hydroxybutyrate were measured gas chromatographically as acetone from the acidic supernatants without thiourea by means of a method described previously [12].

Immediately after the freeze-stop samples had been taken the remaining portions of the livers were excised and transfered to −20°, stored for about 4

weeks, and then homogenized with a Potter–Elvenhjem homogenizer in four parts 0.25 M sucrose containing 1% (v/v) Triton X-100 (BDH Chemicals Ltd., Poole, England). The rate of NAD^+-dependent AcH uptake of the liver homogenates was determined by the gas chromatographic method developed by Marjanen [13] and modified by Koivula *et al.* [14], using an initial AcH concentration of 0.33 mM.

For the statistical correlation analyses the different parameters were intercorrelated within different hepatic ethanol concentration ranges. The theoretical foundation for this procedure is the much debated question about the possible occurrence of different regulatory mechanisms at different ethanol concentrations.

RESULTS

Correlation between the hepatic ethanol and acetaldehyde concentration. Table 1 shows the hepatic AcH concentration means and the individual correlations with the corresponding ethanol concentrations within different ethanol ranges. A graph of the same data may be seen in Fig. 3 of the preceding paper [1]. The female rats displayed significantly lower AcH levels than the male animals at all ethanol concentrations. Significant positive correlations between the hepatic AcH and ethanol concentrations were obtained with both sexes. However, when the ethanol concentration exceeded 30 μmole/g wet wt liver, the positive correlations disappeared.

Correlation between the hepatic redox state and acetaldehyde concentration. To determine the cytosolic redox state in the liver, i.e. the ratio of free NADH/free NAD^+, lactate and pyruvate were measured and the lactate/pyruvate ratio determined. No significant sex differences were found before (means ± S. D.: 8.73 ± 4.44, for males, $n = 4$ and 9.14 ± 5.80, for females, $n = 7$) and during (31 ± 16, for males, $n = 42$ and 36 ± 19, for females, $n = 44$) ethanol oxidation. The lactate/pyruvate ratio did not significantly correlate with the hepatic AcH level. Nor did it correlate with the 3-hydroxybutyrate/acetoacetate ratio which represents the mitochondrial free NADH/free NAD^+ ratio. However, the 3-hydroxybutyrate/acetoacetate ratio correlated significantly with the hepatic AcH level as shown in Table 2. The correlation was negative at ethanol concentrations below 30 μmole/g and positive above this ethanol level. The significance of the positive correlation occurred in male rats stronger within ethanol concentrations above 25 μmole/g ($r = 0.494$, $n = 18$, $p < 0.01$). Significant sex differences occurred, with the female rats displaying higher 3-hydroxybutyrate/acetoacetate ratios before (1.19 ± 0.26, for males, $n = 4$ and 2.06 ± 0.55, for females, $n = 7$) and during (Table 2) the ethanol oxidation.

Correlation between hepatic aldehyde dehydrogenase activity and the acetaldehyde concentration. Table 3 lists the hepatic aldehyde dehydrogenase data. Within ethanol concentrations below 30 μmole/g wet wt liver the aldehyde dehydrogenase activity correlated negatively to the AcH concentration in the liver. This correlation reached significance only when the enzyme activity values of both sexes were combined. This was justified because no sex differences were found in the aldehyde dehydrogenase activities at this ethanol range. At higher ethanol concentrations positive correlation occurred in both male and female rats. When the ethanol exceeded 30 μmole/g wet wt liver, a sex difference occurred, with the male rats displaying a higher aldehyde dehydrogenase activity than the females; consequently in this case the values from both sexes could not be combined.

DISCUSSION

Regulation of the hepatic acetaldehyde level during ethanol oxidation. In the liver during ethanol oxidation AcH is both formed and eliminated. In a "steady state" situation, when the hepatic AcH is neither increasing nor decreasing, a simple equation can be written:

$$\text{AcH formation rate} - \text{AcH elimination rate} = 0. \quad (1)$$

The "AcH formation" is taken to include both the AcH currently being produced in the liver (= ethanol oxidation) and the amount of AcH entering into the liver via the blood (= hepatic AcH input). The latter could be due to AcH that has not been oxidized during circulation, or to extrahepatically oxidized ethanol, or to both, and its value is not exactly known. However, considering the results obtained in the preceding work [1], that more than 95 per cent of the ethanol-derived AcH was oxidized before leaving the

Table 1. Correlation between the hepatic ethanol and acetaldehyde concentration*

Ethanol range (μmole/g wet wt liver)	Number of animals	Acetaldehyde (nmole/g) ♂	Acetaldehyde (nmole/g) ♀	Correlation (r) ♂	Correlation (r) ♀
5–15	17	159 ± 32	136 ± 33†	0.433	0.312
15–30	18	195 ± 24	173 ± 30†	0.074	0.285
<30	35	177 ± 34	155 ± 36†	0.556†	0.570†
>30	13	233 ± 27	205 ± 27†	−0.139	−0.450
Σ	48	192 ± 41	168 ± 40‡	0.683†	0.592†

* Rats were treated and analyses made as described in the Materials and Methods section. Acetaldehyde results are given per wet wt liver as means ± S.D. The *r*-values represent the correlation coefficient between the hepatic ethanol and acetaldehyde concentration. Significance is indicated by † ($p < 0.05$) and ‡ ($p < 0.005$).

Table 2. Correlation between the hepatic acetaldehyde concentration and 3-hydroxybutyrate/acetoacetate ratio*

Ethanol range (μmole/g wet wt liver)	Number of animals	3-hydroxybutyrate/acetoacetate ♂	3-hydroxybutyrate/acetoacetate ♀	Correlation (r) ♂	Correlation (r) ♀
5–15	17	1.94 ± 0.69	2.66 ± 0.57§	−0.278	−0.452†
15–30	18	1.57 ± 0.64	2.45 ± 0.84§	−0.298	−0.187
<30	35	1.74 ± 0.68	2.52 ± 0.70§	−0.386‡	−0.298†
>30	13	2.29 ± 0.74	2.37 ± 0.87	0.378	0.320
Σ	48	1.89 ± 0.73	2.48 ± 0.74§	0.059	−0.170

* Rats were treated and analyses made as described in the Materials and Methods section. The hepatic 3-hydroxybutyrate/acetoacetate ratios are given as means ± S.D. The r values represent the correlation coefficient between these ratios and hepatic acetaldehyde concentrations. Significance is indicated by † ($p < 0.1$), ‡ ($p < 0.05$) and § ($p < 0.005$).

liver and that 50–100 per cent of the remaining hepatic output AcH was eliminated extrahepatically, and the fact that ethanol oxidation is almost exclusively located in the liver, the amount of the hepatic AcH input must be negligible compared with the amount of AcH derived from oxidized ethanol.

The hepatic AcH elimination includes the AcH oxidation and the amount of AcH that leaves the liver with the blood. The rate for the latter amount can be written as:

$$\text{Rate of AcH leaving the liver} = [\text{AcH}] \times \text{hepatic blood flow rate}, \quad (2)$$

where [AcH] is the AcH concentration in the hepatic blood. Since it has been shown that the AcH concentration in the liver approximately equals that of the hepatic blood [15], the hepatic AcH level may be used as an approximation for [AcH]. Thus combining equations (1) and (2) the following equation is obtained:

$$\text{Ethanol oxidation rate} + \text{AcH input rate} - \text{AcH oxidation rate} - [\text{AcH}] \times \text{hepatic blood flow rate} = 0,$$

or

$$[\text{AcH}] = \frac{\text{Ethanol oxidation rate} + \text{AcH input rate} - \text{AcH oxidation rate}}{\text{hepatic blood flow rate}}, \quad (3)$$

in which the rates are for the liver alone.

SUMMARY DISCUSSION OF THE MAIN POINTS OF THE RESULTS

I. When the ethanol concentration is below 30 μmole/g liver, there is:

(A) a positive correlation between the hepatic ethanol and AcH concentration, which could be caused by: (1) increasing hepatic AcH input. The increasing ethanol concentration increases the extrahepatic ethanol oxidation. This is only a theoretical possibility because the AcH input is such an negligible factor in equation (3). (2) increasing hepatic ethanol oxidation rate. If the AcH oxidation rate remains unchanged the small increase in the ethanol oxidation rate can be ADH-mediated, but if the AcH oxidation

Table 3. Correlation between the hepatic acetaldehyde concentration and aldehyde dehydrogenase activity*

Ethanol range (μmole/g wet wt liver)	Aldehyde dehydrogenase activity (μmole/min per g wet wt liver) ♂	Aldehyde dehydrogenase activity (μmole/min per g wet wt liver) ♀	Correlation (r) ♂	Correlation (r) ♀	Correlation (r) ♂ + ♀
5–15	1.29 ± 0.22(14)	1.23 ± 0.22(15)	−0.042	−0.269	−0.099
15–30	1.24 ± 0.17(17)	1.30 ± 0.18(15)	−0.209	−0.287	−0.372†
<30	1.26 ± 0.20(31)	1.26 ± 0.20(30)	−0.150	−0.190	−0.169
>30	1.33 ± 0.27(11)	1.13 ± 0.11(12)‡	0.416	0.498†	
Σ	1.28 ± 0.22(42)	1.23 ± 0.19(42)	0.077	−0.245†	−0.035

* Rats were treated and analyses made as described in the Materials and Methods section. The aldehyde dehydrogenase activities in crude liver homogenate are given as means ± S.D. The r values represent the correlation coefficient between the hepatic acetaldehyde concentration and aldehyde dehydrogenase activity. Significance is indicated by † ($p < 0.1$) and ‡ ($p < 0.05$).

rate increases with increasing ethanol oxidation rate the latter increase would demand a substantial participation from a non-ADH-system, e.g. the MEOS, as suggested by Lieber and DeCarli [16]. (3) decreasing AcH oxidation rate. The increasing ethanol concentration inhibits one or more of the AcH oxidation systems. Nothing of this kind, however, has been reported. (4) decreasing hepatic blood flow rate. This possibility seems unlikely because it has been reported that increasing ethanol concentration if anything increases hepatic blood flow rate [17].

(B) a negative correlation between the mitochondrial free NADH/free NAD^+ ratio and the hepatic AcH concentration, which could be caused by: (1) the mitochondrial free NADH/free NAD^+ ratio reflecting the rate of the AcH oxidation. Lower [AcH] corresponds to faster mitochondrial AcH oxidation, causing a higher mitochondrial redox ratio. The mitochondrial redox state does not regulate the AcH oxidation, but rather reflects the rate of this oxidation, as has previously been reported [7]. (2) the mitochondrial free NADH/free NAD^+ ratio reflecting the rate of the hepatic ethanol oxidation. Lower [AcH] corresponds to lower ethanol oxidation rate, caused by the higher mitochondrial redox ratio. The mitochondrial redox state can regulate the AcH oxidation. These alternatives are supported by the observation that the uncoupler DNP decreases the redox and increases the ethanol and AcH oxidation rates in perfused livers [5].

(C) a negative correlation between the hepatic aldehyde dehydrogenase activity and the AcH concentration, which is caused by: (1) aldehyde dehydrogenase activity regulating the hepatic AcH metabolism, which has previously been shown indirectly [7–9].

II. When the ethanol concentration is above 30 μmole/g liver, there is:

(A) no correlation between the hepatic ethanol and AcH concentration, which could be caused by: (1) no increase in the hepatic AcH input, because the extrahepatic ethanol oxidizing systems are saturated. (2) no increase in the hepatic ethanol oxidation rate, because all ethanol oxidizing systems are saturated. (3) no decrease in the AcH oxidation rate, because the inhibitory effect of ethanol concentration is saturated. (4) no effect upon the hepatic blood flow rate.

(B) a positive correlation between the mitochondrial free NADH/free NAD^+ ratio and the hepatic AcH concentration, which demonstrates that: (1) the conclusion I(B)(1) is not valid here. (2) the AcH oxidation rate might well be regulated by the mitochondrial redox state.

(C) a positive correlation between the hepatic aldehyde dehydrogenase activity and AcH concentration, which demonstrates that: (1) the aldehyde dehydrogenase activity is not necessary regulating the hepatic AcH metabolism, which is supported by conclusion II. (B)(2).

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