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METABOLISM OF ETHANOL AND ACETALDEHYDE IN INTACT RATS DURING PREGNANCY

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Abstract—Ethanol and acetaldehyde contents in the peripheral blood of intact non-pregnant and pregnant rats have been determined after an intraperitoneal injection of ethanol. Determinations have also been made of the ethanol and acetaldehyde contents and the lactate/pyruvate ratios in frozen, clamped livers of intact pregnant and non-pregnant rats with or without a prior injection of ethanol. The *in vitro* activities of the liver alcohol and acetaldehyde dehydrogenase have also been measured. Elimination rate of ethanol *in vivo* was found to be equal in pregnant and non-pregnant rats, but the acetaldehyde content of the peripheral blood after ethanol administration was higher in pregnant than in non-pregnant animals. Because the ethanol and acetaldehyde contents of frozen, clamped livers were similar in magnitude in pregnant and non-pregnant rats and no differences were found in the *in vitro* activities of the liver alcohol and acetaldehyde dehydrogenase between the two animal groups a difference in the extrahepatic metabolism of acetaldehyde is suggested to explain the high acetaldehyde content in the peripheral blood of pregnant rats after ethanol administration. The lactate and pyruvate contents of frozen, clamped livers of pregnant rats without a prior dose of ethanol were higher than those of non-pregnant animals indicating a high rate of glycolysis during pregnancy, but the lactate pyruvate ratios of the livers were equal in the two animal groups both with and without previous ethanol loading.

ALCOHOL has been used for several years for the prevention of premature labor, because of its inhibitory effects on uterine contractions.[1,2] Also, it has been reported in experiments with rhesus monkeys that ethanol does not alter the intensity or frequency of uterine contractions.[3] The activities of the alcohol and acetaldehyde dehydrogenase have been shown to be extremely low in both human and rat fetus.[4–6] Likewise it has been shown that the capacity of human fetal liver to eliminate ethanol and acetaldehyde during liver perfusion experiments is very low.[7] No information, however, is available concerning the maternal metabolism of ethanol or acetaldehyde. The present work was carried out with the aim of studying how the metabolism and the metabolic effects of ethanol and acetaldehyde are modified during pregnancy.

MATERIALS AND METHODS

Female Wistar rats (200–250 g), 7 months in age, were fed an ordinary laboratory diet (Astra-Ewos Ab, Södertälje, Sweden), and tap water *ad lib*. The rats allotted to the pregnant group were housed with males for 12 days with experiments performed on the 20th and 21st days after copulation began. Thus the duration of pregnancy varied largely according to the time when the animals became pregnant. Female non-pregnant rats of the same age served as controls.

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One or two days before the liver experiments, the ethanol and acetaldehyde contents of the peripheral blood were determined after an intraperitoneal injection of 1·2 g ethanol/kg rat body weight as a 10% (w/v) solution. The blood samples were taken from the tip of the tail 30, 60, 90, 120 and 150 min after the injection.

For the liver experiments, the rats were divided into two further groups, each containing both control and pregnant animals. The freeze–stop technique was employed. The livers at one group were freeze-clamped 30 min after being injected with 1·2 g of ethanol/kg rat body weight, whereas the livers of the other were freeze-clamped without any previous ethanol administration. The *in vitro* activities of the alcohol and acetaldehyde dehydrogenase of the livers of this latter group were determined after the liver was freeze-clamped.

In freeze-stop experiments the rats were anaesthetized with 50 mg/kg body weight of pentobarbital (Nembutal®) (40 mg/kg body weight when ethanol was used), and a part of the liver was clamped *in situ* with metal tongs precooled in liquid nitrogen. The frozen liver tissue was crushed to powder in a mortar and homogenized in 10 ml of 0·6 M ice-cold perchloric acid containing 25 mM of thiourea to prevent the spontaneous formation of acetaldehyde from ethanol.[8] After centrifugation, the supernatant was neutralized with 2 M K_2CO_3 to the pH value 7·4.

Ethanol and acetaldehyde were assayed with a Perkin Elmer F 40 gas chromatograph and application of the head-space technique. The samples to be analysed were pipetted into serum bottles, sealed with a rubber stopper, and incubated for 15 min at 65° in a thermostated, sampling turntable; following this, samples were taken automatically by means of an electropneumatic dosing system. For ethanol determinations, *t*-butyl alcohol was used as an internal standard. In place of an internal standard, an acetaldehyde standard solution made of redistilled acetaldehyde from BDH chemicals, Poole, Dorset, England, was used in the acetaldehyde measurements. Lactate and pyruvate were assayed enzymically,[9] direct from the neutralized supernatant of the liver tissue. Enzymes and coenzymes were obtained from C. F. Boehringer, Mannheim, Germany.

For the estimation of ADH activity a 10% liver homogenate was prepared in ice-cold 0·25 M sucrose containing 1% Triton X-100 (Rohm and Haas Co., Philadelphia, Pa., U.S.A.), because 1% Triton X-100 gives maximal ADH activity in liver homogenates.[10] After centrifugation of the homogenate for 60 min at 17,000 *g* (MSE High Speed 18 refrigerator centrifuge MK-2) ADH activity was estimated by the method of Bonnichsen and Brink[11] at pH 8·7 and 22° with a Beckmann DK 1 A recording spectrophotometer. Activity is expressed in the international units recommended by the Commission of Enzymes of I.U.B. (1961)/g wet weight of the liver.[12]

For the estimation of acetaldehyde dehydrogenase activity, a 10% liver homogenate was prepared in ice-cold 0·25 M sucrose. The liver homogenate was filtered through four layers of cheesecloth, and centrifugated for 10 min at 700 *g* (MSE High Speed 18 refrigerator centrifuge Mk-2). After centrifugation the cells of the supernatant were broken by four successive handlings of 30 sec each in an MSE 100 W ultrasonic disintegrator (amplitude 5 μm). Acetaldehyde dehydrogenase activity was estimated in samples incubated at 22° by the method of Marjanen[13] (the incubation mixture contained 2·2 mM pyrazole, 1·5 mM NAD^+, and 41 mM acetaldehyde, with 10% homogenate buffered at pH 7·4 with 0·1 M KH_2PO_4–$Na_2HPO_4.2H_2O$—total volume of 3·075 ml with 50 μl of 10% homogenate) with a Beckmann DK 1 A record-

ing spectrophotometer. The activity is expressed in the international units/g wet weight of the liver.

RESULTS

Figure 1 shows the ethanol and acetaldehyde contents of the peripheral blood in pregnant and non-pregnant rats after an intraperitoneal injection of 1·2 g of ethanol/kg rat body weight. The ethanol contents of the peripheral blood were the same in magnitude, indicating that the capacity of the rat to eliminate ethanol *in vivo* does not change during pregnancy. The standard deviations of the ethanol contents in the peripheral blood were small in both animal groups. The acetaldehyde level of the peripheral blood was clearly higher in pregnant than in non-pregnant rats (analysis of variance: $f = 5{\cdot}26$; $P < 0{\cdot}05$, with 1 and 22 as degrees of freedom), even though the standard deviations in the acetaldehyde contents of the peripheral blood were large in both animal groups.

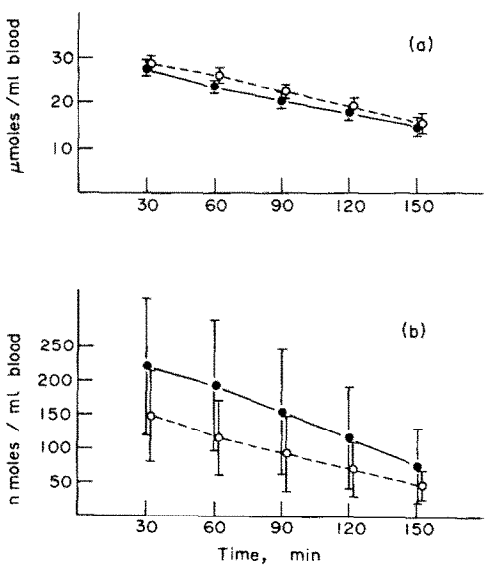

FIG. 1. Ethanol (a) and acetaldehyde (b) contents in the peripheral blood of non-pregnant (○) and pregnant (●) rats at various times after an intraperitoneal injection of 1·2 g ethanol/kg rat body weight. Results are means ± S.D. of 12 different rats.

Table 1 illustrates the ethanol and acetaldehyde contents of the intact liver in pregnant and nonpregnant rats 30 min after an intraperitoneal ethanol injection, and the *in vitro* activities of the liver alcohol and acetaldehyde dehydrogenase. The ethanol content of the livers were equal in magnitude in both pregnant and non-pregnant animals. The *in vitro* activities of the liver alcohol and acetaldehyde dehydrogenase were likewise similar for both animal groups.

Table 2 shows the contents of lactate and pyruvate and the lactate/pyruvate ratios in the intact livers of pregnant and non-pregnant rats with and without previous ethanol administration. Both the lactate ($P < 0{\cdot}025$) and pyruvate ($P < 0{\cdot}01$) contents were higher in the livers of pregnant rats than in those of non-pregnant animals when

TABLE 1. THE CONTENTS OF ETHANOL AND ACETALDEHYDE IN THE INTACT LIVER OF CONTROL AND PREGNANT RATS AFTER AN INJECTION OF ETHANOL, AND THE *in vitro* ACTIVITIES OF THE LIVER ALCOHOL AND ACETALDEHYDE DEHYDROGENASE OF THE CONTROL AND PREGNANT ANIMALS*

Group	Ethanol (μmoles/g fresh liver)	Acetaldehyde (nmoles/g fresh liver)	Alcohol dehydrogenase (units/g wet weight of liver)	Acetaldehyde dehydrogenase (units/g wet weight of liver)
Control	(10) 21·6 ± 3·3	(10) 246·0 ± 40·6	(8) 1·250 ± 0·12	(8) 2·289 ± 0·58
Pregnant	(7) 22·1 ± 1·8	(7) 229·0 ± 36·1	(5) 1·229 ± 0·14	(5) 2·330 ± 0·48

* The results are given as means ± S.D., with the number of experiments in parentheses.

the livers were freeze-clamped without a prior injection of ethanol, but no significant differences could be found in the L/P ratios in pregnant and non-pregnant rats. After ethanol administration the lactate and pyruvate contents as well as the L/P ratio were at the same level in pregnant and control animals.

TABLE 2. THE CONTENTS OF LACTATE AND PYRUVATE AND THE LACTATE/PYRUVATE RATIOS IN THE INTACT LIVER OF CONTROL AND PREGNANT RATS WITH AND WITHOUT A PREVIOUS INJECTION OF ETHANOL*

Group	Ethanol	Lactate (μmoles/g fresh liver)	Pyruvate (μmoles/g fresh liver)	Lactate/pyruvate
Control (7)	–	1·37 ± 0·47	0·082 ± 0·030	17·1 ± 2·3
Control (10)	+	2·09 ± 0·56	0·036 ± 0·011	66·1 ± 29·8
Pregnant (5)	–	2·01 ± 0·46†	0·144 ± 0·032‡	14·7 ± 2·8
Pregnant (7)	+	1·69 ± 0·38	0·035 ± 0·017	62·1 ± 30·4

* The results are given as means ± S.D., with the number of experiments in parentheses.
† $P < 0·025$ for difference between non-pregnant and pregnant rats.
‡ $P < 0·01$ for difference between non-pregnant and pregnant rats.

DISCUSSION

The higher contents of both lactate and pyruvate in the livers of pregnant rats than in those of non-pregnant animals without a prior dose of ethanol suggests a high rate of glycolysis during pregnancy. However, the cytoplasmic redox state of the liver was at the same level in non-pregnant and pregnant animals both with and without previous ethanol administration, indicating that the metabolic events, induced by the change in the redox state of the liver, also take place in the livers of pregnant rats.

The ethanol contents of the peripheral blood were of equal amount in both pregnant and non-pregnant animals. The rate of elimination of ethanol was determined by extrapolating the linear descending limb of the blood–alcohol curve to the time axis. The mean rate of elimination of ethanol was found to be almost identical in both non-pregnant (26·0 mg ethanol/hr/100 g rat body weight) and pregnant (27·0 mg ethanol/hr/100 g rat body weight) animals. This also means that the formation of acetaldehyde during ethanol oxidation was equal in both animal groups. Because the formation of acetaldehyde during ethanol oxidation was similar in magnitude in non-pregnant and pregnant rats, the higher acetaldehyde content in the peripheral

blood of pregnant rats than in that of non-pregnant animals indicates that the capacity of the entire rat body to eliminate acetaldehyde is diminished during pregnancy. A steroid sensitive aldehyde dehydrogenase has been partially purified from the soluble fraction of rabbit liver[14] and found to be inhibited by numerous steroids such as progesterone, testosterone, corticosterone and estrone.[14] No differences could be found in the acetaldehyde content in the livers of pregnant and non-pregnant animals after ethanol administration in the present study. Therefore the steroid sensitive aldehyde dehydrogenase described earlier[14] cannot explain the high acetaldehyde content in the peripheral blood of pregnant rats.

The capacity of various tissues of the female rat to eliminate indole-3-acetaldehyde has been investigated, and the liver was found to be the main site of its oxidation.[15] It was calculated that the aldehyde-oxidizing capacity of the liver alone is double that of kidney, heart, small intestine, gonads, adipose, uterus, lung, brain and adrenal taken together.[15] Among these tissues, the capacity of kidney and uterus together accounts for more than half of the total extrahepatic capacity to eliminate indole-3-acetaldehyde. Whether this finding is also true *in vivo* and for acetaldehyde oxidation after ethanol administration is not known. At any rate, the inhibition in the extrahepatic oxidation of acetaldehyde appears to explain the high acetaldehyde content in the peripheral blood of pregnant rats after ethanol administration. Any difference in the mere physical evaporation of acetaldehyde during respiration could hardly induce such a big difference in the acetaldehyde content of the peripheral blood of pregnant and non-pregnant rats.

It has previously been shown that the activity of the liver alcohol and acetaldehyde dehydrogenase is very low in both human and rat fetus.[4,5] Detectable aldehyde-oxidizing capacity has also been found in kidney, adrenal and gastrointestinal tract of human fetus, even though 90 per cent of the fetal aldehyde-oxidizing capacity is located in the liver.[6] However, the aldehyde-oxidizing capacity of human fetal liver is only about one-tenth to one-fifth that of the adult liver.[6] Thus the fetus is not able to eliminate acetaldehyde that comes in the maternal blood and is exposed to the metabolic influence of ethanol and acetaldehyde during maternal ethanol metabolism. Previously it has been shown that many aldehydes, including acetaldehyde, have sympathomimetic effects producing changes in blood pressure and heart rate.[16] Therefore it is quite possible that during maternal ethanol metabolism the fetus suffers some side-effects.

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