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ETHANOL AND PSYCHOTROPIC DRUG INTERACTION DURING PREGNANCY AND LACTATION*

ARUN K. RAWAT†
Alcohol Research Center, University of Toledo, Toledo, OH 43699, U.S.A.

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Abstract—Prolonged maternal ethanol consumption for 8 days during pregnancy or for five days immediately after birth resulted in 30–46 per cent inhibition in the rate of chlorpromazine metabolism by the rat fetal and neonatal livers respectively. A significant increase in hepatic NADH/NAD and UDPG/ UDPGA ratios was observed in suckling neonatal and maternal livers from the ethanol-fed group. Acute administration of ethanol with chlorpromazine led to about 60 per cent inhibition of the metabolism of chlorpromazine. This inhibitory effect of ethanol on the metabolism of chlorpromazine was largely abolished by preincubation of liver homogenates with pyrazole (2 mM). Lactate (10 mM) addition to liver homogenates resulted in a significant inhibition of chlorpromazine metabolism. It is suggested that maternal ethanol consumption during preganancy and lactation inhibits the hepatic metabolism of drugs such as chlorpromazine which require glucuronidation for their detoxification. This ethanol-mediated inhibition is largely exerted through the decrease in the NAD-dependent conversion of UDP-glucose (UDPG) to UDP-glucuronic acid, (UDPGA).

Recent studies from this and other laboratories have shown that prolonged ethanol exposure during either gestation or lactation exerts a number of injurious and toxic effects on the developing fetus and the newborn [1–7]. In humans, the condition was described by French investigators in 1968 [8]; since then these observations have been confirmed and expanded by several other investigators [9–11], and the condition has been termed "Fetal Alcohol Syndrome".

Although several studies have appeared regarding the interactions between ethanol and metabolism of drugs in the adult mammalian system [12–14], much work is needed about such interactions in the perinatal system.

Adverse effects of several maternally administered drugs on the fetus and the neonate have been observed. In fact, highly lipid soluble drugs cross the placenta in amounts that are directly proportional to the maternal placental blood flow [15]. Nearly all drugs administered to lactating mothers are detectable in maternal milk. Ethanol has been shown to cross from the maternal circulation to the fetus as well as to the suckling newborn [16, 17]. Prolonged maternal alcoholism is associated with the presence of prenatal and postnatal growth retardation, and alterations in the fetal milieu can substantially retard normal growth and development. It was therefore considered relevant to investigate the interactions of ethanol and other drugs in the developing fetus and the newborn. In the present study ethanol and psychotropic drug interaction was studied during pregnancy and lactation.

MATERIALS AND METHODS

Chemicals. UDP-glucuronic acid (ammonium salt), UDP-glucose, NAD, NADH, UDP-*N*-acetylglucosamine, alcohol dehydrogenase, and other enzymes and co-factors were purchased from the Sigma Chemical Co. (St. Louis, MO). Chlorpromazine was obtained locally.

Animals. Pregnant albino rats were obtained from Spartan Research (Haslett, MI). The animals were treated chronically with ethanol in a liquid Sustacal diet. The liquid Sustacal diet consisted of 61% (w/v) commercial Sustacal (Mead Johnson Co., Evansville), in vanilla-flavored 6% (w/v) ethanol or isocaloric sucrose (10.02%, w/v), and water. Sustacal is a nutritionally complete diet and according to its proximate analysis contains (%, w/w): protein, 6.3; fat, 1.3; carbohydrate, 13.0; ash, 1.0; and water, 78.0. Among the ingredients are concentrated sweet skim milk, corn syrup solids, concentrated sucrose, and partially hydrogenated soya-bean protein isolate. It contained vitamins A, B_6, B_{12}, C, D, E, folic acid, thiamin, niacin, biotin and pantothenic acid. In addition, it contains phosphorus, iodine, iron, magnesium, copper, zinc, manganese, potassium, sodium and choline. The daily intake of liquid diet per experimental animal was measured; an equivalent amount of liquid diet containing sucrose was given to control animals. Sustacal contains concentrated sucrose as one of the major sources of carbohydrates. In the control diets, therefore, sucrose addition to make it isocaloric with ethanol diet was considered most suitable.

The average daily intake of liquid diet by pregnant rats was 120 ml per animal. The rats fed well on the liquid diet and both control and ethanol-fed groups

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† Address all correspondence to: Arun K. Rawat, Alcohol Research Center, C.S. No. 10002, University of Toledo, Toledo, OH 43699, U.S.A.

gained weight in parallel. The average daily weight gain by a pregnant animal was 3.0 ± 0.6 g/day (mean ± S.E.M. for fifteen determinations). A significant difference in the weight gain of pregnant rats from the ethanol-fed and sucrose-fed groups was not observed at any stage of gestation. However, the body weights of individual fetuses from the ethanol-fed group were consistently ($P < 0.01$) lower than the corresponding controls. At day 18 of gestation the average weight (± S.E.M.) of the control group was 5.6 ± 0.8 g and of a fetus of the control group was 5.6 ± 0.8 g and of a fetus from the ethanol group, 4.3 ± 0.4 g. There were twenty fetuses in each group. Significant reductions in the brain weights of both the fetuses and the neonates from the ethanol-fed group was observed, compared to corresponding controls.

In this study the ethanol-fed dams consumed ethanol in the range of 9.3 to 12.8 g/kg body weight. Generalized prenatal and postnatal growth retardation was observed in the offspring of the ethanol-fed group. We have previously reported [2] an increase in the neonatal mortality in the ethanol-treated group [1].

Studies with fetal and neonatal livers. To study the effects of acute ethanol administration or chronic maternal alcoholism, 18- to 20-days pregnant rats were used. The abdomens of pregnant rats were opened and the fetuses were dissected while the maternal blood supply was still intact. Fetal liver tissue was obtained by opening the fetal abdomen and freeze clamping the liver by aluminum microclamps precooled in liquid N_2, except in experiments where unfrozen tissue was required. Livers from newborn rats that either had been suckled on liquid Sustacal–ethanol/sucrose diet or had received acute treatment were also removed by liquid N_2 freezing.

Determination of chlorpromazine metabolism. Hepatic chlorpromazine metabolism was studied using liver preparations either from animals that had been exposed to ethanol chronically or preparations to which ethanol was added *in vitro*. In experiments where the effects of prolonged maternal ethanol consumption on hepatic chlorpromazine metabolism were studied, the following procedure was employed. Liver homogenates of fetal, neonatal and maternal livers were incubated with chlorpromazine (200 mg) in phosphate buffer (200 mM), pH 7.4, in a total volume of 2.2 ml. In experiments where effects of *in vitro* additions of ethanol (10 mM), pyrazole (2 mM) or lactate (10 mM) on chlorpromazine metabolism and lactate/pyruvate ratios were studied, the same procedure was followed except that the buffer employed was Krebs–Henseleit bicarbonate (pH 7.4). The incubation was carried out in an environment of CO_2–O_2 (95:5).

In this study no external coenzymes were added to facilitate the metabolism of either chlorpromazine or ethanol. Incubation was carried out for 30 min at 37°. The details of chlorpromazine extraction and determination are described elsewhere [18].

Determination of UDPG. The uridine diphosphate glucose (UDPG) content of the liver preparations was determined by employing UDPG-dehydrogenase as described in detail by Mills and Smith [19].

Determination of UDPGA. Hepatic uridine diphosphate glucuronic acid (UDPGA) content was determined by measuring the formation of *o*-aminophenyl-glucuronide. The details of the method were essentially the same as described by Mills and Smith [19].

Determination of hepatic lactate/pyruvate ratio. Determinations of lactate and pyruvate in the frozen liver preparations or in the incubation medium were carried out enzymatically [20, 21].

RESULTS

We investigated the effects of prolonged maternal ethanol consumption during gestation and lactation on the metabolism of chlorpromazine and on the UDPG/UDPGA and lactate/pyruvate ratios in fetal and neonatal livers (Table 1). Maternal ethanol consumption for 8 days during the second half of the gestation period was followed by about a 37 per cent decrease in the hepatic metabolism of chlorpromazine in the rat fetus. Newborn rats that had been suckled for 5 days immediately after birth by ethanol-fed mothers showed about a 41 per cent decrease in the hepatic metabolism of chlorpromazine, compared with corresponding sucrose-fed controls. In addition, increases in the hepatic

Table 1. Effects of maternal ethanol consumption on hepatic chlorpromazine metabolism and on UDPG/UDPGA and Lactate/Pyruvate ratios*

Age	Treatment	Chlorpromazine metabolism [$\mu g \cdot 30\ min^{-1} \cdot (0.1\ g\ liver)^{-1}$]	Hepatic UDPG/UDPGA ratio	Lactate/ pyruvate ratio
Fetal (−4 days)	Sucrose	6.3 ± 0.5 (8)		24.0 ± 3 (6)
	Ethanol	4.0 ± 0.3† (8)		30.6 ± 4† (6)
Neonatal (+6 days)	Sucrose	86 ± 8.2 (10)	1.49 ± 0.20 (6)	9.8 ± 1 (6)
	Ethanol	51 ± 5.0‡ (10)	2.36 ± 0.20† (6)	24.3 ± 2‡ (6)
Adult (+ 120 days)	Sucrose	103 ± 14.0 (10)	1.40 ± 0.18 (6)	10.0 ± 1 (6)
	Ethanol	58 ± 6.9‡ (10)	3.01 ± 0.20† (6)	31.0 ± 3‡ (6)

* Details of the assays and procedures are given in Materials and Methods. The results are means ± S.E.M. with the numbers of observations given in parenthesis.

† The difference from control value is significant at the $P < 0.01$ level.

‡ The difference from control value is significant at the $P < 0.001$ level.

UDPG/UDPGA and lactate/pyruvate ratios occurred in the newborns from the ethanol-fed group. Maternal adult livers from ethanol-treated rats showed significant inhibition ($P < 0.001$) of hepatic chlorpromazine metabolism and increases in maternal liver UDPG/UDPGA and lactate/pyruvate ratios (Table 1).

Hepatic UDPG/UDPGA ratios in the sucrose-fed control groups of neonates and adults were not significantly different. Ethanol treatment resulted in a greater increase in this ratio in the adult liver compared to the neonates, which is in accordance with the developmental pattern of hepatic alcohol dehydrogenase and hepatic capacity to metabolize ethanol [2]. These observations (Table 2) are also in agreement with the observed highest inhibitory effect of ethanol on chlorpromazine metabolism in the adult liver. The hepatic metabolism of chlorpromazine was lowest in the fetal liver. Newborn livers showed significantly higher ($P < 0.01$) metabolism of chlorpromazine compared with fetal livers. Higher rates of chlorpromazine metabolism by the neonatal livers compared to the fetal livers may be related to a marked surge in the activity of UDP-glucuronosyltransferase activity in the neonatal livers [22]. UDP-glucuronosyltransferase has been suggested to be the rate-limiting enzyme of glucuronidation [23]. Hydroxylation in the 3 and 7 positions and subsequent glucuronide formation represent the principal metabolic pathway of chlorpromazine.

As shown in Table 2, *in vitro* incubation of ethanol (10 mM) with fetal (−4 days), neonatal (+6 days) and adult liver homogenates resulted in inhibition of chlorpromazine metabolism. Addition of pyrazole (2 mM), an inhibitor of alcohol dehydrogenase, reversed the ethanol-mediated inhibition of chlorpromazine (Table 2). Pyrazole alone did not have a significant effect on chlorpromazine metabolism. Addition of lactate (10 mM), which increased the hepatic NADH/NAD ratio (Table 2), also led to an inhibition of chlorpromazine metabolism in all three preparations from fetal, neonatal and adult livers (Table 2).

DISCUSSION

The results presented in this study clearly demonstrate that maternally consumed ethanol, transferred through the placenta to the fetus, is capable of inhibiting the metabolism of chlorpromazine. This inhibitory effect of ethanol is also observed in newborn rats suckled by ethanol-fed mothers. It suggests that ethanol that passed through maternal milk to the newborn can interfere with the hepatic chlorpromazine metabolism of the neonate. As far as I am aware, this is the first report of this effect of maternal alcoholism. Since ethanol intake results in an increase in hepatic NADH/NAD ratio [24–26], the possibility was considered that it may also alter the NAD-dependent conversion of UDPG to UDPGA. If this is true, ethanol could potentially alter the disposition not only of phenothiazines such as chlorpromazine but also of other drugs which are excreted as glucuronic acid-conjugates. The glucuronic formation of the drugs requires the participation of UDPGA in the reaction.

Table 2. Effects of ethanol, pyrazole and lactate addition on the metabolism of chlorpromazine by fetal, neonatal or adult livers*

Age	Additions	Chlorpromazine metabolism [$\mu g \cdot 30\ min^{-1} \cdot (0.1\ g\ liver)^{-1}$]	Lactate/pyruvate ratio
Fetal (−4 days)	None	6.0 ± 0.7 (6)	94 ± 4 (8)
	Ethanol (10 mM)	4.8 ± 0.5† (6)	109 ± 12 (8)
	Pyrazole (2 mM)	5.9 ± 0.5‡ (6)	94 ± 6 (8)
	Ethanol (10 mM) + pyrazole (2 mM)	5.7 ± 0.4‡ (6)	100 ± 10 (8)
	Lactate (10 mM)	4.3 ± 0.4† (6)	186 ± 20 (8)
Neonatal (+6 days)	None	80 ± 8 (6)	36 ± 4 (6)
	Ethanol (10 mM)	56 ± 7§ (6)	71 ± 6 (6)
	Pyrazole (2 mM)	86 ± 7‡ (6)	36 ± 4 (6)
	Ethanol (10 mM) + pyrazole (2 mM)	76 ± 6‡ (6)	53 ± 4 (6)
	Lactate (10 mM)	40 ± 5§ (6)	120 ± 14 (6)
Adult (+120 days)	None	100 ± 11 (6)	30 ± 4 (6)
	Ethanol (10 mM)	60 ± 6§ (6)	86 ± 9 (6)
	Pyrazole (2 mM)	96 ± 9‡ (6)	86 ± 9 (6)
	Ethanol (10 mM) + pyrazole (2 mM)	80 ± 7‡ (6)	72 ± 8 (6)
	Lactate (10 mM)	69 ± 7§ (6)	94 ± 10 (6)

* Incubations were carried out at 37° in the presence of a CO_2–O_2 mixture (95:5). Details of the assay procedures are given in Materials and Methods. Results are means ± S.E.M. of the numbers of observations given in parentheses.

† The difference from control value is significant at the $P < 0.01$ level.

‡ Not significant.

§ The difference from control vaue is significant at the $P < 0.001$ level.

1. UDP-glucose + 2 NAD + H_2O ⟶ UDPGA + 2 NADH + $2H^+$.
2. UDPGA + ROH ⟶ R-*o*-glucuronide + UDP.

UDP-glucuronic acid (1) is formed from UDP-glucose by the NAD-dependent cytoplasmic enzyme UDP-glucose dehydrogenase (EC 1.1.1.22). The microsomal enzyme (2) UDP-glucuronosyltransferase (EC 2.4.1.17) catalyzes the glucuronide formation by glucuronyl transfer from UDPGA.

Hepatic ethanol oxidation via alcohol dehydrogenase has been shown [24–26] to shift the hepatic redox-state toward a reduced state (Table 1). Fetal liver (Table 1) is characterized by a high NADH/NAD ratio and low alcohol dehydrogenase activity [2]. However, even in the fetal liver where glucuronidation of drugs is beginning to develop [22, 23], ethanol causes a small but significant increase in hepatic NADH/NAD ratio [2] and the inhibition of the chlorpromazine metabolism (Table 1).

The NADH/NAD ratio in liver immediately after birth returns to normal levels; ethanol coming through milk, however, elevates this ratio [2]. As shown in Table 1, a simultaneous increase in hepatic UDPG/UDPGA ratio and lactate/pyruvate ratio, which reflects the redox state of the cytoplasmic compartment [24–26], was observed in the neonates suckled by ethanol-fed mothers. Since there is a surge in the activity of UDP-glucuronosyltransferase activity during the neonatal stage [22], the highest rates of chlorpromazine metabolism were observed in the neonatal liver. The neonates had a higher rate of chlorpromazine metabolism than the fetal liver; however, the most pronounced inhibitory effect of ethanol was observed in the adult liver. This may be because the alcohol dehydrogenase activity in the neonatal liver, and its capacity to metabolize ethanol, are only about one-fourth that of the adult liver [2]. These results (Table 1) show that, in fact, by shifting the UDPG/UDPGA ratio in the direction of UDP-glucose, ethanol decreased the available UDP-glucuronic acid for glucuronidation. That the effect of ethanol was exerted through decreased UDP-glucuronic acid synthesis is further supported by studies with pyrazole (Table 2). Pyrazole, a well-known inhibitor of alcohol dehydrogenase, largely abolished the ethanol-mediated inhibition of chlorpromazine metabolism and the changes in hepatic redox-state. Conversely, substrates like lactate, which also increase the hepatic NADH/NAD ratio (Table 2), have an inhibitory effect on chlorpromazine metabolism in fetal, neonatal and adult rat liver preparations. In the liver, lactate is oxidized to pyruvate by NAD-dependent lactate dehydrogenase. Although a relationship between ethanol-mediated changes in chlorpromazine metabolism and the UDPG/UDPGA ratio on the one hand and between UDPG/UDPGA and NADH/NAD ratios on the other has been observed in this study, the possibility that ethanol may also interfere with other minor metabolic pathways of chlorpromazine cannot be ruled out totally. In hepatocytes isolated from adult livers, the presence of low ethanol (10 mM) concentrations has been shown to inhibit the glucuronidation [27] of drugs, e.g. 4-methylumbeniferone, 1-naphthol and phenolphthalein; addition of sorbitol and lactate has an inhibitory effect on glucuronidation. It was further observed in these studies that sulphate conjugation of the drugs was unaffected by ethanol, sorbitol or lactate. Since the effect of ethanol on the hepatic UDPG/UDPGA ratio has been observed under *in vivo* conditions (Table 1), the present study suggests that the ethanol effect on glucuronidation is physiologically significant.

In conclusion, maternal alcoholism during both gestation and lactation led to pronounced inhibition of the metabolism of drugs like chlorpromazine which require glucuronidation for their detoxification. It is suggested that the ethanol-mediated effect is largely exerted through decreased availability of UDP-glucuronic acid for glucuronidation. This effect is abolished by pyrazole and mimicked by lactate.

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