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THE EFFECT OF CHOLINE DEFICIENCY ON THE ACTIVITY OF A PHOSPHATIDYLCHOLINE-REQUIRING ENZYME: ACTIVITY AND PROPERTIES OF UDP-GLUCURONYLTRANSFERASE IN CHOLINE-DEFICIENT RATS

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SUMMARY The effect of choline deficiency on the kinetic properties of the microsomal enzyme UDP-glucuronyltransferase (EC2.4.1.17) was investigated in rats. Animals fed choline-deficient diets, as compared with animals fed a choline-replete diet or standard laboratory chow, showed almost a three-fold increase in enzyme activity when the enzyme was assayed at physiological concentrations of UDP-glucuronic acid (0.25 mM). The increase in activity appeared to be due to an enhanced affinity of the enzyme for UDP-glucuronic acid rather than to an increase in the amount of enzyme. These data indicate that the kinetic properties of tightly bound membrane enzymes are altered by a dietary change that is known to cause liver disease in the rat.

Choline-deficient rats accumulate triglycerides in their livers because of defective secretion of these lipids as plasma very-low-density proteins (1,2). The defect in triglyceride metabolism in livers of choline-deficient rats can be self-limited (3); interestingly, however, there are conditions under which choline deficiency is cirrhogenic in rats (4). In addition, dietary choline can be shown under some circumstances to be essential for maintenance of normal liver function in primates (5-7).

The biochemical basis for the adverse effect of choline deficiency on liver function is not understood. Choline deficiency does lead to diminished activity of a microsomal glycosyltransferase (8). This effect apparently is due to depletion of a pool of microsomal phosphatidylcholine that influences the function of the glycosyltransferase (8). Thus, the ratio of phosphatidyl-

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choline to protein is abnormally low in liver microsomes from choline-deficient rats (9). Moreover, a choline-deficient diet interferes with induction of hepatic cytochrome P450 (10,11) which, in pure form, requires phosphatidylcholine for reconstitution of activity (12). These data suggest that choline deficiency in rats could have general effects on the function of membrane-bound enzymes that have a dependence for activity on interactions with phosphatidylcholine. The microsomal enzyme UDP-glucuronyltransferase is in this category of enzymes (13-15). Since this enzyme is critical for detoxification of a vast array of endogenous and exogenous toxins, defective function could have deleterious effects on the liver. In order to expand the information on the effects of dietary choline deficiency on the function of enzymes that require phosphatidylcholine for activity, we have determined the activity of UDP-glucuronyltransferase in hepatic microsomes from choline-deficient rats.

MATERIALS AND METHODS

UDP-glucuronic acid (ammonium salt), p-nitrophenol, Trizma Base, UDP-N-acetylglucosamine, UDP, UDP-glucose, and NAD were purchased from Sigma Chemical Corp., St. Louis, MO, U.S.A. The vitamin mix, which contained no B12 or choline, was obtained from Calbiochem-Behring Corp., San Diego, CA, U.S.A. Male Wistar rats were purchased from Simonsen Laboratories, Gilroy, CA, U.S.A. They were housed in individual wire-bottom cages in a room with a 12-hour day/night cycle, and were allowed free access to water. The animals were fed artificial granular diets (16). This diet contained, by weight, casein (8 percent), sucrose (72.3 percent), alphacel (5 percent), lard (4 percent), corn oil (4 percent), vitamin mix (2 percent), Hegsted salt mix (4 percent) and cystine (0.5 percent). The diet fed to choline-replete rats (Ch+) contained 0.2 percent choline by weight. No choline was added to the diet fed to choline-deficient rats (Ch-). Animals receiving the choline-deficient and replete diets gained identical amounts of weight. Consumption of diets was the same for both (Ch+) and (Ch-) groups. Five animals were fed standard laboratory chow.

Animals were killed by cervical dislocation, and the livers rapidly removed and placed into chilled 0.25 M sucrose. Livers from the (Ch-) animals showed extensive fatty metamorphosis. Those from the (Ch+) and lab chow groups were normal. The microsomal fraction was collected as previously described (17). All data reported were obtained using microsomes on the day they were prepared. This was necessary because there was activation of UDP-glucuronyltransferase secondary to freeze-thawing. The differences noted below with fresh microsomes were also present in freeze-thawed microsomes; however, because we were interested in changes in the enzyme that had occurred *in vivo*, only fresh microsomes were used. The amounts of microsomal protein per gram body weight were 0.50±0.06 mg (Ch-), 0.49±0.06 (Ch+), and 0.54±0.06 mg (lab chow).

Initial rates of conjugation of p-nitrophenol were determined as previously described (17), except that 1 mM NAD was added to each assay to inhibit nucleotide pyrophosphatase activity. Assay conditions are indicated in the legends to Figures. Proteins were determined by the biuret method (18). All data are expressed as ±S.E.M. Statistics were performed using the student's T test, and statistical significance was achieved when $p \leq 0.05$.

RESULTS AND DISCUSSION

Based on prior findings that phosphatidylcholine, lysophosphatidylcholine and sphingomyelin are the only microsomal phospholipids that reconstitute the activity of delipidated UDP-glucuronyltransferase (13), we expected choline deficiency to decrease the activity of microsomal UDP-glucuronyltransferase. However, the activity of UDP-glucuronyltransferase was greater in liver microsomes from (Ch-) animals as compared with animals fed the (Ch+) diet or lab chow (Table 1). In order to determine the possible cause for the difference in activity of UDP-glucuronyltransferase between animals fed (Ch+) and (Ch-) diets, rates of conjugation of p-nitrophenol were measured as a function of the concentration of UDP-glucuronic acid. Activities as a function of the concentration of UDP-glucuronic acid were the same in rats fed the (Ch+) diet and lab chow. Double-reciprocal plots of the activity of UDP-glucuronyltransferase as a function of the concentration of UDP-glucuronic acid are shown in Fig. 1 for animals fed (Ch-) and (Ch+) diets for two weeks. Similar results were obtained when rats were fed the diets for four weeks (data not shown). The plots in Fig. 1 are non-linear, a finding reported previously.

Two important points are illustrated by the data in Fig. 1. First, the activities at saturating concentrations of UDP-glucuronic acid are identical

Table 1

Effect of Choline Deficiency on
Activity of UDPglucuronyltransferase

Diet	No. Animals	Rate (nmol/min/mg)
Lab Chow	5	0.33±.03
(Ch+)	5	0.32±.01
(Ch-)	5	0.91±.07

Each assay contained 0.25 mM UDP-glucuronic acid; 1 mM $MnCl_2$; 1 mM NAD; 50 mM Tris-HCl, pH 7.5, 30°; and 0.4 mM p-nitrophenol. (Ch-) versus lab chow and (Ch+), $p<0.0001$.

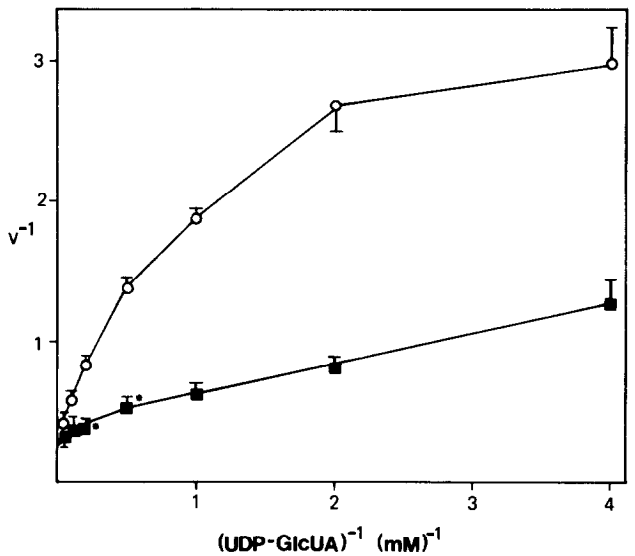

Figure 1: Double-reciprocal plot of the activity of UDP-glucuronyltransferase versus concentration of UDP-glucuronic acid after two weeks of diets. Each assay contained 50 mM Tris-HCl, pH 7.5, 30°; 1 mM $MnCl_2$; 0.4 mM p-nitrophenol; and 1 mM NAD. Rate is expressed in nmol/min/mg protein. Each point is the mean ±S.E. of a minimum of four animals, except for those marked with an *, where only three animals were assayed.

■ Choline-deficient (Ch-)

o Choline-replete (Ch+)

(Ch-) versus (Ch+), $p<0.001$ 0.25, 0.5, 1.0, 5.0, 10.0, 20.0 mM UDP-GlcUA, $p<0.01$ 2 mM UDP-GlcUA.

for enzyme derived from (Ch+) and (Ch-)-fed groups of animals. Second, the differences in enzymatic activity in vitro for rats fed (Ch+) and (Ch-) diets are greatest at low concentrations of UDP-glucuronic acid. Since the concentration of UDP-glucuronic acid in intact liver is about 0.2 mM (20), it seems that the activity in vivo of the form of UDP-glucuronyltransferase assayed in Fig. 1 will be higher in (Ch-) versus (Ch+)-fed rats.

Previous investigators have shown that the activity of UDP-glucuronyltransferase in microsomes can be altered by feeding rats a low-protein or high-fat diet (21,22). It also was shown by the data in (21) that microsomes from rats fed a low-protein diet contain greater than normal concentrations of lysophosphatides,which are activators of UDP-glucuronyltransferase (15,23). We were interested, therefore, in the possibility that increased activity of endogenous phospholipase A_2

effected the observed changes in the activity of UDP-glucuronyltransferase in (Ch-) versus (Ch+)-fed animals. This question is open to experimental verification via kinetic studies of UDP-glucuronyltransferase because there are distinctive phospholipase A_2-induced changes in the properties of the enzyme. These include enhanced susceptibility to end-product inhibition by UDP, loss of specificity for the binding of UDP-sugars, and lack of response to activation by UDP-N-acetylglucosamine (24). UDP (1,2,5, and 10 mM, respectively) was added to assays containing microsomes from rats fed (Ch-) or (Ch+) diets. The extent of product inhibition was the same for enzyme from rats fed either diet. Selectivity of binding at the UDP-glucuronic acid site was tested by adding UDP-glucose to microsomes from choline-deficient rats. UDP-glucose at concentrations as high as 20 mM did not inhibit the activity of UDP-glucuronyltransferase from (Ch-) rats. Moreover, we found that UDP-N-acetylglucosamine stimulated the activity of UDP-glucuronyltransferase in microsomes from (Ch-) rats (Table 2). These results demonstrate that the change in UDP-glucuronyltransferase in (Ch-) versus (Ch+) rats cannot be due to increased in vivo activity of phospholipase A_2.

Table 2

Effect of UDP-N-Acetylglucosamine
on the Activity of UDPglucuronyltransferase

Diet	Additions to Assay	Rate (nmol/min/mg)
(Ch+)	None	0.53 ± 0.07^{a}
(Ch+)	UDP-N-acetylglucosamine	3.60 ± 0.46^{b}
(Ch-)	None	1.07 ± 0.09^{a}
(Ch-)	UDP-N-acetylglucosamine	5.20 ± 0.48^{b}

The composition of the assay is described in Table 1. The concentration of UDP-N-acetylglucosamine was 2 mM. Each data point is the average for three rats fed the specified diet for four weeks.

[a] Difference (Ch+) versus (Ch-), $p<0.025$

[b] Difference (Ch+) versus (Ch-), p NS

We do not know as yet how choline deficiency leads to enhanced activity of UDP-glucuronyltransferase. We think it is important to point out, however, that a simple change in the amount of UDP-glucuronyltransferase(s) in (Ch-) as compared with (Ch+) groups cannot explain the data in Figure 1. These data must reflect a difference in the kinetic properties of UDP-glucuronyltransferase in the two groups of animals. We cannot exclude, of course, the possibility that choline deficiency leads to induction of a unique form of UDP-glucuronyltransferase and suppression of others such that the activity at $V_{max}^{apparent}$ remains unchanged. We think, however, that there is a more likely explanation for the data. We want to propose that the choline-deficient diet changes the nature of the phospholipids intimately associated with UDP-glucuronyltransferase. We favor this idea because a change of this type can alter the kinetic properties of purified forms of UDP-glucuronyltransferase that conjugate p-nitrophenol (15,25,26). For example, studies of pure, delipidated forms of UDP-glucuronyltransferase indicate that the chain length and degree of unsaturation of the fatty acids of the phosphatidylcholine used to reconstitute activity determine the kinetic properties of reconstituted enzyme (25,26). Hence, it is reasonable to expect alterations within the microsomes of the species and relative abundance of different choline-containing phospholipids could alter the activity of UDP-glucuronyltransferase. It is known in this regard that choline deficiency leads not only to a decrease in choline-containing phosphatides, but alters as well the fatty acid composition of microsomal phospholipids (27). Subtle changes in the composition of the phospholipids of cellular membranes can lead to alterations in the properties of many membrane-bound enzymes, some of which could be critical to the normal function of cells. Further work is warranted in order to explore these possibilities.

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