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EFFECTS OF L-ASPARAGINE AND RELATED COMPOUNDS ON THE HEPATIC FATTY INFILTRATION AND NECROSIS INDUCED BY ETHIONINE AND CARBON TETRACHLORIDE

NICHOLAS M. ALEXANDER, ROBERT SCHEIG and GERALD KLATSKIN

Departments of Internal Medicine and Biochemistry,
Yale University School of Medicine,
New Haven, Conn., U.S.A.

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Abstract—1. In rats given ethionine or carbon tetrachloride, intraperitoneal injection of L-asparagine fully protected against fatty infiltration of the liver. L-Asparagine did not prevent CCl_4-induced hepatic necrosis. L-Glutamine and L-methionine also prevented fatty infiltration after ethionine or CCl_4, but L-aspartic and α-ketoglutaric acids did not.

2. Chromatographic analysis of the hepatic lipids after CCl_4 administration revealed that the intraperitoneal injection of L-asparagine had prevented the expected alterations in the fatty acid pattern indicative of lipid mobilization from adipose tissue.

3. The implications of these findings are discussed.

ETHIONINE produces a marked increase in hepatic triglycerides,[1, 2] with little [3] or no[4] hepatic necrosis, an effect seen in female but not in male rats. This increase in hepatic triglycerides is prevented by the injection of methionine,[1] ATP, and ATP precursors.[5–7] In contrast, carbon tetrachloride (CCl_4) gives rise to both fatty infiltration and centrilobular necrosis of the liver, and affects both male and female animals.[8] The increase in hepatic triglyceride concentration produced by CCl_4 is prevented by ATP but not by its precursors.[7]

Since both ATP[7] and L-asparagine[9] have been found to prevent the fatty liver induced by ethanol, the present investigation was carried out to determine whether the amino acid amide also protected rats from the effects of ethionine and CCl_4. Evidence is presented to show that L-asparagine and some other amino acids protect against the accumulation of fat in the liver produced by ethionine and CCl_4 but not against the hepatic necrosis.

MATERIALS AND METHODS

Sprague–Dawley-type female rats, 125–200 g, obtained from the Charles River Rat Farm, North Wilmington, Mass., were housed in individual cages at constant temperature and humidity and maintained on Purina lab chow and water *ad libitum* until 16 hr before an experiment, when food but not water was withdrawn.

The total dose of DL-ethionine was 100 mg (0·6 m-mole)/100 g body weight and was administered either by intraperitoneal injection in two or five doses or by gastric tube as a single dose.

CCl_4 was administered subcutaneously in a total dose of 0·20 or 0·26 ml/100 g body weight. In both instances CCl_4 was given in two equal doses, 2 hr apart.

The protective effects of L-asparagine were investigated at total dose levels ranging from 80 mg (0·6 m-mole) to 400 mg (3·0 m-moles) given either i.p. in multiple doses or as a single dose by gastric tube. The results were compared with those obtained with i.p. injected saline and several other amino acids. Details of these experiments are given in the tables.

Animals were sacrificed by decapitation 5, 5·5, or 24 hr after initial injection. Livers were removed promptly and, after excising a small wedge from each for histologic study, the remainder was homogenized for 1 min with 9 vol. of ice-cold 0·067 M phosphate buffer at pH 7·0 in a Waring blender. Hepatic triglyceride concentration was determined by the method of Butler *et al.*,[10] but glycerol instead of corn oil was used as a standard. Fatty acids were analyzed by gas-liquid chromatography.[11] Results are expressed as means ± S.E.M. The unpaired *t* test was used in calculating the statistical significance of differences between experimental groups.[12]

The sources of the materials used in these experiments were as follows: L-asparagine, L-aspartic acid, and L-methionine from Nutritional Biochemicalr Corp., Cleveland, Ohio; glycine and CCl_4 from Fisher Scientific Co., Fairhaven, N.J.; L-lysine from Eastman Organic Chemicals, Rochester, N.Y.; α-ketoglutaric acid from Sigma Chemical Co., St. Louis, Mo.; and all other amino acids from Mann Research Laboratories, Inc., New York, N.Y. All other chemicals were reagent grade.

L-Asparagine was dissolved in water with the aid of heat, if necessary, and cooled to 37° before injection. Aqueous solutions of L-aspartic acid and α-ketoglutaric acid were adjusted to pH 7·4 with sodium hydroxide prior to use.

RESULTS

Ethionine

Intraperitoneally injected DL-ethionine, in a dose of 100 mg (0·6 m-mole)/100 g body weight, produced a two- to fivefold increase in hepatic triglycerides within 5 hr (Tables 1–3), which persisted for at least 24 hr. Orally administered ethionine was equally effective in raising the hepatic triglyceride level (Table 2).

Table 1 shows that L-asparagine, injected i.p. at a dose of 200–400 mg (1·5 to 3·0 m-moles)/100 g body weight, completely prevented the fatty infiltration of the liver induced by i.p. ethionine, and partially prevented it at a dose of 160 mg (1·2 m-moles)/100 g body weight. L-Asparagine alone had no effect on hepatic triglyceride concentration. Of interest, but not explored further, was the observation that i.p. injection of hypertonic (0·375 M) saline alone raised the hepatic triglyceride concentration significantly (Table 1).

Since the asparagine solutions injected i.p. were hypertonic (0·30–0·75 M), and invariably produced ascites, one possibility considered was that the osmotic action of asparagine interfered with the absorption of ethionine from the peritoneum, and thus prevented fatty infiltration of the liver. However, this appeared unlikely, since (1) the injection of hypertonic (0·375 M) sodium chloride solution failed to prevent the increase in hepatic triglyceride seen after i.p. injection of ethionine (Table 1), and (2) i.p. injected asparagine significantly decreased fatty infiltration of the liver not only when the ethionine was injected i.p. but also when it was administered orally (Table 2). Moreover, the oral administration of L-asparagine, at a dose of 200 mg

(1·5 m-moles) per 100 g body weight, also significantly diminished the fatty infiltration of the liver induced by ethionine given i.p.

Table 3 shows that, like asparagine, L-glutamine, glycine, and L-methionine (in half the amount) given i.p. were also effective in preventing the expected rise in hepatic

TABLE 1. HEPATIC TRIGLYCERIDE LEVELS OF FEMALE RATS RECEIVING INTRAPERITONEAL INJECTIONS OF DL-ETHIONINE AND L-ASPARAGINE

DL-ethionine (mg)	L-asparagine (mg)	Saline (M)	Liver triglycerides (μmoles/g liver)	P
0 (7)	0	0·155	11·3 ± 1·3	
0 (8)	0	0·375	21·8 ± 1·7	
0 (3)	80	0	7·3 ± 1·5	
0 (6)	400	0	11·5 ± 1·6	
100 (18)	0	0	40·1 ± 1·7	
100 (5)	0	0·375	50·8 ± 7·8	
100 (7)	400	0	11·8 ± 2·4	<0·001
100 (4)	320	0	9·0 ± 0·9	<0·001
100 (3)	200	0	10·4 ± 0·9	<0·001
100 (4)	160	0	27·9 ± 1·9	<0·01
100 (3)	80	0	30·3 ± 7·3	>0·05

Separate i.p. injections of 2 ml/100 g body weight of each substance were administered after a 16-hr fast. The injections were repeated in 2 hr and the animals were sacrificed 3 hr later. The total number of milligrams of each substance given is indicated in the table; P values were obtained by comparison with the group that received only ethionine. The numbers in parentheses indicate the number of animals in each group.

TABLE 2. HEPATIC TRIGLYCERIDE LEVELS OF FEMALE RATS RECEIVING ORAL AND INJECTED DOSES OF DL-ETHIONINE AND L-ASPARAGINE

Treatment		Liver triglycerides (μmoles/g liver)
Oral	Intraperitoneal	
Water (4)	Saline	11·7 ± 2·5
Saline (5)	Ethionine	57·6 ± 4·2*
Ethionine (9)	Saline	54·2 ± 3·1*
Asparagine (6)	Ethionine	30·3 ± 3·2†
Ethionine (6)	Asparagine	27·9 ± 5·5†

Single oral doses of each substance were 4 ml/100 g body weight; by i.p., injection this volume was equally divided and given at 0, 30, 60, 120, and 180 min after the stomach-tube feeding. Sacrifice of the animals occurred 5·5 hr after the initial injection. The total amount given by either route was 200 mg L-asparagine and 100 mg DL-ethionine/100 g body weight. Saline was 0·155 M. The numbers in parentheses refer to the number of animals in each group.

* P value < 0·001 when compared with group receiving water orally and saline i.p.

† P value < 0·01 when compared with the appropriate ethionine-treated group receiving saline instead of asparagine.

triglycerides after injection of ethionine. However, similar doses of L-aspartic acid, L-lysine, L-threonine, and α-ketoglutaric acid afforded no protection against ethionine-induced fatty infiltration of the liver.

Carbon tetrachloride

Within 5 hr after s.c. administration of CCl_4, there was a 2·5-fold increase in hepatic triglycerides (Table 4). This effect was prevented by i.p. injection of L-asparagine, L-glutamine, L-glutamic acid, or DL-methionine, but not by i.p. injection of L-aspartic acid or glycine.

TABLE 3. HEPATIC TRIGLYCERIDE LEVELS OF FEMALE RATS INJECTED WITH DL-ETHIONINE AND VARIOUS AMINO ACIDS

DL-ethionine (0·155 M)	Amino acid (0·311 M)	Saline (0·155 M)	Liver triglycerides (μmoles/g liver)	P
0 (6)	0	+	14·1 ± 2·6	
+ (6)	0	+	22·1 ± 2·5	
+ (7)	L-glutamine	0	11·1 ± 2·2	<0·05
+ (4)	L-methionine (0·155 M)	0	14·1 ± 1·9	<0·05
+ (8)	glycine	0	15·1 ± 2·9	<0·05
+ (10)	L-aspartic acid	0	19·1 ± 1·6	>0·05
+ (4)	L-lysine	0	28·6 ± 2·8	>0·05
+ (4)	L-threonine	0	23·3 ± 1·5	>0·05
+ (4)	None + α-keto-glutarate	0	20·3 ± 1·7	>0·05

Separate i.p. injections of 2 ml/100 g body weight of DL-ethionine plus 4·9 ml/100 g body weight of the materials listed in the other columns were given with different syringes. Injections of each substance were repeated in 2 hr and the animals were killed 5 hr after the first injection. The animals not receiving ethionine were given instead 2 ml/100 g body weight of 0·075 M NaCl. The numbers in parentheses refer to the number of animals in each group and the P values were obtained by comparison with the group receiving ethionine plus saline.

TABLE 4. HEPATIC TRIGLYCERIDE LEVELS OF FEMALE RATS INJECTED WITH CCL_4 AND VARIOUS AMINO ACIDS

CCl_4	Amino acid (0·311 M)	Saline (0·155 M)	Liver triglyceride (μmoles/g liver)	P
0 (6)	0	+	14·1 ± 2·6	
+ (7)	0	+	34·7 ± 2·5	
+ (8)	L-asparagine	0	18·5 ± 1·9	<0·01
+ (5)	L-glutamine	0	20·6 ± 2·6	<0·01
+ (5)	L-glutamic acid	0	16·0 ± 3·1	<0·01
+ (4)	DL-methionine	0	16·9 ± 4·2	<0·01
+ (5)	L-aspartic acid	0	29·3 ± 5·5	>0·05
+ (5)	Glycine	0	27·1 ± 4·7	>0·05

CCl_4 (0·1 ml) was administered s.c. and 4·9 ml/100 g body weight of all other materials was given at the same time by i.p. injection after a 16-hr fast. Injections of all substances were repeated in 2 hr and the animals were killed 5 hr after the first injection. The numbers in parentheses refer to the number of animals in each group. P values were obtained by comparison with the group receiving CCl_4 plus saline.

The results of gas-liquid chromatographic analysis of hepatic fatty acid composition shown in Table 5 were consistent with previous reports[13] that the triglycerides which accumulate in the liver after CCl_4 administration are synthesized principally from fatty acids mobilized from adipose tissue. The changes to be expected from such

mobilization—increases in the proportion of oleic, linoleic, and palmitic acids (fatty acids that predominate in adipose tissue) and decreases in the proportions of stearic and arachidonic acids—were evident after CCl_4 administration (Table 5). It can further be seen from the table that the i.p. injection of L-asparagine prevented those changes in the hepatic fatty acid pattern induced by CCl_4.

TABLE 5. FATTY ACID COMPOSITION OF LIVER AFTER CCl_4 AND ASPARAGINE

Treatment	Fatty acids in total lipid extract (% of total fatty acids)					
	Palmitic	Stearic	Oleic	Linoleic	Arachidonic	Other
Saline	19 ± 0·3	25 ± 0·7	11 ± 2·3	13 ± 0·7	21 ± 1·0	11 ± 0·7
CCl_4 and saline	21 ± 1·0	19 ± 0·4	19 ± 0·7	15 ± 0·3	14 ± 0·5	12 ± 0·6
CCl_4 and L-asparagine	19 ± 0·7	22 ± 0·3	14 ± 0·7	13 ± 0·4	19 ± 0·5	13 ± 0·3

The per cent distribution of the five major fatty acids present in the total liver lipid from rats given saline, CCl_4, and CCl_4 plus asparagine as described in Table 4 is shown. Data are the means ± S.E. of four animals in each group. Total liver lipids were extracted and fatty acids were separated as their methyl esters by gas chromatography in a 6 ft × 4 mm glass U tube packed with 17 per cent EGSS-X coated on Gas Chrom P, 80–100 mesh. Column temperature: 173°, detector: 200°.[11]

TABLE 6. THE EFFECT OF L-ASPARAGINE ON LIVER INJURY AFTER CCl_4 ADMINISTRATION

Treatment	Degree of necrosis	Liver triglycerides (μmoles/g liver)
CCl_4 and saline (10)	++++	93·0 ± 9·9
CCl_4 and L-asparagine (10)	++++	52·9 ± 3·7 ($P < 0·01$)

Female rats, fasted for 16 hr, were given s.c. 0·26 ml/100 g body weight of a solution containing equal volumes of CCl_4 and paraffin oil At the same time 4·9 ml/100 g body weight of either 0·155 M NaCl or 0·311 M L-asparagine was injected i.p. Each pair of injections was repeated in 2 hr and the animals were sacrificed 24 hr after the first injection. The numbers in parentheses refer to the number of animals in each group.

Twenty-four hr after s.c. injection of CCl_4 there was still a significant increase in hepatic triglycerides, and microscopically the liver showed typical centrilobular necrosis (Table 6). Simultaneous i.p. administration of L-asparagine reduced fat accumulation but failed to prevent hepatic necrosis. Asparagine did not completely prevent hepatic lipid accumulation in this experiment, perhaps because the animals were fasted for a longer period and 32 per cent more CCl_4 was administered.

DISCUSSION

It has been suggested that the accumulation of fat in the liver after the administration of either ethionine or CCl_4 may be the result of a defect in the removal of

fat from the liver, a functional disturbance attributed to inhibition of protein (including lipoprotein) synthesis.[14–19] Farber *et al.*[5, 18] have indicated that the depressed protein synthesis induced by ethionine is secondary to depressed hepatic ATP levels because ATP can be trapped as S-adenosyl ethionine. However, some observations are not explained by this hypothesis. (1) While ethionine lowers hepatic ATP in both male and female rats,[17] it produces fatty infiltration of the liver in females only.[1, 2] (2) Glutamine does not prevent the fall in hepatic ATP that follows ethionine administration,[18] yet we have found it to be effective in preventing the accompanying accumulation of fat. (3) Asparagine was similarly effective and, in preliminary experiments, failed to prevent the fall in hepatic ATP. These discrepancies suggest that the disturbance in protein synthesis and lipid transport observed in ethionine-treated animals may not be dependent only upon the associated reduction in hepatic ATP. Indeed, the administration of precursors of ATP, which raise hepatic ATP levels after CCl_4, do not prevent the development of fatty livers.[7]

Neither the mode nor the site of action of asparagine and glutamine is evident from the data presented. Conceivably, these compounds play a role in regulating triglyceride metabolism, and are blocked by ethionine and CCl_4. Asparagine may have a peripheral effect on lipid mobilization from adipose tissue rather than, or in addition to, a direct effect on lipid metabolism within the liver. This possibility is suggested by our observation that asparagine prevents the characteristic change in the hepaiic fatty acid pattern induced by CCl_4 as a consequence of lipid mobilization from the depots. However, the alternative possibility, that such fatty acids are preferentially cleared from the liver under conditions of normal hepatic lipid metabolism, cannot be excluded.

It is unlikely that asparagine protects against CCl_4-induced fatty infiltration of the liver by impairing the absorption or by enhancing the detoxification of CCl_4, since, as shown in the present study, it does not prevent hepatic necrosis. This observation also supports other evidence indicating that the accumulation of fat and the occurrence of necrosis in the liver after the administration of hepatotoxins depend on different mechanisms.[20]

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Note added in proof. In a more extensive study employing two different methods for ATP analysis, Dr. B. K. Chew in our laboratory has found that L-asparagine does indeed reverse the depressed hepatic ATP levels that are produced by ethionine. Moreover, CCl_4 injected S.C. does not alter the hepatic levels even though the triglyceride levels were markedly elevated.