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INCREASE IN ACETYLATION OF SPERMIDINE IN RAT LIVER EXTRACTS BROUGHT ABOUT BY TREATMENT WITH CARBON TETRACHLORIDE

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SUMMARY Within three hours of the administration of hepatotoxic doses of carbon tetrachloride to rats there was a substantial increase in the ability of liver extracts to catalyze the accumulation of monoacetylspermidine when incubated with spermidine and acetyl-CoA. This increase was maximal by six hours and correlated with the period in which there was a pronounced fall in hepatic spermidine and concomitant increase in putrescine. During this time there is a large increase of the conversion of labeled spermidine into putrescine in the liver. These results therefore suggest that this conversion requires the prior acetylation of spermidine.

INTRODUCTION

Spermidine is synthesized by the action of an aminopropyltransferase which transfers the aminopropyl group from decarboxylated S-adenosylmethionine to putrescine (1). Although the question has not been tested rigorously this reaction is thought to be effectively irreversible. However, even in normal rat liver evidence obtained using labeled spermidine suggests that a small degree of conversion to putrescine takes place (2). This conversion is greatly enhanced on treatment of rats with the hepatotoxin, carbon tetrachloride (3). The mechanism by which the degradation of spermidine to putrescine takes place is not well understood. A polyamine oxidase capable of carrying out the oxidative cleavage of spermidine yielding putrescine and 3-aminopropionaldehyde has been purified from rat liver but activity required the addition of high concentrations of aldehydes not likely to be present in physiological materials (4). Furthermore, no increase in the activity of this enzyme could be detected following treatment with doses of carbon tetrachloride that produced maximal enhancement of putrescine production from spermidine (4).

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Several laboratories have reported that polyamine acetylase activity is present in rat liver (5-7). The monoacetylated derivatives may be degraded by de-acetylases back to the polyamine (5,8) but also may be substrates for polyamine oxidase (4) or monoamine oxidase (5,9,10). The present paper describes the changes brought about by pretreatment with carbon tetrachloride in the ability of rat liver extracts to catalyze the accumulation of acetylated spermidine when incubated with acetyl-CoA and spermidine. Such treatment produced a substantial increase in this activity at a time similar to that at which the conversion of spermidine to putrescine was maximal. These results therefore, support the concept that acetylation of spermidine is required for its conversion to putrescine.

METHODS

Reagents and Animals. Polyamines and other biochemicals were obtained from Sigma Chemical Co., St. Louis, MO. [Tetramethylene, 1,4-^{14}C]Spermidine (10 mCi/mmol) and [1-^{14}C]acetyl-coenzyme A (48.3 mCi/mmol) were purchased from New England Nuclear, Boston, MA. Female Sprague-Dawley rats weighing 150-250 grams were used in all experiments. Carbon tetrachloride was injected intraperitoneally at a dosage of 0.2 ml per 100 gram body weight.

Polyamine Analysis. Polyamine content and labeling after injection of [^{14}C] spermidine was measured following electrophoretic separation and ninhydrin staining as previously described (11) or by the method of Seidenfeld and Marton (12). Both methods gave essentially the same results.

Preparation of ultracentrifuged supernatant extracts. Livers were excised and chilled in several volumes of ice-cold 0.25 M sucrose containing 50 mM tris-HCl, pH 7.5, 25 mM KCl and 5 mM $MgCl_2$. They were then weighed, blotted and homogenized with two volumes of the same solution. The homogenate was centrifuged at 100,000 x g for 60 min and the supernatant used for enzyme assay.

Preparation of chromatin. Chromatin was isolated as described by Blankenship and Walle (6). The isolated chromatin was swollen overnight in distilled water, centrifuged at 30,000 x g for 30 minutes and the supernatant extract used for enzyme assay.

Assay of spermidine acetylase activity. The activity was measured by the modified method of Libby (7). Enzyme was incubated with 0.3 µmol of spermidine, 10 µmol tris-HCl (pH 7.8) and 40 nCi of [^{14}C]-acetyl CoA in a final volume of 100 µl at 30°C for 10 min. The chromatin extract was incubated with Na-bicine buffer (pH 8.8) instead of tris-HCl buffer. The reaction was terminated by chilling and the addition of 20 µl of 1M $NH_2OH.HCl$; the reaction mixture was placed in a boiling water bath for 3 min. Aliquots of 50 µl of the reaction mixture were spotted on a 2.3 cm disc of cellulose phosphate paper. The discs were washed with tap water and then five times with 1 ml aliquots of water followed by three washes with 1 ml aliquots of ethanol on a sintered-glass filter. After drying under a heat lamp, the discs were counted in a Beckman LS-3133T liquid scintillation spectrometer in vials containing 10 ml of formula 949 counting fluid (New England Nuclear, Boston, MA).

Table I. Effect of carbon tetrachloride on polyamine content and on conversion of spermidine to putrescine in rat liver.

Treatment	Polyamine content (nmol/gram) Putrescine	Spermidine	Spermine	Putrescine labeling (dpm/gram)
Control	8 ± 2	517 ± 42	585 ± 33	37 ± 7
CCl_4, 3 hr	57	487	560	N.D.
CCl_4, 6 hr	273 ± 29	205 ± 22	375 ± 89	2702 ± 314
CCl_4, 12 hr	546 ± 85	265 ± 47	320 ± 47	592 ± 138
CCl_4, 24 hr	139 ± 32	564 ± 130	292 ± 31	225 ± 62
CCl_4, 72 hr	23	1088	516	N.D.

Results for polyamine content are shown as means ± S.D. for four or more estimations and as the mean for less than four determinations. Conversion of [^{14}C]spermidine into [^{14}C]putrescine was determined following intraperitoneal injection of 1 μCi (0.1 μmol) of [^{14}C]spermidine in 0.9% (w/v) NaCl per 100 grams body weight 2 hr before death. N.D., not determined.

RESULTS

Table I shows the content of polyamines in rat liver following administration of carbon tetrachloride. This led to a transient but pronounced fall in hepatic spermidine within 6-12 hours amounting to a 50-60% decrease. Spermine content also decreased but more slowly reaching about 50% of the control level by 24 hours. At the same time there was a substantial increase in hepatic putrescine content which rose more than 50-fold reaching a peak at 12 hours and then declining. When hepatic spermidine was labeled by intraperitoneal injection of [^{14}C]spermidine, treatment with carbon tetrachloride greatly enhanced the formation of [^{14}C]putrescine over the same period suggesting that the decline in spermidine is due at least in part to its conversion to putrescine (Table I). These results are in good agreement with those of Hölttä et al. (3).

When rat liver cytosol extracts were incubated with spermidine and [^{14}C]acetyl-CoA the formation of [^{14}C]acetylspermidine took place. Treatment with carbon tetrachloride led to a substantial increase in the ability of these extracts to catalyze the accumulation of acetylspermidine (Figure 1). The enhancement was maximal by six hours and declined towards control values

by 15 hours after administration of carbon tetrachloride. Other workers have reported that they were unable to detect spermidine acetylase activity in rat liver cytosol but that activity was present in chromatin (5-7). As shown in Figure 1, we also observed that spermidine acetylase activity was present in chromatin fractions and this activity was enhanced by treatment with carbon tetrachloride although not to the same extent as the cytosolic activity. It should be noted that the activity of the liver cytosol extracts in forming acetylspermidine was not proportional to the protein content (see below); comparisons in Figure 1 were made using equal amounts of tissue extract from the control and the treated animals. Although the chromatin derived extracts had a higher specific activity than those from the cytosol the total activity present in the supernatant was much greater since only a small fraction of the liver protein was present in the solubilized chromatin preparation. However, the relative activities could not be estimated precisely because of the lack of proportionality between protein added and acetylspermidine obtained when cytosolic extracts were used.

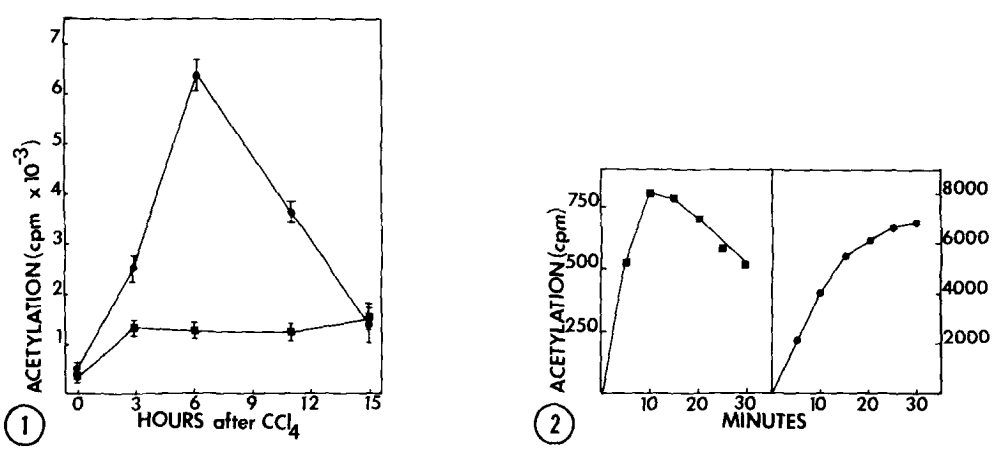

Figure 1. Stimulation of spermidine acetylation by pretreatment with carbon tetrachloride. Assays were carried out as described under methods using either 0.14 mg of protein from the ultracentrifuged supernatant (●) or 0.025 mg of protein from the solubilized chromatin preparation (■). Results are shown ± S.D.

Figure 2. Time course of accumulation of acetylspermidine by cytosolic extracts from control (■) and carbon tetrachloride (●) pretreated (6 hours) rats. Assay conditions were as for Figure 1.

Table II. Effect of carbon tetrachloride on acetylation of polyamines by rat liver cytosolic extracts.

Enzyme Source	Acetylase Activity (c.p.m./10 min incubation)		
	Putrescine	Spermidine	Spermine
Control	236	362	145
CCl_4, 6 hr	103	4,436	1,550

The assays contained 3 mM putrescine, spermidine or spermine as shown, 0.14 mg protein of a liver supernatant from control or carbon tetrachloride treated rats, 100 mM tris-HCl, pH 7.8 and 8.3 μM [^{14}C]acetyl-CoA in a total volume of 0.1 ml.

It is probable that deacetylation or other metabolism of acetylspermidine catalyzed by enzymes present in the crude cytosolic extracts limits the extent of reaction, accounting for the time course observed (Figure 2) and resulting in the lack of proportionality between product accumulation and added protein. As shown in Figure 2, a decrease in acetylspermidine accumulation was observed when control cytosol extracts were incubated for longer than 10 minutes. In contrast the extracts from rats pretreated with carbon tetrachloride continued to accumulate acetylspermidine over a 30 minute incubation albeit at a decreasing rate. The apparent discrepancy between our findings and other reports of the lack of acetylase activity in control supernatants may be explained by differences in the time of incubation and amount of protein added.

The cytosolic spermidine acetylase activity also was tested with putrescine and spermine as substrates (Table II). Putrescine acetylation was not enhanced by carbon tetrachloride treatment but that of spermine was elevated by at least 10-fold when assayed under standard reaction conditions. Acetylation of spermidine and spermine by the cytosolic extracts was similar at pH 8.8 and 7.8.

DISCUSSION

The present results support the hypothesis of Seiler (5,9,13) that mono acetylation of polyamines provides an important route in their further metabolism. The substantial rise in spermidine acetylation paralleling the increased conversion of spermidine into putrescine following carbon tetra-

chloride treatment suggests that such acetylation may be the rate limiting step in this conversion. It will be of considerable interest to ascertain whether partial hepatectomy, treatment with thioacetamide or growth hormone which also enhance the conversion of spermidine into putrescine (3) increase cytosolic spermidine acetylase activity. Such experiments are currently in progress.

Rat liver spermidine acetylase has been shown to form both the N^1- and the N^8-monoacetylspermidine isomers (7,14). The mechanism by which monoacetylspermidine might serve as a precursor of putrescine requires further investigation but it should be noted that N^1-acetylspermidine is a substrate for highly purified polyamine oxidase yielding putrescine (4). The acetylated derivative may be a more physiologically relevant substrate than the Schiff base between aromatic aldehydes and the unconjugated polyamine. Very recently Blankenship (15) has shown that N^1-acetylspermidine is converted to putrescine by 100,000 x g supernatant fractions from rat liver. The N^8-acetylspermidine isomer is an excellent substrate for the rat liver deacetylase and may be degraded to regenerate spermidine (8).

Other workers who have reported on polyamine acetylase have noted that the activity was chromatin bound (5-7). Putrescine acetylase activity also was present in microsomal preparations (5). Although we were able to detect some activity in cytosolic fractions of control rats, only livers of carbon tetrachloride treated rats contained appreciable activity in this fraction. There was a smaller but significant and more prolonged enhancement of chromatin-bound acetylase activity after treatment with the hepatotoxin. The means by which the enhanced activity is brought about could involve an increased release of acetylase from a latent membrane-bound form or an activation or increased synthesis of the enzyme. At present we cannot rule out the possibility that inhibition of a deacetylase also contributes substantially to the apparent increase observed in polyamine acetylase. However, the enhanced activity in chromatin extracts presumably free of deacetylase (5,6) implies

that some increase in acetylase is taking place. The exact relationship between the chromatin and soluble enzymes can only be clarified after substantial purification of the latter.

Our results also raise the interesting possibility that enhanced acetylation of spermidine and spermine caused by carbon tetrachloride may in some way contribute to the development of liver damage by this agent.

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