FORMATION OF N\textsuperscript{1}-ACETYLSPERMIDINE IN RAT LIVER AFTER TREATMENT WITH CARBON TETRACHLORIDE

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Summary

A single intraperitoneal injection of carbon tetrachloride produced a significant increase in the concentration of N\textsuperscript{1}-acetylspermidine in rat liver. The concentration of N\textsuperscript{1}-acetylspermidine was maximal at the same time after injection at which other workers reported maximal conversion of spermidine to putrescine and maximal acetylase activity in liver extracts. N\textsuperscript{1}-acetylspermidine was not detectable in livers of untreated animals and at 45 hours after injection with carbon tetrachloride. These findings support the hypothesis that monoacetylation of polyamines precedes their degradation by polyamine oxidases. Spleen, lungs and erythrocytes of untreated animals contained detectable amounts of the monoacetyl polyamines. Treatment with carbon tetrachloride did not produce changes in the concentrations of the monoacetyl polyamines in these tissues.

The synthesis and accumulation of polyamines in mammalian tissues is greatly increased after stimulation of growth by various agents. The biosynthetic pathways of the polyamines in these tissue have been extensively studied (1). However, the catabolic pathways have received very little attention. The degradation of polyamines by various oxidase has been recognized for a number of years as an important catabolic pathway (2). The acetylation of polyamines has also been demonstrated in some tissues (3). Indeed, the monoacetyl derivatives of spermidine and putrescine represent the major urinary excretory products of the polyamines in man (4). Seiler et al. (5) have proposed that the monoacetylation of the polyamines is an important step in their further metabolism.

Hölttä et al. (6) have demonstrated that injection of carbon tetrachloride, a hepatotoxic agent, produces changes in polyamine metabolism similar to those observed in tissue regeneration. In this system, an increase in the conversion of spermidine to putrescine was observed at the time of maximal accumulation of putrescine. Recently, Matsui and Pegg (7) reported that treatment with carbon tetrachloride produced an increase in the ability of liver extracts to catalyze the formation of monoacetylspermidine from spermidine and acetyl-CoA in vitro. This effect was maximal at the time when maximal conversion of spermidine to putrescine was observed in vivo. These results support the hypothesis of Seiler et al. (5) that acetylation of the polyamines precedes their oxidation by polyamine oxidases. The present paper describes the changes in polyamine and acetyl polyamine concentrations in rat tissues after treatment with carbon tetrachloride. Injection of carbon tetrachloride produced a sharp increase in the concentration of N\textsuperscript{1}-acetylspermidine in the liver.
Experimental

Animals and Preparation of Tissue Extracts. Female Sprague-Dawley rats (150-200 g) were used in all experiments. Carbon tetrachloride (0.2mL/100 g) was injected intraperitoneally. Liver, spleen and lungs were homogenized with 3-4 volumes of 0.4 N perchloric acid using a Polytron® tissue grinder. The 20,000 Xg supernatant of the tissue homogenate was stored at -20 °C until analysis. Blood was collected by cardiac puncture. The plasma and buffy coat were separated and plasma stored at 4 °C until analysis. The packed red blood cells were washed three times by resuspending in an equal volume of normal saline and centrifugation. The packed cells were stored at 4 °C until analysis.

Polyamine Analysis. A 100 µL aliquot of the tissue extract or packed red blood cells was used for the determination of the polyamine content as described by Newton et. al. (8). Duplicate samples were analyzed for each tissue extract. The separation of the dansyl polyamines was carried out on a Micropak CN-10 column (250 X 2.5 mm; Varian, Palo Alto, CA, U.S.A.) with a solvent composed of n-hexane/2-propanol (100:3) as solvent A and n-hexane/methylene chloride/2-propanol (10:5:1) as solvent B. The sample was eluted with a programmed solvent gradient using the concave gradient curve number 5. The gradient changed from 100% of solvent B in 15 min. at a flow rate of 3 ml/min. The elution was continued in the isocratic mode with solvent B for additional 5 min. The column eluant corresponding to the dansyl monoacetyl polyamines was collected. The column was allowed to reequilibrate with solvent A for 5 min. before a second sample was injected.

Standard solutions containing various concentrations of putrescine, spermidine, spermine and N1-acetylspermidine were analyzed concomitantly with the tissue samples. The ratios of the peak heights for each of the amines to that of the internal standard were calculated. The correlation graphs were obtained by regression analysis to determine the line of best fit for the data points.

The separation of the dansyl monoacetyl polyamines was carried out on a silica gel column (Ultrasphere-Si, 150 X 4.4 mm, packed with 5 micron silica gel, Altex, Berkeley, CA, U.S.A.). The sample was eluted in the isocratic mode with a solvent composed of chloroform/2-propanol (100:6) with a programmed flow rate using the concave gradient curve number 9. The flow rate changed from 0.5 ml/min. to 1.5 ml/min. in 10 min. and maintained at 1.5 ml/min. for an additional 10 min.

Results

Figure 1 represents the chromatographic separation of the dansyl derivatives of the polyamines in liver extracts obtained from rats after a single intraperitoneal injection of carbon tetrachloride. The monoacetyl polyamines were not present in detectable quantities in extracts from livers of untreated animals (Fig 1A.) A peak with a retention volume equal to that of N1-acetylspermidine was observed in the chromatograph of extracts of livers obtained at 6 hrs. after injection with carbon tetrachloride (Fig 1B). The chromatograph on a silica gel column of the material eluted in peak 4 (Fig 1B) is shown in Fig 1C. A component with the same retention volume as N1-acetylspermidine was the only dansyl polyamine present in detectable quantity. Other peaks seen in Fig. 1C are artifacts which were also observed in chromatographs of authentic samples of N1-acetylspermidine treated in the same manner as tissue extracts.