SYNTHESIS AND ACCUMULATION OF POLYAMINES IN RAT LIVER REGENERATING AFTER TREATMENT WITH CARBON TETRACHLORIDE

E. Hölttä, Riitta Sinervirta and J. Jänne

Department of Medical Chemistry, University of Helsinki, SF-00170 Helsinki 17, Finland

Received July 17,1973

SUMMARY: A single intraperitoneal injection of carbon tetrachloride into rats resulted within 12 hours in a marked accumulation of putrescine in liver with a concomitant decrease in the concentration of spermidine. The accumulation of putrescine apparently was partly due to an immense stimulation of ornithine decarboxylase activity occurring at the same time. However, in addition it was found that during the maximal accumulation of putrescine there was a marked incorporation of radioactivity from labelled spermidine to liver putrescine in vivo. The conversion of spermidine to liver putrescine was hardly detectable in control animals. Besides the treatment with carbon tetrachloride, increased conversion of radioactive spermidine to liver putrescine in vivo also occurred after treatment with growth hormone, after partial hepatectomy and after treatment with thioacetamide, i.e. under circumstances characterized by a stimulation of ornithine decarboxylase activity and an increased accumulation of putrescine.

The formation of putrescine in mammalian tissues is catalyzed by L-ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17),almost exclusively located in the cytosol fraction of various cell types. Besides this straight decarboxylation of L-ornithine no other reaction has been demonstrated as yet to synthesize putrescine in cell free extracts from various mammalian tissues.

The synthesis and accumulation of putrescine in various mammalian tissues is greatly increased after application of certain growth stimuli. Well known examples are regenerating rat liver after partial hepatectomy (1-3), various target organs following appropriate hormone treatment such as rat liver after treatment with growth hormone (4), the ventral prostate of castrated rats following treatment with testosterone (5). The activity of ornithine decarboxylase is also enhanced in cell cultures due to purified epidermal growth factor (6).

Usually the first change in polyamine metabolism after appropriate growth stimulus is an immense increase in the activity of ornithine decarboxylase with a concomitant accumulation of putrescine (7). The accumul-

ation of putrescine is followed by a relatively slow increase in the concentration of spermidine (1,4,7). In 1967 Siimes (8) reported that, in addition to the interconversions between spermidine and spermine, putrescine can be formed from spermidine as judged by the incorporation of radioactivity from intraperitoneally injected ^{14}C-spermidine to liver putrescine. The conversion of exogenous spermidine to putrescine was negligible in normal liver but greatly increased at early liver regeneration (8). However, even during liver regeneration the incorporation of label from spermidine to putrescine was relatively feable and the contribution of this conversion to the accumulation of putrescine remained open.

In the present study we found that injection of carbon tetrachloride, a common hepatotoxic agent, into rats offers an useful model for studying the changes in polyamine metabolism during tissue regeneration. In principal, the changes in the synthesis and accumulation of putrescine, spermidine and spermine were similar or comparable to those found in rat liver regenerating from partial hepatectomy (7,9,10) or following the treatment with growth hormone (4,11).

Shortly after a single injection of CCl_4 there was an immense stimulation in the activity of ornithine decarboxylase and a remarkable accumulation of liver putrescine. At the same time there was a striking transient decrease in the concentration of liver spermidine. Later the concentration of spermidine increased above the control values. When radioactive spermidine was injected intraperitoneally to control animals there was hardly any incorporation of the radioactivity to liver putrescine, however, at the time of maximal putrescine accumulation following carbon tetrachloride injection it appeared that a marked portion of the dose was converted to liver putrescine. In addition to carbon tetrachloride, growth hormone, partial hepatectomy and thioacetamide also caused an enhanced conversion of intraperitoneally injected radioactive spermidine to liver putrescine.

MATERIALS AND METHODS

Female rats of the Wistar strain (weighing 100-130 g) were used in all experiments.

Unlabelled and labelled S-adenosylmethionine were prepared as described earlier (12). Putrescine-1,4-^{14}C (specific radioactivity 17.5 mCi/mmole) and spermidine-1,4-^{14}C (specific activity 10.22 mCi/mmole) were purchased from the New England Nuclear Corporation and purified on Dowex 50-H^+ columns before use (7). Carbon tetrachloride and thioacetamide were obtained from E. Merck AG (Darmstadt, Germany). Carbon tetrachloride was

dissolved in olive oil and thioacetamide in physiological saline and injected intraperitoneally. Porcine growth hormone (Somacton^{R}, Ferring, Malmö, Sweden) was dissolved in 0.04 M acetic acid and injected intraperitoneally.

The activities of ornithine decarboxylase, S-adenosylmethionine decarboxylase and spermidine synthase were assayed using undialyzed cytosol fractions of liver, as described earlier (13-15).

The concentrations of putrescine, spermidine and spermine were measured by the method of Raina and Cohen (16). To ensure that the radioactivity derived from 1,4-^{14}C-spermidine really was in putrescine, the residues after butanol extraction were subjected to ascending paper chromatography with authentic putrescine standard in the following solvent systems: n-butanol-glacial acetic acid-water (50:25:25), n-butanol-glacial acetic acid--pyridine-water (40:10:10:20) and n-propanol-concentrated HCl-water (30:10:10). In each case the only radioactive fraction, in addition to spermidine and spermine, was putrescine.

Protein was measured by the method of Lowry et al. (17).

Partial hepatectomy was performed under light ether anaesthesia by the method of Higgins and Anderson (18).

RESULTS

The effect of a single intraperitoneal injection of carbon tetrachloride (0.2 ml / 100 g body weight) on the concentrations of polyamines and the activities of ornithine and S-adenosylmethionine decarboxylases is illustrated in Fig. 1. The injection of carbon tetrachloride resulted in a rapid accumulation of putrescine in the liver. The concentration of putrescine reached its maximum at 12 h after the injection of the drug. At the same time also the activity of ornithine decarboxylase was maximal. Furthermore, there was also a striking, although transient, drop in the concentration of liver spermidine. Spermidine concentration reached a minimum also at 12 h after carbon tetrachloride whereafter it sharply increased to values markedly exceeding the control values. The concentration of spermine decreased until 24 h after the injection and thereafter slowly increased towards the control values. Surprisingly, at 12 h after the injection of carbon tetrachloride putrescine was the main polyamine in the liver. Its concentration exceeded 0.5 μmoles per g wet wt, which is about ten times the putrescine content in normal rat liver. On the other hand, at the same time the concentration of spermidine was only about one fourth of the normal. As seen in the figure, there was a transient decrease (about 50%) in the activity of S-adenosylmethionine decarboxylase at 4 h after the injection of

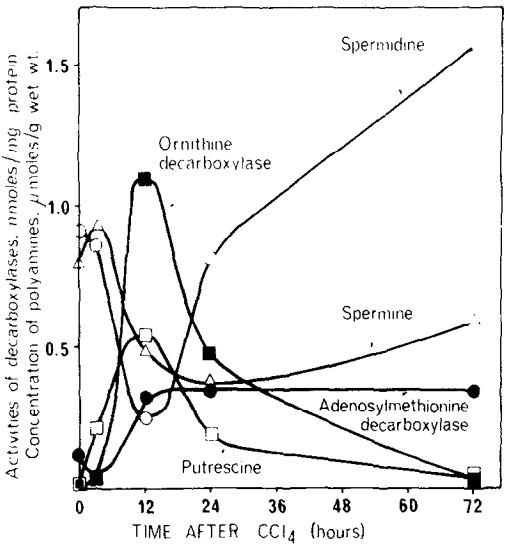

Fig. 1 Effect of carbon tetrachloride on the concentrations of polyamines and on the activities of ornithine and S-adenosylmethionine decarboxylases in rat liver. The animals received a single intraperitoneal injection of 0.2 ml of CCl_4 per 100 g body wt. and were killed at time points indicated. The concentrations of polyamines and the enzyme activities were measured as described in the text. Three animals in each group.

carbon tetrachloride. Thereafter the activity of S-adenosylmethionine decarboxyl ase swiftly increased. At 12 h, the activity was about 3-fold the control values and remained at this higher level for the whole period of observation. The activity of spermidine synthase did not change after carbon tetrachloride treatment.

The rapid decrease in the concentration of spermidine within the first 12 h after the injection of carbon tetrachloride suggested that there might have been an enhanced degradation of this polyamine after the injection of the drug. This was especially plausible in the light of the observations that, at least exogenous spermidine has a rather long half-life in rat liver, i. e. several days (8, 19). In fact, the concomitant accumulation of liver putrescine, though at least partly due to the greatly increased ornithine decarboxylase activity, awaked the idea that spermidine might have been converted to putrescine under these conditions.

As shown in Table 1, there was indeed a drastically increased conversion of intraperitoneally injected radioactive spermidine to putrescine at 12 h after the treatment with carbon tetrachloride. When labelled spermidine was injected into the control animals there was hardly any detectable radioactivity in the putrescine fraction. In fact, the radioactivity of putrescine was about half of that of liver spermidine at 4 h after the injection of radioactive spermidine in carbon

Table 1

Effect of carbon tetrachloride on the conversion of radioactive spermidine to liver putrescine in vivo

Treatment	Activity of ornithine decarboxylase pmoles/mg protein per 30 min	Conversion of ^{14}C-spermidine to putrescine cpm/g liver	Concentration of putrescine nmoles/g liver	spermidine	spermine
Control	50±16	360±53	49±6	810±52	650±11
CCl_4	2 150±880	13 100±5 290	540±220	370±82	510±2

The rats received carbon tetrachloride (0.2 ml per 100 g body wt.) 12 h and ^{14}C-spermidine (1 μC, 0.05 μmoles) 4 h before sacrifice. The concentrations of polyamines and the activity of ornithine decarboxylase were measured as described in the text. The values are means ± standard deviations obtained from three rats in each group.

tetrachloride-treated rats. As also seen in Table 1, there was a marked accumulation of putrescine, decrease in the concentration of spermidine and remarkable stimulation of ornithine decarboxylase activity in the treated animals. It might also be worth of mentioning that the excretion of radioactivity from spermidine into urine remained unchanged during the treatment of carbon tetrachloride (results not shown).

To further evaluate the universality of the conversion of spermidine to putrescine a few other situations, characterized by an accumulation of putrescine and stimulation of ornithine decarboxylase activity, were studied. As seen in Table 2, the conversion of radioactive spermidine to liver putrescine greatly increased after treatment with thioacetamide. In each case an increase in putrescine concentration and the stimulation of the activity of ornithine decarboxyase was also observed. However, as opposed to the carbon tetrachloride treatment, the concentration of spermidine remained practically unchanged.

Attempts to demonstrate the conversion of spermidine to putrescine *in vitro* using liver homogenates from carbon tetrachloride-treated rats have so far been unsuccessful.

DISCUSSION

A single injection of carbon tetrachloride into rat is known to cause

Table 2

Effect of growth hormone, partial hepatectomy and thioacetamide on the conversion of radioactive spermidine to liver putrescine in vivo

Treatment	Activity of ornithine decarboxylase pmoles/ mg protein per 30 min	Conversion of ^{14}C-spermidine to putrescine cpm/g liver	Concentration of putrescine n moles/g liver	spermidine	spermine
Control	37±16	230± 76	51±6	650±54	540±21
Growth hormone	1 560± 410	710±79	150±17	690±42	570±14
Partial hepatectomy	680± 230	2 910±1 620	140±37	710±57	590±33
Thioacetamide	2 110± 280	4 880±1 240	410±81	640±29	510±14

The rats received growth hormone (5 I.U. per 100 g body wt.) 4 h or thioacetamide (15 mg per 100 g body wt.) 24 h before killing, or were partially hepatectomized 12 h before sacrifice. ^{14}C-spermidine (1 μC, 0.05 μmoles) was injected intraperitoneally 3 h before sacrifice. The concentration of polyamines and the activity of ornithine decarboxylase were measured as described in the text. The values are means ± standard deviations obtained from four rats in each group.

an acute liver necrosis followed by a rapid regenerative process (20). The changes in the metabolism of liver polyamines after an injection of carbon tetrachloride were roughly comparable to those found, for instance, after partial hepatectomy or growth hormone treatment. It seems that several different phases can be distinguished in the changes of polyamine metabolism after the poison. The first phase appears to involve a dramatic accumulation of putrescine as a result of increased ornithine decarboxylase activity, a decrease in adenosylmethionine decarboxylase activity (block in the synthesis of spermidine) and obviously also an increased conversion of spermidine to liver putrescine. At the time of maximal putrescine accumulation, the concentration of spermidine decreased to its minimum, possibly due to the increased conversion to putrescine. The second phase, starting after 12 h, involved a rapid rise in the concentration of liver spermidine, steadily elevated levels of S-adenosylmethionine decarboxylase activity and rapid decrease in the concentration of putrescine and in the activity of ornithine decarboxylase. The

concentration of spermine decreased for about two days thereafter starting to increase slowly. It is possible that during this phase spermine is converted to spermidine at increased rate (8).

The finding that during the accumulation of putrescine there is an increased conversion of spermidine to liver putrescine, at least in vivo, is interesting in several respects. Firstly, it might turn out to be a novel reaction in polyamine metabolism catalyzed by enzyme(s) that is inducible under circumstances there is a stimulation of ornithine decarboxylase activity. Secondly, it appears that putrescine might have some specific function, in addition to serve as a precursor for the synthesis of spermidine, during tissue growth. Nothing is known as yet of the enzymic mechanism of the conversion of spermidine to putrescine. It is possible that the pathway used for the synthesis of spermidine, i. e. the transfer of the propylamine group from decarboxylated S-adenosylmethionine (S-methyladenosylhomocysteamine) to putrescine to yield spermidine, thiomethyladenosine and a proton, might be reversible under some special circumstances. This, however, should be coupled with an energy transfer because of the formation of a high energy sulphonium compound (decarboxylated S-adenosylmethionine). It is also fairly possible that in mammalian tissues there are spermidine oxidizing enzyme activities of the type found in Pseudomonas which converts spermidine to putrescine and aminopropylaldehyde in the presence of oxygen (21). In fact, it has been reported that human blood contains aminopropylaldehyde, and that amine oxidase from hog kidney is capable to form putrescine and aminopropylaldehyde from spermidine (22).

The evaluation of the importance of the conversion of spermidine to putrescine as well as attempts to characterize the enzymic pathway(s) are currently started in this laboratory.

ACKNOWLEDGEMENTS

The skilful technical assistance of Mrs. Sirkka Kanerva is gratefully acknowledged.

REFERENCES

1. Jänne, J., Acta Physiol. Scand., Suppl. 300, 1 (1967).
2. Jänne, J., and Raina, A., Acta Chem. Scand., 22, 1349 (1968).
3. Russell, D., and Snyder, S.H., Proc. Natl. Acad. Sci., U.S., 60, 1420 (1968).
4. Jänne, J., Raina, A., and Siimes, M., Biochim. Biophys. Acta, 166, 419 (1968).
5. Pegg, A.E., Lockwood, D.H., and Williams-Ashman, H.G., Biochem. J., 117, 17 (1970).

6. Stastny, M., and Cohen, S., Biochim. Biophys. Acta, 204, 578 (1970).
7. Raina, A., Jänne, J., Hannonen, P., and Hölttä, E., Ann. N.Y. Acad. Sci., 171, 697 (1970).
8. Siimes, M., Acta Physiol. Scand., Suppl. 298, 1 (1967).
9. Hannonen, P., Raina, A., and Jänne, J., Biochim. Biophys. Acta, 273, 84 (1972).
10. Hölttä, E., and Jänne, J., FEBS Letters, 23, 117 (1972).
11. Raina, A., and Hölttä, E., in "Growth and Growth Hormone", Pecile, A., and Müller, E.E. (eds.) Excerpta Medica International Congress Series No. 244. Amsterdam, p. 143 (1972).
12. Pegg, A.E., and Williams-Ashman, H.G., J. Biol. Chem., 244, 682 (1969).
13. Jänne, J., and Williams-Ashman, H.G., J. Biol. Chem., 246, 1725 (1971).
14. Jänne, J., and Williams-Ashman, H.G., Biochem. Biophys. Res. Commun., 42, 222 (1971).
15. Raina, A., and Hannonen, P., FEBS Letters 16, 1 (1971).
16. Raina, A., and Cohen, S.S., Proc. Natl. Acad. Sci., U.S., 55, 1587 (1966).
17. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. Biol. Chem., 193, 265 (1951).
18. Higgins, G.H., and Anderson, R.M., Arch. Pathol., 12, 186 (1931).
19. Russell, D.H., Medina, V.J., and Snyder, S.H., J. Biol. Chem., 245, 6732 (1970).
20. Villela, G.G., Biochem. Pharmacol., 13, 665 (1964).
21. Padmanabhan, R., and Kim, K., Biochem. Biophys. Res. Commun., 19, 1 (1965).
22. Quash, G., and Taylor, D.R., Clin. Chim. Acta, 30, 17 (1970).