Stimulation of Spermidine Synthesis in the Regenerating Rat Liver: Relation to Increased Ornithine Decarboxylase Activity

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In our previous studies on liver regeneration [1,2] we have demonstrated a marked increase in the incorporation of radioactivity from ^{14}C-methionine into spermidine in the rat liver following partial hepatectomy. In contrast, we could not show any stimulation of the synthesis of spermidine from ^{14}C-putrescine during the early period of regeneration.[3,4] This finding was somewhat unexpected, especially in view of the fact that Dykstra and Herbst [5] have reported increased incorporation of ^{3}H-putrescine into spermidine almost immediately after partial hepatectomy. The changes in the rates of incorporation of putrescine and methionine into spermidine during liver regeneration, although at first sight contradictory, become understandable in the light of the observation that there is a marked accumulation of endogenous putrescine in the regenerating liver.[4] We believe that the stimulated synthesis of spermidine, seen as increased incorporation of ^{14}C-methionine into this polyamine, may be largely due to the marked accumulation of putrescine in the liver. The present results suggest that the accumulation of putrescine is due to a sharp increase in the activity of ornithine decarboxylase in the regenerating liver. Further, we shall show that the synthesis of spermidine in the normal rat liver can be increased by administration of large doses of putrescine or methionine.

Materials and methods. Young female rats of the Wistar strain were used. Partial hepatectomy was performed according to Higgins and Anderson.[6] The radioactive isotopes were purchased from the New England Nuclear Corporation (Boston, Mass., U.S.A.). The following isotopes were used: DL-methionine-2-^{14}C, specific activity 1.92 mC/mmole, putrescine-1,4-^{14}C, sp.act. 5.22 mC/mmole, and L-ornithine-U-^{14}C, sp.act. 172 mC/mmole. In the experiments *in vivo* the isotopes were administered intraperitoneally either as such or diluted with unlabelled carrier as indicated.

The polyamines were quantitatively analysed, after paper electrophoretic separation, spermidine and spermine with amido black,[7] putrescine with ninhydrin.[8] The radioactivities of the polyamines were determined from paper strips in a Packard Tri-Carb Liquid Scintillation Spectrometer with an efficiency of about 70 %.

Ornithine decarboxylase activity was assayed from the 100 000 *g* supernatant of the liver homogenate made in 0.25 M sucrose-1 mM 2-mercaptoethanol. The $^{14}CO_2$ released from ornithine-U-^{14}C was trapped into hyamine and assayed for radioactivity as described earlier.[9] After incubation, the radioactivity in the putrescine fraction was analysed from the acidified medium as described above.

Results. Table 1 demonstrates the effect of large amounts of exogenous putrescine on the incorporation of label from ^{14}C-methionine into spermidine. In the normal liver, a dose of 75 μmoles elevated the hepatic concentration of putrescine by about tenfold, while at the same time the synthesis of spermidine from ^{14}C-methionine was considerably stimulated. Further, after partial hepatectomy there was a threefold increase in the concentration of endogenous putrescine and the synthesis of spermidine was increased over fivefold. In partially hepatectomized rats exogenous putrescine caused some additional increase in the synthesis of spermidine. Table 1 further shows that the radioactivities in the acid-soluble and acid-insoluble fractions of the liver, which, to some extent at least, reflect the uptake of radioactive methionine, are significantly higher in partially hepatectomized rats. Obviously, the increased uptake of ^{14}C-methionine contributes to the higher specific activity of spermidine in regenerating livers as compared with the unoperated controls. However, it is probable that the accumulation of putrescine is the main reason for the stimulated synthesis of spermidine in the regenerating liver.

As shown in Table 2, not only exogenous putrescine, but also methionine markedly increased the synthesis of spermidine in the normal rat liver. The increased incorporation of ^{14}C-putrescine into spermidine was also seen with a large "saturating" dose of the isotope. In the regenerating liver exogenous methionine only moderately increased the synthesis of spermidine from ^{14}C-putrescine when the latter was used at

Table 1. Effect of exogenous putrescine on the incorporation of ^{14}C-methionine into spermidine in normal and regenerating rat liver.

One-month-old rats, average weight 65 g, were used. Partial hepatectomy was performed 12 h before sacrifice. Putrescine or an equal volume of saline was administered intraperitoneally 1.5 h before analysis. Five μC of DL-methionine-2-^{14}C was given one hour before sacrifice. Three animals in each group.

Group	Putrescine treatment μmoles	Concentration of putrescine μmoles/g wet wt.	Spermidine synthesis cpm/μmole	Total distribution of radioactivity cpm/g wet wt. Acid-soluble	Acid-insoluble
Normal	—	137	1 690	86 100	237 000
Normal	75	1 220	5 990	83 500	233 000
Regener.	—	355	9 730	157 000	357 000
Regener.	75	1 200	14 700	168 000	344 000

Table 2. Effect of exogenous methionine on the incorporation of ^{14}C-putrescine into spermidine in normal and regenerating rat liver.

One-month-old rats, average weight 81 g, were used. Partial hepatectomy was performed 12 h before sacrifice. DL-Methionine or an equal volume of saline was administered intraperitoneally 1.5 h before analysis. Three μC (0.32 μmole) or 5^1μC (100 μmoles) of putrescine-1,4-^{14}C was given one hour before sacrifice. Three animals in each group.

	Dose of ^{14}C-putrescine μmoles	Dose of DL-methionine μmoles	Spermidine synthesis cpm/μmole	Total distribution of radioactivity cpm/g wet wt. Acid-soluble	Acid-insoluble
A. Normal liver	0.32	—	9 870	99 000	14 600
	0.32	200	30 400	100 000	13 000
	100	—	4 270	165 000	7 150
	100	200	7 840	178 000	7 130
B. Regenerating liver	0.32	—	12 700	128 000	11 100
	0.32	200	11 500	138 000	14 100
	100	—	4 100	177 000	6 890
	100	200	5 080	178 000	7 510

saturating levels. A comparison of the data for the hepatectomized animals and the control group after large doses of both precursors does not indicate any increase, but rather a decrease, in the capacity of the regenerating liver to synthesize spermidine from putrescine.

As reported earlier,[4] partial hepatectomy causes a rapid and marked increase in the synthesis of putrescine from ^{14}C-ornithine *in vivo*. We have now demonstrated the formation of putrescine from ^{14}C-ornithine, obviously through a direct decarboxylation reaction, *in vitro*, using the 100 000 *g*

Table 3. Ornithine decarboxylase activity in normal and regenerating rat liver.

1.5-month-old rats weighing about 105 g were used. Partial hepatectomy was performed 8 h before sacrifice. Ornithine decarboxylase activity *in vitro* was assayed by determining the $^{14}CO_2$ released and ^{14}C-putrescine formed from uniformly labelled L-ornithine. Each 50-ml Erlenmeyer flask with a central well contained 2.0 ml 100 000 *g* supernatant of liver homogenate (six livers were pooled per sample), 200 μmoles of glycylglycine, pH 7.4, 2 μmoles of EDTA, 0.008 μmole of pyridoxal-5-phosphate and 2 μmoles of L-ornithine-U-^{14}C (specific activity 0.5 mC/mmole) in a final volume of 2.23 ml. Incubation time was 60 min. The $^{14}CO_2$ released and ^{14}C-putrescine formed are expressed as μμmoles of product per 60 min and per mg protein.

Group	$^{14}CO_2$ released	^{14}C-putrescine formed
Control	33	35
Regener.	1 260	1 260

supernatant fraction of rat liver homogenate as the source of the enzyme (Table 3). As seen in Table 3, a stimulation of about fortyfold in the formation of putrescine was observed in the supernatant obtained from 8 h-regenerating liver. ^{14}C-putrescine and $^{14}CO_2$ were formed in stoichiometric amounts. The direct decarboxylation of ornithine, rather than the formation of putrescine *via* arginine and agmatine, is further supported by the fact that two enzyme systems catalysing the synthesis of citrulline are located in the mitochondrial fraction.[10] We were not able to demonstrate any formation of radioactive agmatine in this system, either. We have also observed that ornithine decarboxylase activity in the rat liver is increased after growth hormone treatment (unpublished observation). It may be mentioned that ornithine decarboxylase activity has also been demonstrated in the supernatant fraction of rat ventral prostate and that this activity is increased after administration of testosterone (Pegg, personal communication).

In conclusion, our results seem to indicate that the increased production of putrescine in the regenerating liver, leading to an increased tissue concentration of this compound, is the main cause of the increased synthesis and accumulation of spermidine. However, our data do not preclude the possibility that enzymes catalysing the formation and transfer of the propylamine group from methionine are also stimulated. The mechanism of stimulation of spermidine synthesis by exogenous putrescine or increased endogenous putrescine could be explained by the recent observation of Pegg and Williams-Ashman,[11] who showed that putrescine markedly stimulates S-adenosylmethionine decarboxylase from rat liver and prostate *in vitro*, spermidine being the ultimate reaction product.

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