ORNITHINE DECARBOXYLASE ACTIVITY AND THE ACCUMULATION OF PUTRESCINE AT EARLY STAGES OF LIVER REGENERATION

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1. Introduction

The intensive stimulation of ornithine decarboxylase (EC 4.1.1.17, L-ornithine carboxylase) activity after partial hepatectomy of the rat belongs to the earliest biochemical changes in the regenerating liver remnant [1–4]. Enhanced ornithine decarboxylase activity results in a concomitant accumulation of liver putrescine [1, 5, 6] which in turn is supposed to stimulate the synthesis of spermidine [1] probably by activating the decarboxylation of S-adenosyl-L-methionine (Ado-met), the reaction needed for the synthesis of spermidine [7].

The activity of ornithine decarboxylase is almost maximally stimulated as early as at about 4 hr after partial hepatectomy and remains elevated for several days [2, 6]. It has been reported that hypophysectomy, but not adrenalectomy, castration, or thyroidectomy, considerably delays, although does not prevent, the stimulation of ornithine decarboxylase after partial hepatectomy [8]. This latter observation might indicate the involvement of some humoral factor(s) in the regulation of putrescine synthesis in the regenerating rat liver.

In the present communication we have studied more closely the synthesis and accumulation of putrescine at early stages of liver regeneration. The results of several series of rats revealed that during the first day of regeneration the stimulation of ornithine decarboxylase appeared to occur in two phases, the first peak of the enzyme activity invariably occurring at 4 hr after the operation independently of the age of the animal. The stimulation of ornithine decarboxylase was closely followed by an accumulation of putrescine. Evidence is also presented indicating that the accumulation of putrescine plays a critical role in regulating the activity of putrescine-activated Ado-met decarboxylase and thence the synthesis of spermidine.

2. Materials and methods

Female rats of the Wistar or Sprague-Dawley strain were used in all experiments. Partial hepatectomy was performed by the method of Higgins and Anderson [9]. The animals were fasted for 6 hr before the sacrifice.

DL-ornithine-1-^{14}C (specific radioactivity 37 mCi/mmole) was purchased from the Radiochemical Centre (Amersham) and treated before use as described earlier [10, 11]. Unlabelled or labelled Ado-met (Ado-met-^{14}COOH and Ado-met-2-^{14}C) was prepared enzymatically and purified according to Pegg and Williams-Ashman [7]. Putrescine-1,4-^{14}C (specific radioactivity 17.5 mCi/mmole) was purchased from the New England Nuclear Corporation and purified before use on a Dowex 50-H^+ column [6].

The cytosol fraction of rat liver, homogenized with two volumes of 0.25 M sucrose–1 mM 2-mercaptoethanol–0.3 mM EDTA (or 100 mM KCl–0.3 mM EDTA in the lyophilization experiments), was prepared by centrifuging the homogenates for 60 min at 100,000 g_{max}. The 100,000 g supernatant fractions were used as the source of enzymes as such, desalted by passing them through a Sephadex G-25 column, or lyophilized at −70° before use. The recovery of the enzyme activities or of the polyamines after the lyophilization was essentially 100%.

The activity of ornithine decarboxylase was assayed in the presence of 2 mM L-ornithine-1-^{14}C [10, 11]

and that of Ado-met decarboxylase in the presence of 0.2 mM Ado-met-^{14}COOH with or without 2.5 mM putrescine [12, 13]. Spermidine synthase activity and the synthesis of spermidine from Ado-met-2-^{14}C was assayed as described earlier [12–14].

The concentration of putrescine was measured by the method of Raina and Cohen [15] and the protein measurements were made by the method of Lowry et al. [16].

3. Results

Fig. 1A shows the stimulation of ornithine decarboxylase activity during the first day after partial hepatectomy of rats at different ages. The activity rose rapidly after the operation and reached a definite peak (or shoulder) as early as at 4 hr postoperatively independently of the age of the animal (sham operation 4 hr before the sacrifice produced only about a 10% increase over the basal level). Thereafter the activity decreased to rise again after a lag period apparently dependent on the age of the animal. In young animals the second peak occurred almost immediately after the first peak (curve 1 in fig. 1A) whereas in older animals there was a delay of several hours before the second peak. The delay in the occurrence of the second phase was roughly related to the age of the animal (curves 2–4 in fig. 1A).

Two enzymes are needed for the synthesis of spermidine from putrescine and Ado-met: i) Ado-met decarboxylase and ii) spermidine synthase. Based on the specific activities of these enzymes in liver cytosol fraction the decarboxylation of Ado-met is the rate limiting reaction in the synthesis of spermidine [17]. Fig. 1 shows the corresponding changes of Ado-met decarboxylase activity, as assayed in the presence of saturating concentrations of putrescine, after partial hepatectomy. With one exception, there was a decrease of about 50% in the activity of putrescine-activated Ado-met decarboxylase between 2 and 8 hr after partial hepatectomy. At 24 hr postoperatively Ado-met decarboxylase activity was already at or over the control level in all groups of animals. The changes of Ado-met decarboxylase activity were rather similar in all groups with no definite correlation to the age of the animal. The activity of spermidine synthase (not shown) remained essentially unchanged during the first 24 hr after partial hepatectomy.

It has been reported that there is a close correlation

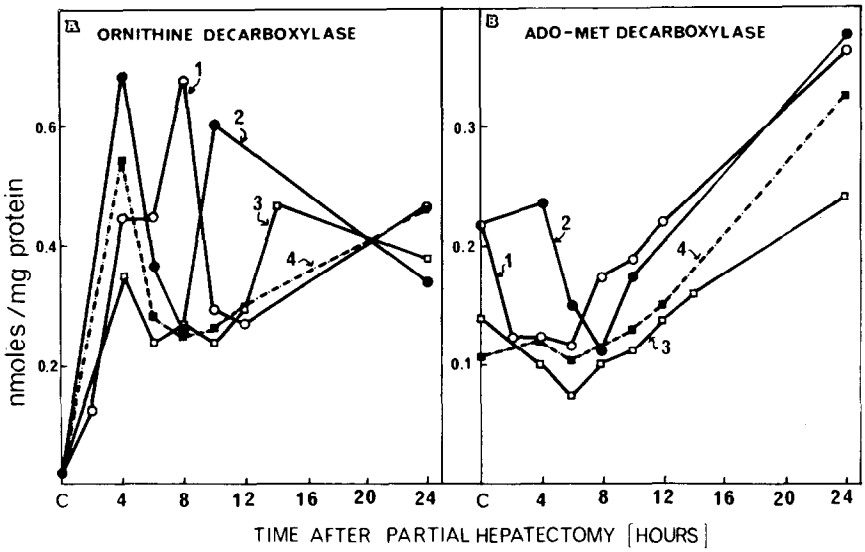

Fig. 1. Stimulation of ornithine decarboxylase and Ado-met decarboxylase activities after partial hepatectomy in rats of different ages. Ornithine decarboxylase activity (A) was assayed under standard incubation conditions as described in the text. Ado-met decarboxylase activity (B) was assayed in the presence of 2.5 mM putrescine from the same animals. Each value represents a pooled sample from 3 to 4 rats. The activities are expressed as nmoles of $^{14}CO_2$ released per mg protein per 30 min. The weights of the animals were as follows: 1, 105–120 g; 2, 140–160 g; 3, 161–180 g; 4, 181–200 g.

Table 1
Ornithine decarboxylase activity, putrescine accumulation and Ado-met decarboxylase activity in regenerating rat liver after partial hepatectomy.

Time after partial hepatectomy (hr)	Ornithine decarboxylase activity (pmoles of $^{14}CO_2$ per mg protein per 30 min)	Putrescine concentration (nmoles/g liver wet wt)	Ado-met decarboxylase activity (pmoles of $^{14}CO_2$ per mg protein per 30 min)		
			−putrescine (a)	+putrescine (b)	b/a
Control	17	34	22	218	9.9
4	687	150	97	238	2.5
6	363	117	63	151	2.4
8	256	93	46	114	2.5
10	607	293	111	171	1.5
24	340	75	122	378	3.1
48	356	72	160	438	2.7

Female rats (140–160 g) were partially hepatectomized at times indicated. Ornithine decarboxylase and Ado-met decarboxylase activities were assayed as described in the text using undialysed cytosol fractions as the source of the enzymes. Each value is obtained from a pooled sample, 3 livers in each.

between ornithine decarboxylase activity and the accumulation of putrescine in the regenerating rat liver [6]. Table 1 lists the activity of ornithine decarboxylase, the concentration of putrescine in liver homogenate as well as the activity of Ado-met decarboxylase assayed in the absence or presence of exogenous putrescine using undialyzed cytosol fraction as the source of the enzyme. As shown, the accumulation of putrescine followed very closely the activity of ornithine decarboxylase in regenerating liver. Ado-met decarboxylase activity, when assayed in the absence of any added putrescine (the only putrescine in the incubation mixture was that present in the undialyzed enzyme preparation) also paralleled closely to the activity of ornithine decarboxylase and putrescine accumulation at the early stages of liver regeneration. The stimulation achieved by the addition of putrescine (2.5 mM) to the reaction mixture was about 10-fold in the control liver but declined to only 1.5-fold at 10 hr postoperatively when the accumulation of putrescine in the liver was maximal. It must not be forgotten that under the assay conditions used the dilution of the tissue putrescine concentration was more than 5-fold in the final incubation mixture. After passing the cytosol fractions through Sephadex G-25 columns the magnitude of the fluctuations in Ado-met decarboxylase activity in the absence of exogenous putrescine decreased but did not disappear.

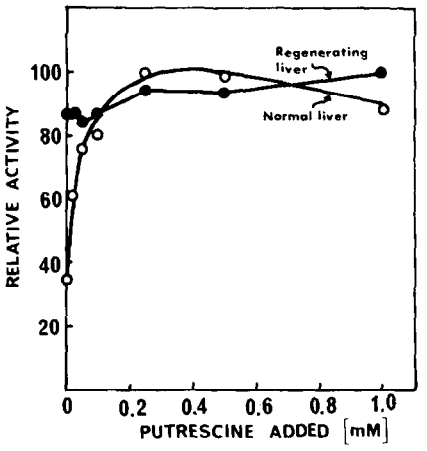

Fig. 2. Effect of exogenous putrescine on Ado-met decarboxylase activity from normal and regenerating rat liver. Liver cytosol fractions from 3 (control) or 5 (regenerating) livers were prepared as described under Materials and methods. After lyophilization the residues were dissolved in a small volume of water so that the actual concentration of the cytosol fractions in the final incubation mixture corresponded to that in the liver tissue. Ado-met decarboxylase activity was assayed under standard incubation conditions except employing an incubation time of 10 min. The concentration of exogenous putrescine was varied as indicated in the figure.

This might indicate that Ado-met decarboxylase binds endogenous putrescine very tightly. Based on the data presented in table 1 it appears that in the regenerating liver at the time of maximal putrescine accumulation the endogenous putrescine concentration was sufficient to saturate Ado-met decarboxylase reaction. Fig. 2 attempts to prove this directly. In this experiment the cytosol fractions from both normal and regenerating liver were lyophilized and the residues were dissolved in a specified volume of water so that in the final incubation mixture the concentration of the enzyme solution corresponded to that in the living cell. In other words, the concentration of endogenous putrescine present in the final reaction mixture was calculated to be the same as in the cytosol fraction *in vivo*. As shown in the figure, even under these conditions the control liver was not saturated by putrescine since an addition of putrescine up to 1 mM resulted in about 3-fold stimulation of the decarboxylation of Ado-met. On the contrary, the cytosol fraction prepared from 12 hr regenerating liver appeared to be practically saturated by endogenous putrescine. Extensive dialysis of the cytosol fraction from regenerating liver restored its response to putrescine. In a comparable experiment (not tabulated), the synthesis of spermidine from Ado-met-2-^{14}C was followed in the absence or presence of exogenous putrescine. Again the regenerating liver was fully saturated by endogenous putrescine whereas the addition of putrescine (1 mM) to control liver preparations resulted in a many-fold increase in the synthesis of spermidine from Ado-met-2-^{14}C. Using the same technique it was also observed that the synthesis of spermidine, as measured by the incorporation of ^{14}C-putrescine into spermidine, was not saturated by endogenous Ado-met, either. However, the degree of saturation was equal both in control and regenerating liver preparations. This might indicate that the concentration of endogenous Ado-met remains unchanged during the regeneration, at least as far as the saturation of spermidine synthesis is concerned.

4. Discussion

In the majority of previous reports on the stimulation of ornithine decarboxylase activity in rat liver after partial hepatectomy the analyses have been made at rather long time intervals, i.e. at 4, 8, 12, 24 hr etc. after the surgery. However, the apparent half-life of ornithine decarboxylase in rat liver is extremely short, 10 to 20 min [18]. Thus the changes in ornithine decarboxylase activity are likely to take place very rapidly. The much closer analysis of the present study revealed that the activity of ornithine decarboxylase in the regenerating liver indeed seems to fluctuate in a manner suggesting the involvement of at least two different phases. It has been shown before that partial hepatectomy of previously hypophysectomized rats caused a markedly delayed response in ornithine decarboxylase activity [8]. The removal of any other endocrine gland prior to the partial hepatectomy, i.e. adrenalectomy, castration, or thyroidectomy, did not alter the response of ornithine decarboxylase after partial hepatectomy as compared with intact rats [8]. Growth hormone is known to greatly enhance ornithine decarboxylase activity in the liver of normal rats and the stimulation occurs at about 4 hr after a single injection of the hormone [19]. Interestingly, the stimulation evoked by growth hormone seems to always occur at the same time independently of the age of the animal [20]. It is possible that the first peak of ornithine decarboxylase activity after partial hepatectomy observed in this study is due to the release of endogenous growth hormone. The second phase might be the "regenerative response" of the liver tissue itself. Further work is going on in this laboratory to solve the role of growth hormone secretion in the regulation of tissue putrescine synthesis.

It is obvious that the activation of ornithine decarboxylase and the accumulation of putrescine are of major importance in the regulation of spermidine synthesis in regenerating rat liver. Ado-met decarboxylase, most probably the rate limiting enzyme in the synthesis of spermidine in the liver, is intensively stimulated by putrescine in all rat tissue studied [7] even after its resolution from spermidine synthase activity [12, 13, 17]. As seen in the present results it appears that in the regenerating liver the concentration of putrescine is sufficient to cause maximal stimulation of Ado-met decarboxylase and also of the synthesis of spermidine from Ado-met and putrescine. The accumulation of putrescine after partial hepatectomy occurred far before any stimulation of the activities of Ado-met decarboxylase or spermidine synthase could be seen. This probably indicates that the trigger for

increased synthesis of spermidine in the regenerating rat liver is the activation of ornithine decarboxylase.

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