REGULATION OF ORNITHINE DECARBOXYLASE ACTIVITY BY PUTRESCINE AND SPERMIDINE IN RAT LIVER

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Received September 30,1974

SUMMARY: The marked enhancement of the activity of ornithine decarboxylase (EC 4.1.1.17) in rat liver at 4 h following partial hepatectomy or the treatment with growth hormone could be almost completely prevented by intraperitoneal administration of putrescine. A single injection of putrescine to partially hepatectomized rats caused a remarkably rapid decline in the activity of liver ornithine decarboxylase with an apparent half-life of only 30 min, which is almost as rapid as the decay of the enzyme activity after the administration of inhibitors of protein synthesis. Under similar conditions putrescine did not have any inhibitory effect on the activity of adenosylmethionine decarboxylase (EC 4.1.1.50) or tyrosine aminotransferase (EC 2.6.1.5). Spermidine given at the time of partial hepatectomy or 2 h later also markedly inhibited ornithine decarboxylase activity at 4 h after the operation and, in addition, also caused a slight inhibition of the activity of adenosylmethionine decarboxylase.

Partial hepatectomy of the rat causes an immense stimulation of ornithine decarboxylase activity in the regenerating liver remnant (1,2,3). The stimulation of ornithine decarboxylase activity as well as the accumulation of liver putrescine after partial hepatectomy appears to occur in several phases, the time course of which is somewhat dependent on the age of the animal (4,5). It has been suggested that different types of stimuli are involved in the response of ornithine decarboxylase to partial hepatectomy (4).

Based on the data published until now, it appears that the activity of mammalian ornithine decarboxylase is regulated through changes in the rate of the synthesis (and/or degradation) of the enzyme protein rather than by any low molecular weight effectors (6,7,8). Putrescine, a very feable product inhibitor of the enzyme (6), has been shown to inhibit ornithine decarboxylase activity in stimulated human lymphocytes in micromolar concentrations (9), i. e. in concentrations that are several orders of magnitude lower than those required for a direct inhibition of the enzyme activity in vitro.

In a further attempt to elucidate the mechanism(s) of the rapid fluctuations of the activity of liver ornithine decarboxylase after various growth stimuli, we have studied the effect of intraperitoneally administered

putrescine or spermidine on the activity of ornithine decarboxylase in regenerating rat liver. According to the present results, it appears that tissue concentrations of putrescine which do not markedly exceed those found, for instance, in regenerating rat liver can cause a marked decrease in ornithine decarboxylase activity. The effect of putrescine, and also that of spermidine, seems to be specific for ornithine decarboxylase, and might involve both transcriptional and post-transcriptional control of the enzyme synthesis.

MATERIAL AND METHODS

Female rats of the Wistar strain weighing about 100 g were used in all experiments.

Partial hepatectomy was performed by the method of Higgins and Anderson (10). Neutralized putrescine · 2 HCl and spermidine · 3 HCl were administered intraperitoneally.

Ornithine decarboxylase (6) and adenosylmethionine decarboxylase activities (11,12) were assayed as described earlier using undialyzed cytosol fraction as the source of the enzyme. The activity of tyrosine aminotransferase was assayed by the method of Diamondstone (13). Protein was measured by the method of Lowry et al. (14), and polyamines as described by Raina and Cohen (15).

RESULTS

Fig. 1 illustrates the effect of a single injection of putrescine, given at different times before and after partial hepatectomy or treatment with porcine growth hormone, on the activities of ornithine decarboxylase, adenosyl methionine decarboxylase and tyrosine aminotransferase. Partial hepatectomy (Fig. 1 A) caused an intensive stimulation (about 150-fold) of the activity of liver ornithine decarboxylase at 4 h after the operation. Administration of putrescine one hour before the operation did not have any effect on the enzyme activity at 4 h postoperatively. However, putrescine given at the time of the operation partially prevented the stimulation of the enzyme activity. If administered later, at 30 or 60 min after the operation, the inhibitory effect of putrescine on the ornithine decarboxylase activity appeared to diminish, but an injection of putrescine at 2 h after the operation, i.e. 2 h before death of the animals, completely abolished the stimulation of ornithine decarboxylase activity. The dose of putrescine, used in this experiment (Fig. 1 A) caused a transient elevation in liver putrescine that was roughly comparable to that occurring after partial hepatectomy alone.

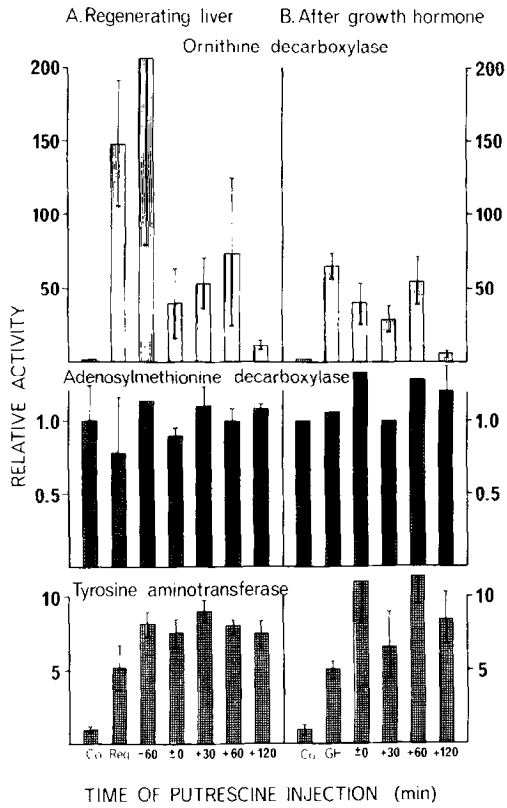

Fig. 1. Effect of putrescine, administered at various times, on the activities of ornithine decarboxylase, adenosylmethionine decarboxylase and tyrosine aminotransferase in relation to partial hepatectomy or treatment with growth hormone.

A: Partially hepatectomized rats. B: Growth hormone-treated rats. The enzyme activities were assayed 4 h following the operation or hormone injection. There were three animals in each group. The vertical lines represent the standard deviation of the means. Co; intact animals, Reg; partially hepatectomized animals, GH; growth hormone-treated animals.

The concentration of putrescine in liver tended to return to normal values in less than 2 h. It should be noted that the maximal tissue concentration of putrescine did not exceed 1 mM. Under the conditions used for the assay of the activity of ornithine decarboxylase (in the presence of 2 mM L-ornithine), 10 mM putrescine in the assay system decreased the enzyme activity only by 35 %.

It is possible that an injection of putrescine could prevent the increase in the activity of ornithine decarboxylase at 4 h after partial hepatectomy at different sites; the first being at the time of the operation and the second close to the sacrifice of the animals. The activities of adenosyl methionine decarboxylase and tyrosine aminotransferase, used as "reference" enzymes

because of their short half-lives (16,17), were practically unchanged after the treatment with putrescine, as shown in Fig. 1 A. Similar inhibition of the activity of ornithine decarboxylase by putrescine could also be seen in growth hormone treated rats, as illustrated in Fig. 1 B. Putrescine given at the time of the hormone injection caused a slight decrease in the enzyme activity at 4 h after growth hormone. A more clear decrease in the enzyme activity was found when putrescine was administered 30 min after growth hormone (Fig. 1 B). The decreases, however, were not as marked as seen in the regenerating liver. If putrescine was injected 2 h after growth hormone, i. e. 2 h before the death of the animals, the stimulation of ornithine decarboxylase activity was completely abolished. There were practically no changes whatsoever in the activity of adenosylmethionine decarboxylase either by growth hormone, putrescine or the combination of both. If anything, putrescine appeared to stimulate the activity of tyrosine aminotransferase when given after growth hormone (Fig. 1 B).

It looked possible, based on the very short half-life of ornithine decarboxylase as well as on the rather transient elevation of tissue putrescine concentration, that the total prevention of the increase in ornithine decarboxylase activity by the administration of putrescine shortly before the sacrifice of the animal, might have been due to a post-transcriptional action of the diamine on the synthesis of the enzyme.

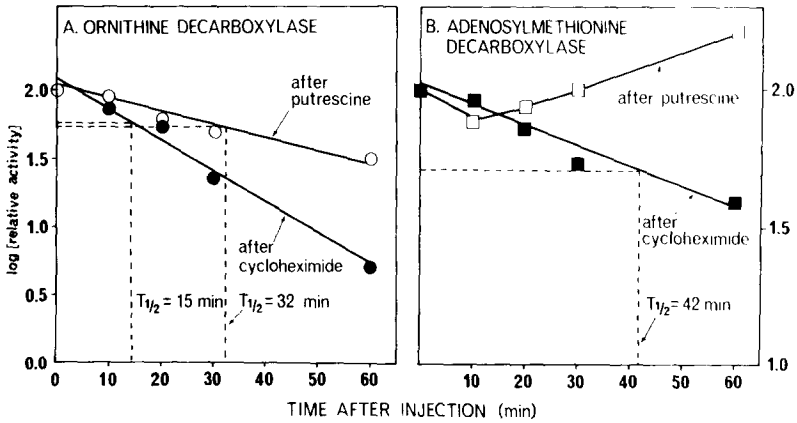

Fig. 2. Effect of intraperitoneal administration of putrescine or cycloheximide on the activities of ornithine decarboxylase and adenosylmethionine decarboxylase in regenerating rat liver.

A: Ornithine decarboxylase B: Adenosylmethionine decarboxylase

Rats were partially hepatectomized 24 h before the administration of putrescine (75 μmoles/100 g body weight) or cycloheximide (0.8 mg/100 g body weight). The enzyme activities were assayed as described in the text at time points indicated. The lines were plotted by the least squares method.

TABLE 1. Effect of injections of putrescine and cycloheximide on the activity of ornithine decarboxylase, adenosylmethionine decarboxylase and tyrosine aminotransferase in regenerating rat liver. The animals were partially hepatectomized 24 hours before sacrifice and received injections of putrescine (75 μmoles) or cycloheximide (0.8 mg) 120 min before death. The enzyme activities were assayed as described in the text. Three animals in each group. The activities are expressed as nmoles product formed per mg protein per 30 min (± S.D.).

Time after injection (min)	Ornithine decarboxylase activity		Adenosylmethionine decarboxylase activity		Tyrosine aminotransferase activity	
	Putrescine	Cycloheximide	Putrescine	Cycloheximide	Putrescine	Cycloheximide
Control	0.720 ± 0.230 (100%)		0.179 ± 0.027 (100%)		405 ± 150 (100%)	
120	0.048 ± 0.033 (6%)	0.001 ± 0.000 (0.1%)	0.179 ± 0.008 (104%)	0.015 ± 0.004 (8%)	756 ± 342 (186%)	177 ± 39 (43%)

When partially hepatectomized rats were treated with cycloheximide, the activity of ornithine decarboxylase rapidly decreased, as shown in Fig. 2 A, with an apparent half-life of 15 min in this particular experiment. In the same figure one can also see that an injection of putrescine also resulted in a swift decline in the enzyme activity. The half-life of ornithine decarboxylase after putrescine was about 30 min (Fig. 2 A). The decay of the activity of adenosylmethionine decarboxylase after cycloheximide was about 40 min, whereas the administration of putrescine rather increased than decreased the enzyme activity (Fig. 2 B).

As seen in Table 1, the activity of ornithine decarboxylase decreased by more than 90% at 2 h after the injection of putrescine, whereas there was no change in the activity of adenosylmethionine decarboxylase, and a stimulation in the activity of tyrosine aminotransferase. In another experiment it was shown that putrescine did not change the decay of adenosylmethionine decarboxylase or tyrosine aminotransferase activity after cycloheximide. These experiments obviously show that the effect of putrescine on the activity of ornithine decarboxylase is very rapid, almost comparable to that of cycloheximide, and the effect also appears to be specific for ornithine decarboxylase.

In addition to putrescine, an injection of spermidine at the time of partial hepatectomy or 2 h later resulted in a decrease in the activity of ornithine decarboxylase at 4 h after the operation. Furthermore, spermidine might have had a slight inhibitory effect on the activity of adenosylmethionine decarboxylase when given at the time of partial hepatectomy (Table 2), whereas the activity of tyrosine aminotransferase increased by the injection of spermidine.

DISCUSSION

The mechanism of the stimulation of ornithine decarboxylase after the application of a variety of different stimuli is not known. There is, however, an increasing body of indirect (18,19,20,21) and also direct (8) evidence supporting the idea that the stimulation of ornithine decarboxylase activity is attributable to *de novo* synthesis of the enzyme, and is not due to an activation of pre-existing enzyme molecules. This is in contrast to the ornithine decarboxylase activity from *Escherichia coli* which is profoundly regulated by guanosine nucleotides including the polyphosphates (22,23).

The very central role of putrescine in the regulation of polyamine synthesis in mammalian tissues has been realized for some time. Putrescine, in addition to being a weak product inhibitor of ornithine decarboxylase (6,24), greatly and specificially enhances the activity of adenosylmethionine decarboxy

TABLE 2. Effect of spermidine on ornithine decarboxylase, adenosylmethionine decarboxylase and tyrosine aminotransferase activities in regenerating rat liver. The rats were partially hepatectomized 4 hours before sacrifice and the injections of spermidine given at time points indicated. Three animals in each group. The activities are expressed as nmoles product formed per mg protein per 30 min (± S.D.). Unoperated control; rats with intact liver not receiving spermidine. Regenerating control; partially hepatectomized rats not receiving spermidine.

Time of spermidine injection (min after operation)	Ornithine decarboxylase activity	Adenosyl-methionine decarboxylase activity	Tyrosine aminotransferase activity
Unoperated control	0.010 ± 0.005	0.062 ± 0.022	92 ± 24
Regenerating control	0.713 ± 0.112	0.045 ± 0.012	741 ± 105
± 0	0.149 ± 0.073	0.031 ± 0.007	927 ± 63
+ 120	0.166 ± 0.105	0.041 ± 0.029	909 ± 99

ase (11, 25, 26) and, on the other hand, inhibits the spermine synthase reaction *in vitro* (27, 28). Furthermore, it has been reported that the activity of ornithine decarboxylase in stimulated lymphocytes can be inhibited by minute concentrations of putrescine or spermidine (9). Inhibition of ornithine decarboxylase activity in regenerating rat liver *in vivo* by relatively high doses of putrescine has been also reported by Schrock *et al.* (20). As seen in the present study, moderate elevations of the putrescine concentration in rat liver by an intraperitoneal injection of the diamine also markedly inhibited the stimulation of the ornithine decarboxylase activity following partial hepatectomy or the treatment with growth hormone. The peculiar time dependence of the inhibition of ornithine decarboxylase by putrescine (in relation to the operation) suggests that putrescine (and/or spermidine) might control the enzyme activity at both transcriptional and post-transcriptional level. Regulation through the latter mechanism is supported by the finding that an injection of putrescine to partially hepatectomized rats led to a swift decay of the enzyme activity which was almost comparable to that observed after the injection of cycloheximide.

In any case, it appears that the activity of ornithine decarboxylase in rat liver conceivably can be regulated by changes in tissue putrescine concentrations through a mechanism that is not due to a direct inhibition of the enzyme activity by the product.

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