REGULATION OF ORNITHINE DECARBOXYLASE BY DIAMINES IN REGENERATING RAT LIVER

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Received 11 October 1976
Revised version received 22 November 1976

1. Introduction

Unlike the inducible enzymes in bacteria, many of the mammalian enzymes, which regulate the flow of substrates through metabolic pathways rapidly changing their activity in response to a variety of stimuli, are labile proteins with short molecular half-lives [1]. Ornithine decarboxylase (EC 4.1.1.17), rate controlling enzyme in putrescine, spermidine and spermine synthesis seems an example of such enzymes, with a half-life of about 10 min in rat liver [2]. The activity of mammalian ornithine decarboxylase is probably not regulated by any low molecular weight physiological effectors [3,4], its activity controlled mainly by changes in the synthesis and/or degradation of the enzyme protein [4]. This is supported by indirect evidence obtained with inhibitors of nucleic acid and protein synthesis [4] as well as demonstrations that stimulation/decay of ornithine decarboxylase activity accompanies similar change in amount of immunoreactive enzyme protein [5,6]. By analogy with the inducible enzymes in prokaryotes, mammalian ornithine decarboxylase could be controlled with a repression type mechanism by its immediate product, putrescine, or by spermidine and other related amines, both in cell culture [7–9] and rat liver in vivo [10–13]. The inhibitory effect exerted by the amines on the accumulation of the enzyme is rather complex, probably involving both transcriptional and post-transcriptional sites of action [9,11,14].

Here we extend our studies on control of ornithine decarboxylase in regenerating rat liver, by investigating the mode of 1,3-diaminopropane action, a potent repressing agent of mammalian ornithine decarboxylase [12–14]. It appears that the control of ornithine decarboxylase occurs on at least two levels of gene expression. During the very early periods of liver regeneration the effect of diaminopropane on the enzyme resembled that of α-amanitin, i.e., the compound apparently prevented gene activation evoked by partial hepatectomy. The administration of the diamine during later periods of liver regeneration resulted in a swift decay of enzyme activity, with an apparent half-life of 12 min, compared with half-life of 14 min following injection of cycloheximide. The decay rate of ornithine decarboxylase induced by diaminopropane, was uninfluenced by simultaneous injection of cycloheximide or α-amanitin.

2. Materials and methods

Wistar or Sprague-Dawley male rats, 75–100 g, were used in all experiments. Partial hepatectomy was performed under ether anaesthesia by the method of Higgins and Anderson [15].

D,L-[1-^{14}C]Ornithine (specific radioactivity 59 mCi/mmol) was purchased from the Radiochemical Centre (Amersham). *S*-Adenosyl-L-[1-^{14}C]methionine was prepared enzymically from L-[1-^{14}C]methionine (Radiochemical Centre) as described earlier [16]. α-Amanitin was purchased from Boehringer (Mannheim) and cycloheximide (Acti-Dione) from the Nutritional Biochemicals Corp. (Cleveland). The activities of

ornithine decarboxylase [3], adenosylmethionine decarboxylase (EC 4.1.1.50) [17] and tyrosine aminotransferase (EC 2.6.1.5) [18] were measured by methods described earlier.

Diaminopropane, putrescine, spermidine and spermine were measured from tissue extracts after alkaline–butanol extraction and paper electrophoresis [19]. The conditions for the electrophoretic separation of diaminopropane and putrescine are described elsewhere [13,19]. Protein was measured by the method of Lowry et al. [20] using bovine serum albumin as the standard.

3. Results

As illustrated in fig. 1A, the stimulation of ornithine decarboxylase activity 4 h after partial hepatectomy could be prevented by an injection of 1,3-diaminopropane, cycloheximide or α-amanitin, given at the time of operation (time point 0). The enhancement in ornithine decarboxylase activity could still be prevented with diaminopropane or cycloheximide given 3 h after the operation, i.e., 1 h before the death of the animals. However, an injection of α-amanitin at this time was without any inhibitory effect (fig.1A).

The activity of adenosylmethionine decarboxylase, required for the synthesis of spermidine and spermine, was not stimulated in response to partial hepatectomy, nor did diaminopropane depress the enzyme activity when given at the time of the operation (fig.1B). Cycloheximide and α-amanitin partially inhibited the enzyme activity when administered at the time of partial hepatectomy (fig.1B). Adenosylmethionine decarboxylase activity was mildly inhibited by an injection of cycloheximide or diaminopropane, 3 h after the operation (fig.1B). An injection of α-amanitin, 3 h after the operation did not inhibit adenosylmethionine decarboxylase activity. The behavior of tyrosine aminotransferase, reference enzyme with short half-life, closely resembled that of adenosylmethionine decarboxylase, except diaminopropane stimulated enzyme activity when given at the time of operation (fig.1C).

As shown in table 1, changes in liver putrescine content after each treatment followed the activity of ornithine decarboxylase, whereas the concentrations of spermidine and spermine showed insignificant changes. The hepatic concentration of 1,3-diaminopropane reached a maximum, about 1.7 mM, 1 h after the injection and appeared to be metabolized quickly (table 1).

When the experiment in fig.1 and table 1 was repeated 24–28 h after the operation, the effect of

Table 1
Effect of 1,3-diaminopropane, cycloheximide and α-amanitin on the concentrations of polyamines in 4 h regenerating rat liver

Treatment	Time of injection (h after operation)	Putrescine (nmoles/g ± S.D.)	Spermidine (nmoles/g ± S.D.)	Spermine (nmoles/g ± S.D.)	Diaminopropane (nmoles/g ± S.D.)
Normal liver	–	73 ± 11[a]	1070 ± 94	678 ± 48	–
4 h regenerating liver	–	199 ± 15	921 ± 113	614 ± 62	–
Diaminopropane	0	109 ± 11[a]	830 ± 35	612 ± 33	600 ± 164
Cycloheximide	0	90 ± 9[a]	994 ± 122	623 ± 51	–
α-Amanitin	0	87 ± 26[a]	850 ± 25	621 ± 91	
Diaminopropane	3	82 ± 8[a]	789 ± 102	560 ± 36	1670 ± 246
Cycloheximide	3	122 ± 12[a]	804 ± 75	608 ± 41	–
α-Amanitin	3	194 ± 64	858 ± 118	571 ± 43	–

[a] $p < 0.001$

Significant differences as compared with the 4 h regenerating liver.
For experimental details see fig.1.

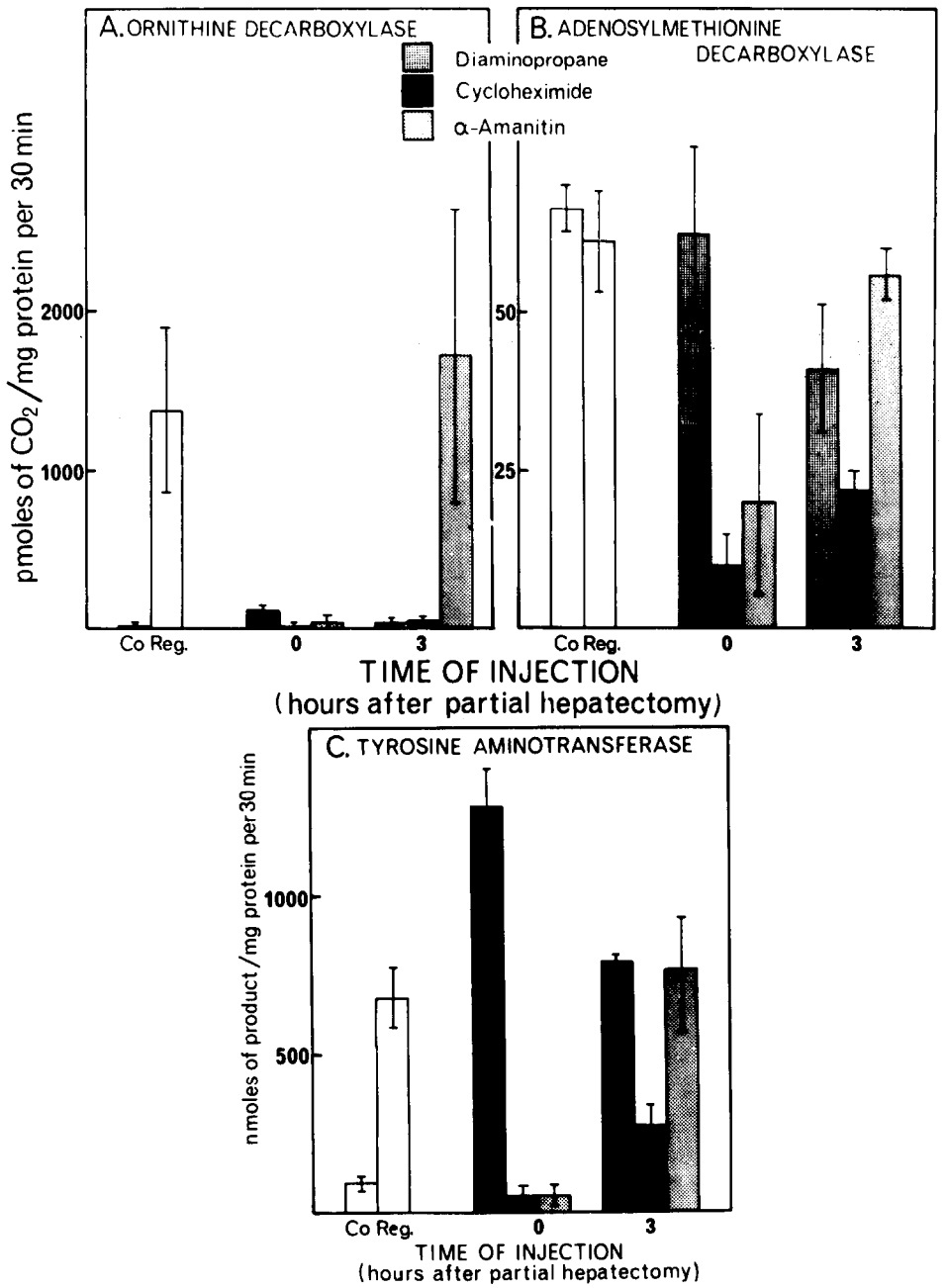

Fig.1. Effect of 1,3-diaminopropane, cycloheximide and α-amanitin on ornithine decarboxylase (A), adenosylmethionine decarboxylase (B) and tyrosine aminotransferase (C) activities in regenerating rat liver. Partially hepatectomized rats received diaminopropane (75 μmol/100 g body wt), cycloheximide (0.8 mg/100 g) or α-amanitin (0.1 mg/100 g) at time points indicated and killed 4 h after partial hepatectomy. Each group consisted of four animals. The vertical bars represent standard deviations of the means. Co, unoperated controls. Reg., 4 h regenerating liver (without any treatment).

diaminopropane and α-amanitin on ornithine decarboxylase was strikingly different from that observed during the early hours of liver regeneration. Unlike the beginning of the regenerative process, an injection of α-amanitin given 4 h before death insignificantly depressed ornithine decarboxylase activity (table 2). Similarly, diaminopropane tended to increase the enzyme activity when given 4 h before death (table 2). This differed from the complete inhibition of enzyme activity observed in fig.1A. An injection of diaminopropane 1 h before death again abolished enzyme activity (table 2). The inhibition of ornithine decarboxylase activity by α-amanitin became less pronounced when the injection was given closer to death (table 2) whereas the compound significantly inhibited adenosylmethione decarboxylase activity when given 4 h or 1 h before death (table 2). Diaminopropane depressed adenosylmethionine decarboxylase activity only if administered 1 h before death. The inhibition at 4 h did not reach the level of statistical significance (table 2).

The activity of tyrosine aminotransferase was inhibited by α-amanitin while diaminopropane again enhanced the enzyme activity (table 2).

When the decay rates of these three enzyme activities after α-amanitin administration were calculated, the following half-lives were obtained: 6 h for ornithine decarboxylase, 3.9 h for adenosylmethionine decarboxylase and 3.8 h for tyrosine aminotransferase. These values might give some idea of the turnover rate of the mRNA of these rapidly vanishing enzymes.

To explore the mechanism of action of diaminopropane on ornithine decarboxylase further, we measured the decay rate of the enzyme in 24 h regenerating liver after an injection of diaminopropane, cycloheximide and their combination. As shown in fig.2, the activity of ornithine decarboxylase decayed with an apparent half-life of 12 min after an intraperitoneal injection of diaminopropane, and half-lives of 14 min and 15 min after cycloheximide after a combined injection of diaminopropane and cycloheximide. It thus appears that (i) the effect of this diamine on ornithine decarboxylase is as rapid as that of inhibitor of protein synthesis (ii) there is no additive effect between the two compounds and hence (iii) diaminopropane evidently does not enhance the breakdown of ornithine decarboxylase. α-Amanitin given under similar experimental conditions did not exert an

Table 2
Effect of 1,3-diaminopropane and α-amanitin on the activities of ornithine decarboxylase, adenosylmethionine decarboxylase and tyrosine aminotransferase in 24 h regenerating rat liver

Treatment	Time of injection (h before death)	Ornithine decarboxylase activity (pmoles/mg protein ± S.D.)	Adenosylmethionine decarboxylase (pmoles/mg protein ± S.D.)	Tyrosine aminotransferase (nmoles/g protein ± S.D.)
Normal liver	–	48 ± 14[a]	135 ± 44[a]	102 ± 30[b]
24 h regenerating liver	–	1520 ± 501	344 ± 33	768 ± 294
Diaminopropane	4	2260 ± 732	282 ± 97	1540 ± 132[b]
α-Amanitin	4	997 ± 235	168 ± 63[b]	367 ± 84
Diaminopropane	1	82 ± 24[b]	176 ± 52[b]	888 ± 66
α-Amanitin	1	1380 ± 138	282 ± 26[a]	636 ± 330

[a] $p < 0.05$
[b] $p < 0.01$
[c] $p < 0.001$

Significant differences as compared with the 24 h regenerating liver.
24 h Partially hepatectomized rats, received either 1,3-diaminopropane (75 μmoles/100 g) or α-amanitin (0.1 mg/100 g) at the times indicated. Four animals in each group.

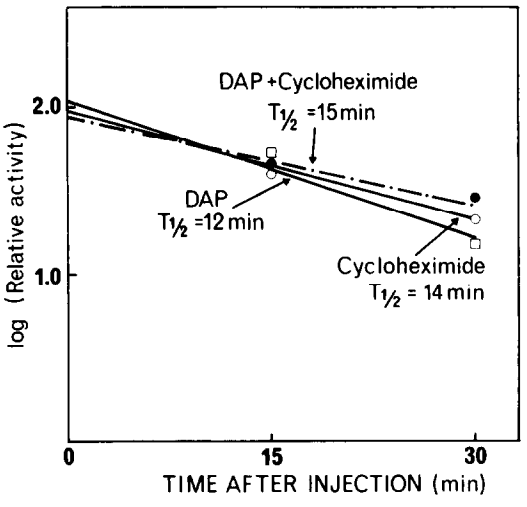

Fig.2. Effect of 1,3-diamino-propane, cycloheximide and their combination on ornithine decarboxylase activity in regenerating rat liver. 24 h Partially hepatectomized rats, were injected with 1,3-diaminopropane (75 μmol/100 g body wt) cycloheximide (0.8 mg/100 g) or their combination (75 μmol + 0.8 mg/100 g) and ornithine decarboxylase activity measured at time points indicated. Four animals in each group. The regression lines were computed by the least squares method.

influence on ornithine decarboxylase activity nor did it modify the inhibitory action of diaminopropane. This indicates that continuation of RNA synthesis was not required for the inhibitory action of diaminopropane.

It has been recently reported that inhibition of ornithine decarboxylase by putrescine in rat hepatoma cells [21] and regenerating rat liver [22] is partly based upon a protein-like macromolecule exerting an inhibitory influence on ornithine decarboxylase. In order to investigate whether diaminopropane and putrescine induced formation of such inhibitory macromolecules, rats were treated with diaminopropane and putrescine (75 μmol/100 g body wt) and killed one hour after injection of amine. The cytosol fractions were dialyzed and used for mixing assays of ornithine decarboxylase activity. (The extracts obtained from regenerating liver were combined with similar extracts from rats previously treated with diaminopropane or putrescine [21].) Under these conditions an equal amount of liver cytosol from amine-treated rats inhibited ornithine decarboxylase activity of cytosol (obtained from untreated partially hepatectomized rats) by 4.5–10.8%, compared with 70–93% inhibition of enzyme activity in cytosol-extracts, from amine-treated rats. Addition of liver cytosol from unoperated animals (exhibiting very low ornithine decarboxylase activity) to that obtained from partially hepatectomized rats, resulted in ~ 5% inhibition of enzyme activity. It thus appeared that undialysable inhibitory factor(s) contributed minimally to the rapid decay of ornithine decarboxylase activity.

4. Discussion

These experiments are consistent with the concept that ornithine decarboxylase activity is controlled in regenerating rat liver by mechanisms involving a set of different regulatory sites and/or levels. It appears that partial hepatectomy of the rats evokes immediate gene activation of ornithine decarboxylase. This is supported by the following experimental evidence: An injection of α-amanitin (inhibitor of nucleoplasmic RNA synthesis) at the time of the operation completely prevented the 4 h post-operative rise in the enzyme activity (fig.1A). Russell and Snyder [2] found an injection of actinomycin D, 1 h after partial hepatectomy, did not prevent the 4 h post-operative rise in ornithine decarboxylase activity. Fausto [23] and Kay et al. [24,25] reached similar conclusions employing different experimental systems.

α-Amanitin administration at the time of operation completely suppressed ornithine decarboxylase activity 4 h post-operatively. When administered 1 h or 4 h before death, during later regeneration times (table 2), α-amanitin effected no suppression of ornithine decarboxylase activity. However, as pointed out by Marinozzi and Fiume [26], α-amanitin brings about early changes in the nuclear morphology of the rat and thus might have secondary effects. This makes the interpretation of the present results on the timing of ornithine decarboxylase mRNA synthesis more difficult.

After the gene activation of ornithine decarboxylase is complete the mRNA of the enzyme might remain relatively stable. After initial gene activation the enzyme activity is probably controlled at a post-transcriptional level. This concept is supported by the following experimental evidence:

(i) α-Amanitin induced a rather slow decay of ornithine decarboxylase activity when given during the second day of regeneration (table 2). A calculation based upon the decay of enzyme activity after α-amanitin administration, gives an approximate half-life of 6 h for ornithine decarboxylase mRNA. This half-life appears to be somewhat longer than computed for adenosylmethionine decarboxylase and tyrosine aminotransferase (table 2).

(ii) Injections of various amines resulted in a rapid decrease in enzyme activity with an apparent half-life comparable to that found after inhibition of protein synthesis.

(iii) After the first 4 h post-operative peak, the activity of ornithine decarboxylase in regenerating rat liver fluctuates with additional peaks [27–29]. This fluctuation might be a manifestation of a transient repression of ornithine decarboxylase by increased levels of tissue putrescine and/or spermidine.

The post-transcriptional regulatory mechanisms may include formation of inhibitory macromolecules to ornithine decarboxylase [21,22] although it appears that the formation of these inhibitors is a slow process, requiring high concentrations of the amines [22]. It is thus unlikely that these inhibitors are primarily (or entirely) responsible for the rapid decay of ornithine decarboxylase activity following the injection of putrescine [11], diaminopropane (fig.2 [14]) or other amines [14]. It is however, tempting to speculate that the post-transcriptional control of ornithine decarboxylase by the amines occurs through a specific inhibition of the enzyme protein translation process, resulting in formation of incomplete peptide chains, the accumulation of which would later directly inhibit ornithine decarboxylase. It has been reported that the inhibitory protein-like compounds induced by the administration of amines are much smaller than ornithine decarboxylase itself.

The finding that diaminopropane exerted an inhibitory effect on adenosylmethionine decarboxylase raises the interesting possibility that both decarboxylases are coordinately regulated.

Acknowledgements

The skillful technical assistance of Miss Kristina Bjugg and Mrs Riitta Sinervirta is gratefully acknowledged. One of us (H.P.) received financial support from the Emil Aaltonen Foundation (Tampere, Finland). Dr Giuseppe Scalabrino is a visiting scientist from the Institute of General Pathology, University of Milan and recipient of a NATO research fellowship.

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