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Regulation of Ornithine Decarboxylase and *S*-Adenosyl-L-Methionine Decarboxylase in Regenerating Rat Liver by Various Amines: Evidence for Translational Control

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The activity of ornithine decarboxylase in regenerating rat liver could be completely or partially inhibited *in vivo* by a single intraperitoneal injection of various amines. Unphysiological, 1,3-diaminopropane depressed most effectively the activity of ornithine decarboxylase. It depressed also the activity of adenosylmethionine decarboxylase, which was not inhibited by other amines. The activity of tyrosine aminotransferase was invariably stimulated by injection of the amines.

Cycloheximide caused a rapid decay of the activity of liver ornithine decarboxylase (half-life 15 min) and also a decay of the activity of adenosylmethionine decarboxylase (half-life 36 min). 1,3-Diaminopropane inhibited the activity of ornithine decarboxylase (half-life 13 min) and to lesser extent also the activity of adenosylmethionine decarboxylase (half-life 120 min). On the contrary, α-amanitin did not have any effect on the activity of the decarboxylases.

These experiments are consistent with the view that diamines and spermidine might conceivably control the activity of ornithine decarboxylase in regenerating rat liver *in vivo* at steps beyond transcription. It is also possible that 1,3-diaminopropane similarly controls the activity of adenosylmethionine decarboxylase thus suggesting that the synthesis of ornithine and adenosylmethionine decarboxylases may be coordinatively regulated in liver.

The first two enzymes in the biosynthetic pathway of putrescine and the higher polyamines, spermidine and spermine, are in vertebrate tissues: (i) a soluble ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17) that catalyzes the decarboxylation of L-ornithine to yield putrescine and CO_2,[1,2] and (ii) a soluble *S*-adenosyl-L-methionine decarboxylase (*S*-adenosyl-L-methionine carboxy-lyase, EC 4.1.1.50) that catalyzes the decarboxylation of *S*-adenosyl-L-methionine to yield *S*-adenosylmethylhomocysteamine (decarboxylated adenosylmethionine) and CO_2[3–6]

Ornithine decarboxylase (ODC) is the rate controlling enzyme in the synthesis of polyamines and may be associated somehow with the control of cell growth.[7,8] ODC possesses the shortest biological half-life of only 10 – 20 min known for enzymes in animal tissues.[9,10] The activity of the enzyme is greatly stimulated under conditions of rapid growth, such as in rat liver after partial hepatectomy [1,2] or after treatment with growth hormone.[11]

In bacteria the activity of ODC appears to be controlled directly by low molecular weight modulators [12,13] but in higher organisms such effector(s) have not yet been found.[7,14] It thus appears that the activity of eukaryotic ODC is largely regulated through changes in the rate of the synthesis and/or degradation of the enzyme protein.[7,15] There is considerable amount of indirect evidence suggesting that the activity of ODC in mammalian tissues is controlled *in vivo* by a repression type inhibition. In regenerating rat liver [16–18] and in cultured mammalian cells [19,20] exogenous putrescine, spermidine or 1,3-diaminopropane rapidly depressed ODC activity. It has been proposed that the control of ODC by polyamines occurs at some posttranscriptional, possibly at translational level.[17,21]

The enhanced activity of ODC rapidly results in a concomitant increase in liver put-

rescine [2] and an accumulation of spermidine somewhat later.[23,24] Adenosylmethionine decarboxylase (AMDC) in animal tissues and in yeast is strongly stimulated by putrescine and by other diamines.[3,5,6,25] Putrescine thus directly regulates the activity of the second enzyme of the biosynthetic pathway of polyamines. AMDC has also very short half-life (about 35 min) in regenerating rat liver [10] indicating that its activity is likely to be influenced by changes in protein synthesis.

In the present paper I have investigated the efficacy and specificity of various amines to inhibit ODC and AMDC *in vivo*. The most potent inhibitor seemed to be 1,3-diaminopropane which also depressed the activity of AMDC.

Based on comparison of the effect of diaminopropane, cycloheximide and α-amanitin on the activity of ODC and AMDC it is proposed that 1,3-diaminopropane controls the synthesis of ODC and AMDC at translational level.

MATERIAL AND METHODS

Chemicals. Unlabelled *S*-adenosyl-L-methionine was synthesized by the method originally described by Cantoni *et al.*[26] and modified by Pegg and Williams – Ashman.[3]

(Carboxyl-^{14}C)*S*-adenosyl-L-methionine (sp. radioactivity 60 mCi/mmol) was purchased from New England Nuclear Corp. (Dreieichenhain, West-Germany) and DL-ornithine-1-^{14}C (sp. act. 51 mCi/mmol) from the Radiochemical Centre (Amersham, Bucks, England). Putrescine, spermidine and spermine (as their hydrochlorides) were obtained from Calbiochem (San Diego, Calif., U.S.A.). 1,3-Diaminopropane was the product of Fluka AG (Buchs SG, Switzerland) and cadaverine hydrochloride was obtained from Nutritional Biochemicals Corp. (Cleveland, Ohio, U.S.A.). Methylglyoxal bis(guanylhydrazone) was purchased from Aldrich Chemicals (Milwaukee, Wis., U.S.A.). Cycloheximide (Acti-Dione®) was from Nutritional Biochemicals Corp. and α-amanitin from Boehringer (Mannheim, West-Germany). Amines were neutralized before use. Cycloheximide and α-amanitin were dissolved in 0.9 % NaCl just before injections.

Experiments in vivo. Male rats of the Wistair strain weighing 65 – 150 g (95 – 105 g in Table 1; 130 – 150 g in Table 2; 65 – 85 g in Figs. 1 and 2) were used. The animals were partially hepatectomized 24 or 4 h before death. They received the compounds as an intraperitoneal injection 60 min before death (Table 1 and Table 2) or at the time points indicated in Figs. 1 and 2. The amount of amines was 75 μmol per 100 g of body wt., that of cycloheximide 0.8 mg per 100 g of body wt. and that of α-amanitin 100 μg per 100 g of body wt.

Partial hepatectomy was performed under light ether anaesthesia by the method of Higgins and Anderson.[27]

Preparation of liver extracts. After decapitation of the rats, the livers were removed and immediately homogenized with 2 volumes of cold 25 mM Tris-HCl buffer, pH 7.4, containing 0.1 mM EDTA and 5mM dithiothreitol. The homogenates were centrifuged at 105 000 g_{max} for 30 min at 2 °C. The enzyme activities were assayed as quickly as possible using undialyzed supernatant fractions as the source of enzymes.

Analytical methods. The activity of ODC was measured by the method of Jänne and Williams-Ashman,[14] that of AMDC as described by Jänne and Williams-Ashman,[4] and that of tyrosine aminotransferase (EC 2.6.1.5) by the method of Diamondstone.[28] The enzyme activities are expressed as nmol of product formed per mg protein per 30 min.

Quantitative polyamine measurements were carried out by the method of Raina and Cohen.[29] Protein was measured by the method of Lowry *et al.*[30] The decay lines of the enzyme activities were computed by the least-squares method.

RESULTS

The effect of various amines on ODC and AMDC activities. A single intraperitoneal injection (75 μmol/100 g body weight) of putrescine, spermidine, 1,3-diaminopropane, cadaverine, methylglyoxal bis(guanylhydrazone) (MGBG) or MGBG with putrescine into partially hepatectomized (24 h earlier) rats one hour before death clearly depressed the activity of ODC (Table 1). 1,3-Diaminopropane was the most potent inhibitor of ODC decreasing the enzyme activity by more than 90 %. 1,3-Diaminopropane also inhibited the activity of AMDC. The latter enzyme was unaffected by other amines although MGBG, which is the specific inhibitor of AMDC *in vitro*,[31] appeared to inhibit the enzyme activity in undialyzed cytosol fractions. In fact, putrescine, spermidine and cadaverine appeared to even slightly stimulate AMDC (Table 1).

The inhibition of ODC by MGBG might partly be due to the fact that the latter compound inhibits AMDC and hence prevents the conversion of putrescine to spermidine. Thus intracellular concentration of putrescine is expected to rise rapidly after the administration

Table 1. Effect of various amines on the activity of liver ODC, AMDC, tyrosine aminotransferase (TAT) and on the concentration of putrescine, spermidine and spermine in partially hepatectomized rats. The animals, partially hepatectomized 24 h earlier, were treated as described in the text. Four animals in each group. The percentages in the parentheses indicate the relative enzyme activities in comparison with controls.

Treatment	Enzyme activities ±S.D. ODC	AMDC	TAT	Polyamine concentrations (nmol/g of liver ±S.D.) Putrescine	Spermidine	Spermine
Controls	1.1(3) (100 %)	0.11(5) (100 %)	537(76) (100 %)	248(39)	869(139)	298(85)
Putrescine	0.4(3) (33 %)	0.18(9) (170 %)	805(112) (150 %)	410(85)	864(143)	278(7)
Spermidine	0.2(1) (19 %)	0.16(4) (146 %)	798(66) (148 %)	276(69)	1130(130)	255(10)
Diaminopropane	0.10(2) (7 %)	0.07(3) (63 %)	916(186) (170 %)	–	884(213)	275(23)
Cadaverine	0.5(3) (47 %)	0.17(7) (160 %)	864(78) (161 %)	222(44)	830	294(62)
MGBG + Putrescine	0.15(7) (14 %)	0.03(1) (32 %)	746(188) (140 %)	717(188)	851(77)	259(45)
MGBG	0.3(2) (31 %)	0.02(1) (17 %)	817(116) (152 %)	352(49)	853(49)	256(23)

of MGBG. The increased putrescine might, in turn, influence the activity of ODC.

Table 1 also illustrates that the concentrations of spermidine and spermine did not change after injection of the amines, except when spermidine was injected. MGBG alone appeared to elevate, as expected, the concentration of putrescine. After an injection of diaminopropane, it was impossible to measure the concentration of putrescine because the separation of putrescine from diaminopropane is very difficult or impossible by the electrophoretic method employed.

It also appears that the inhibition of ODC by amines and AMDC by diaminopropane is specific since the activity of tyrosine aminotransferase which was used as "reference" enzyme because its short half-life [32] was not inhibited by the amines. In fact, the activity of tyrosine aminotransferase was invariably stimulated after injection of these compounds (Table 1).

1,3-Diaminopropane also inhibited the activity of ODC and AMDC at 4 h after partial hepatectomy when injected 1 h before death. The inhibition of AMDC was in this particular experiment smaller at 4 h (18 %) than at 24 h (37 %) after partial hepatectomy (Table 2).

The inhibition of ODC and AMDC after partial hepatectomy at 4 and 24 h suggests that diaminopropane might influence the synthesis of both decarboxylases during early and later phases of liver regeneration in a coordinative manner.

Table 2. Comparison of the effect of 1,3-diaminopropane on the activity of liver ODC and AMDC. The animals, partially hepatectomized 4 h or 24 h earlier, were treated as described in the text. Other details as in Table 1.

Treatment	4 h regenerating liver ODC activity	AMDC activity	24 h regenerating liver ODC activity	AMDC activity
Controls	0.7(1) (100 %)	0.05(1) (100 %)	0.6(2) (100 %)	0.18(8) (100 %)
Diamino-propane	0.05(2) (7 %)	0.042(8) (82 %)	0.045(5) (12 %)	0.13(8) (63 %)

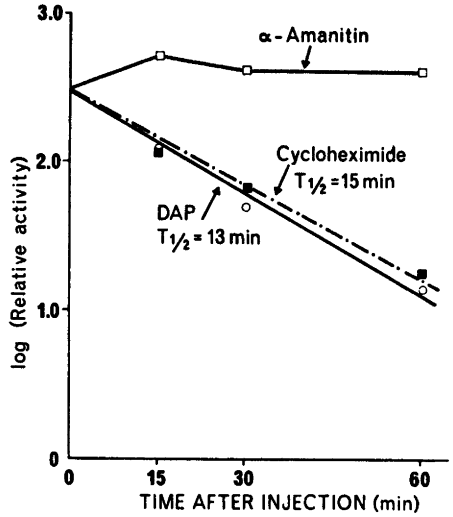

Fig. 1. Effect of 1,3-diaminopropane (DAP), cycloheximide and α-amanitin on liver ODC. The animals, partially hepatectomized 24 h earlier, were treated as described in the text. Three animals in each group.

Comparison of the effect of 1,3-diaminopropane, cycloheximide and α-amanitin on the activity of ODC and AMDC. When partially hepatectomized (24 h earlier) rats were treated with cycloheximide, which is a known inhibitor of eukaryotic protein synthesis,[33] or with diaminopropane, the activity of ODC rapidly decreased with an apparent half-life of 13 min after diaminopropane and 15 min after cycloheximide. α-Amanitin which is a specific inhibitor of nucleoplasmic RNA-polymerase,[34] did not have any effect on the enzyme activity during the whole period of observation (Fig. 1).

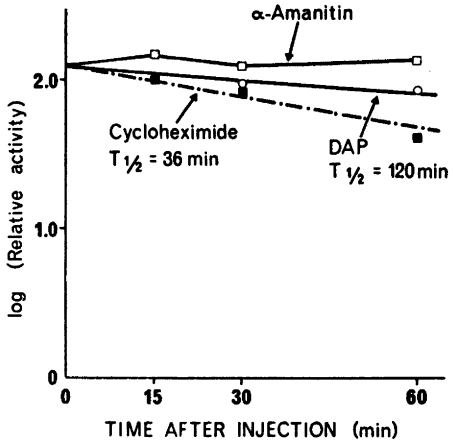

Fig. 2. Effect of 1,3-diaminopropane (DAP), cycloheximide and α-amanitin on liver AMDC. Details as in Fig. 1.

The activity of AMDC was also decreased after treatment with cycloheximide with an apparent half-life of 36 min and also diaminopropane caused an inhibition of AMDC, the half-life being now about 120 min. As in the case of ODC, α-amanitin did not have any effect on the enzyme activity, suggesting that also AMDC has a stable mRNA like ODC (Fig. 2).

It seems possible, even likely, that diaminopropane exerted its effect on the synthesis of ODC mainly at translational level because the enzyme activity was after diaminopropane administration decaying as rapidly as after cycloheximide (Fig. 1).

DISCUSSION

Various compounds have been tested to serve as specific inhibitors of polyamine biosynthesis in mammalian tissues in order to unravel the physiological function(s) of natural polyamines. MGBG was the first and probably the most extensively studied inhibitor of putrescine-activated AMDC and hence the synthesis of spermidine and spermine. It has been reported that millimolar concentrations of MGBG inhibited DNA and protein synthesis when added to cultures of activated human lymphocytes. However, it should be mentioned that these concentrations of MGBG were at least ten times higher than those needed for a complete inhibition of AMDC under these conditions.[35] MGBG, though being a specific inhibitor of putrescine-activated AMDC *in vitro*, appeared also to stabilize the enzyme against intracellular degradation *in vivo*[36] resulting in large increases in the enzyme activity which made it quite impossible to prevent the accumulation of spermidine after partial hepatectomy using sublethal doses of the compound.[37]

A few congeners of ornithine, inhibiting the activity of ODC *in vitro*, have also been tested for their capacity to stop the synthesis of polyamines *in vivo*. It has been reported that the administration of α-hydrazino-δ-valeric acid

depressed ODC activity, inhibited putrescine accumulation and resulted in a concomitant decrease in DNA synthesis in mouse parotid gland.[38] Moreover, Relyea and Rando showed that "unsaturated" ornithine (dehydroornithine) inhibited polyamine synthesis and cell division in chick embryo muscle cultures.[39]

In addition to the use of these kinds of inhibitors, structurally resembling more or less closely L-ornithine or *S*-adenosylmethionine, there is another approach to inhibit polyamine synthesis *in vivo*, namely by directly influencing the synthesis of ODC. This kind of inhibition is based on the fact that the activity of ODC *in vivo* appears to be regulated by polyamines *via* a repression-type mechanism.[17–19,22] 1,3-Diaminopropane, which is not found in mammalian tissues in any appreciable amounts, clearly inhibited the activity of ODC and also prevented the accumulation of spermidine in the regenerating liver remnant when given every 3 h after partial hepatectomy.[18] Interestingly, chronic treatment with diaminopropane resulted in a concomitant block of the synthesis of DNA which normally is greatly enhanced during the second day of liver regeneration.[19] Whether the prevention of the stimulation of DNA synthesis is causally related to the inhibition of polyamine synthesis by diaminopropane remains to be proved.

The present investigation shows that 1,3-diaminopropane is the most effective inhibitor among a number of other amines tested. A remarkable advantage of diaminopropane is the fact that the compound is unphysiological and cannot replace putrescine in the synthesis of higher polyamines.[18] It should be mentioned that it does not inhibit ODC in high concentrations *in vitro*.[18] So it is "fooling" by its structural resemblance, the control mechanisms responsible for the transcription and/or translation of the mRNA for ODC. The type of inhibition caused by diaminopropane resembles that exerted by isopropyl thiogalactoside (not attacked by β-galactosidase) on *E. coli* lac operon.[40]

This investigation also shows that diaminopropane might control the activity of ODC at steps beyond the transcription. The time dependence of the inhibition of ODC by diaminopropane was comparable to that observed after the injection of cycloheximide. Jänne and Hölttä [17] have shown that the decay rate of ODC activity after putrescine injection was of the same order of magnitude. α-Amanitin did not have any effect on the activity of ODC obviously indicating that mRNA of the enzyme is rather stable. The mRNA of ODC is most probably synthesized at the beginning of the liver regeneration which means that the control of ODC through transcription is not possible after the very early period of regeneration. By comparing the effect of polyamines and that of the inhibitors of transcription and translation on the activity of ODC in 3T3 cells, Clark and Fuller [22] likewise suggested that the control of polyamines could occur at translational level.

The physiological significance of the inhibition of ODC *in vivo* by various amines is not known yet, although it has been suggested that the typical phasic fluctuations [41] of ODC activity in regenerating rat liver could be due to putrescine affecting the enzyme activity after its concentration has risen to sufficient levels during the liver regeneration.[41] 1,3-Diaminopropane can also depress the activity of ODC but is not, in contrast to putrescine, converted to higher polyamines.[18]

The use of these compounds also appears to offer a convenient means to investigate the general regulatory mechanisms of protein synthesis in eukaryotic organisms using ODC and AMDC as marker enzymes. However, the most intriguing opportunity offered by a specific inhibitor of polyamine biosynthesis, like diaminopropane, is the possibility finally to solve the physiological function(s) of the natural polyamines, especially during periods of rapid growth.

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