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STABILIZATION OF ORNITHINE DECARBOXYLASE IN RAT LIVER

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Summary

The response of ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17) activity to cycloheximide has been measured in rat liver after various stimuli known to enhance the enzyme activity. While a single injection of cycloheximide almost totally (>95%) inhibited the activity of ornithine decarboxylase in livers of growth-hormone treated rats at 45 min after the administration of the drug, this inhibition was much less striking in livers of partially hepatectomized (80%), of thioacetamide-treated (60%) and especially of carbon-tetrachloride treated (15%) animals thus suggesting a stabilization of the enzyme after the latter treatments.

The measurement of the apparent half-life of ornithine decarboxylase after cycloheximide revealed a substantial decrease in the decay rate of the enzyme in livers of thioacetamide-treated rats. The apparent half-life of *S*-adenysyl-L-methionine decarboxylase (EC 4.1.1.50) likewise appeared to be longer in rats previously treated with thioacetamide or carbon tetrachloride than in partially hepatectomized animals.

Injection of thioacetamide also resulted in an initial stabilization of liver ornithine decarboxylase against 1,3-diaminopropane, a potent indirect inhibitor of the enzyme. However, 30 min after diaminopropane injection the enzyme activity started to fall extremely rapidly. This sudden drop in ornithine decarboxylase activity following diaminopropane could be largely prevented with cycloheximide. This finding clearly indicates that a protein-synthesis dependent formation of macromolecular inhibitors of ornithine decarboxylase may also be an early event after amine treatments. The striking stabilization of ornithine decarboxylase activity following these treatments, which are known to produce an initial liver necrosis (especially carbon tetrachloride and thioacetamide), may indicate that the system(s) responsible for the rapid inactivation of liver ornithine decarboxylase is more sensitive to tissue necrosis than the enzyme itself.

Introduction

L-Ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17) has two properties which make it as a unique representative among mammalian enzymes: (i) ornithine decarboxylase has the far shortest half-life of only about 10 min (1), and (ii) a great number of diverse agents produce dramatic stimulations of the enzyme activity [2,3], the extent and rapidity of which are quite unusual for eukaryotic enzymes. These two properties of the enzyme may conceivably be causally related as recently pointed out [4].

Mammalian ornithine decarboxylase does not appear to have any low molecular weight effectors of physiological occurrence [5]. In the majority of the instances, the stimulation of the enzyme activity can be prevented with inhibitors of nucleid acid and/or protein synthesis [2,3] thus indicating that the enzyme activity is mainly regulated by the rate of the synthesis of new enzyme protein. It has been shown indeed that the stimulation of ornithine decarboxylase activity is associated with a similar increase in the amount of immunoreactive protein in all cases studied so far [6—9]. Furthermore, the swift decay of the enzyme activity following an injection of cycloheximide was associated with a decrease in the amount of immunoreactive protein [6,8,9] although the half-life of the antigen appears to be slightly longer than that of the enzyme activity [8].

The stimulation of ornithine decarboxylase activity in some cell cultures, in which proliferation is initiated upon addition of serum [10], growth factors [11], asparagine or [12] or changes in osmolality [13], appears to involve a su substantial lengthening of the half-life of the enzyme. Marked increases in the half-life of mammalian ornithine decarboxylase have also been observed following addition of competitive inhibitors of the enzyme such as α-hydrazinoornithine [14] and α-methylornithine [15] or following infection of animal cells with transforming viruses [16]. On the contrary, the half-life of ornithin decarboxylase in rat liver appears to remain relatively constant regardless of the intense stimulation of the enzyme activity in response to partial hepatectomy, for instance [1]. Obenrader and Prouty [8] quite recently observed that the half-life of ornithine decarboxylase they obtained in livers of thioacetamide-treated rats was somewhat longer than previously reported for the enzyme either in normal or regenerating liver [1], although no direct comparisons were made.

We have now found that a treatment with thioacetamide and especially with carbon tetrachloric results in an apparent stabilization of liver ornithine decarboxylase as revealed by markedly reduced sensitivity of the enzyme activity to cycloheximide. The treatment with carbon tetrachloride likewise markedly stabilized liver adenosylmethionine decarboxylase against cycloheximide. Similar initial stabilization of ornithine decarboxylase was also evident after injection of 1,3-diaminopropane even though the later decay of the enzyme activity produced by this diamine clearly differed from that observed after cycloheximide.

Materials and Methods

Animals and treatments

Male rats of the Sprague-Dawley strain (weighing 150—200 g) were used in all experiments. Partial hepatectomy was performed under light ether anaesthesia by the method of Higgins and Anderson [17]. All the compounds used were injected intraperitoneally as neutralized solutions.

Chemicals

DL-[1-^{14}C]ornithine (specific radioactivity 59 Ci/mol) was purchased from the Radiochemical Centre (Amersham, Bucks., U.K.) and *S*-adenosyl-L-[1-^{14}C]-methionine was prepared as described by Pegg and Williams-Ashman [18]. Cycloheximide was obtained from the Sigma Chemical Company (St. Louis, Mo.), carbon tetrachloride and thioacetamide from E. Merck (Darmstadt, W. Germany) and porcine growth hormone from Ferring AB (Malmö, Sweden). 1.3-Diaminopropane was purchased from Fluka AG (Buchs SG, Switzerland) and neutralized before use.

Analytical methods

The activities of ornithine decarboxylase [5] and adenosylmethionine decarboxylase [19] were assayed by the methods routinely used in this laboratory. Antibody to rat liver ornithine decarboxylase (partially purified from livers of thioacetamide-treated animals) was raised in rabbits, absorbed and used for the inhibition of the enzyme as described earlier [9].

Results

Inhibition of liver ornithine decarboxylase by cycloheximide after various treatments

A single injection of cycloheximide usually results in a remarkably swift decay of ornithine decarboxylase activity in rat liver [1]. However, when liver ornithine decarboxylase activity was measured at 45 min after an injection of cycloheximide there appeared to be striking differences in the remaining activity depending on the means used to stimulate the enzyme. While cycloheximide produced more than 95% decrease in ornithine decarboxylase activity stimulated by growth hormone, it only marginally inhibited the enzyme activity enhanced by an injection of carbon tetrachloride (Table I). The extent of cycloheximide inhibition of ornithine decarboxylase activity stimulated by thioacetamide or by previous partial heptectomy fell between these two extremes, being less pronounced in the livers of thioacetamide-treated rats (Table I). This result apparently indicates that some of the treatments produced a distinct stabilization of the enzyme activity. The sensitivity of liver adenosylmethionine decarboxylase to cycloheximide likewise appeared to be reduced especially in rats treated with carbon tetrachloride (Table I).

Inhibition of ornithine decarboxylase by anti-ornithine decarboxylase serum

Ornithine decarboxylase stimulated by any of these agents was equally well inhibited by anti-ornithine decarboxylase serum (Table II) raised against

TABLE I

INHIBITION OF ORNITHINE AND ADENOSYLMETHIONINE DECARBOXYLASE ACTIVITIES BY CYCLOHEXIMIDE IN RAT LIVER AFTER VARIOUS TREATMENTS

The animals received growth hormone (2.5 I.U./100 g; 4 h before death), thioacetamide (15 mg/100 g; 20 h before death), carbon tetrachloride (0.2 ml/100 g; 14 h before death) or were partially hepatectomized (24 h before death). The rats then received cycloheximide (0.8 mg/100 g) or saline 45 min before killing. The enzyme activities are expressed as pmol CO_2 formed per mg protein per 30 min (±S.E.M.). Relative activities are given in parentheses. There were three to four animals in each group. The significance of the differences produced by cycloheximide was * $P < 0.05$, ** $P < 0.01$, *** $P < 0.01$.

Treatment	Ornithine decarboxylase activity		Adenosylmethionine decarboxylase activity	
Growth hormone	1820 ± 105	(100%)	93 ± 12	(100%)
Growth hormone plus cycloheximide	87 ± 50 ***	(5%)	38 ± 4 **	(41%)
Partial hepatectomy	775 ± 114	(100%)	118 ± 9	(100%)
Partial hepatectomy plus cycloheximide	178 ± 53 **	(22%)	62 ± 2 **	(53%)
Thioacetamide	1780 ± 173	(100%)	167 ± 22	(100%)
Thioacetamide plus cycloheximide	733 ± 27 **	(41%)	79 ± 7 *	(47%)
Carbon tetrachloride	3980 ± 1990	(100%)	217 ± 7	(100%)
Carbon tetrachloride plus cycloheximide	3490 ± 866	(87%)	183 ± 28	(84%)

partially purified enzyme from livers of thioacetamide-treated rats thus making it unlikely that the observed differences in the stability of the enzyme would be based upon an appearance of immunologically different isoenzymes.

Apparent half-life of ornithine decarboxylase activity in livers of partially hepatectomized and thioacetamide-treated rats

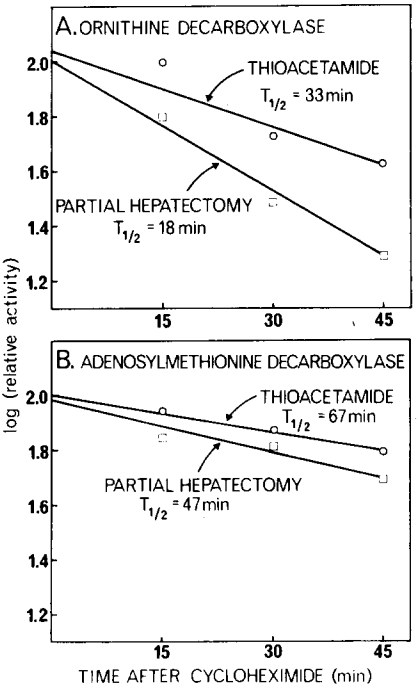
In a more detailed analysis we consistently found that the apparent half-life of ornithine decarboxylase activity was just about doubled in livers of thioacetamide-treated rats as compared with partially hepatectomized animals (Fig. 1A). The difference between the treatment was statistically significant both at 30 min ($P < 0.05$) and 45 min ($P < 0.01$) after cycloheximide injection. Interestingly, the half-life of liver adenosylmethionine decarboxylase

TABLE II

INHIBITION OF ORNITHINE DECARBOXYLASE ACTIVITY STIMULATED BY VARIOUS TREATMENTS BY ANTI-ORNITHINE DECARBOXYLASE SERUM

Rat liver cytosol fractions obtained after various treatments (for experimental details see Table I) were diluted with buffer as to give approximately equal activities and mixed with antiserum. After an incubation of the cytosol fractions with antiserum for 2 h at 3°C residual ornithine decarboxylase activity was assayed. Ornithine decarboxylase activity is expressed as pmoles of CO_2 liberated per 30 min.

Treatment	Antiserum	Ornithine decarboxylase activity	
Growth hormone	—	2770	(100%)
	+	847	(31%)
Partial hepatectomy	—	2850	(100%)
	+	882	(31%)
Thioacetamide	—	2380	(100%)
	+	699	(29%)
Carbon tetrachloride	—	2890	(100%)
	+	932	(32%)



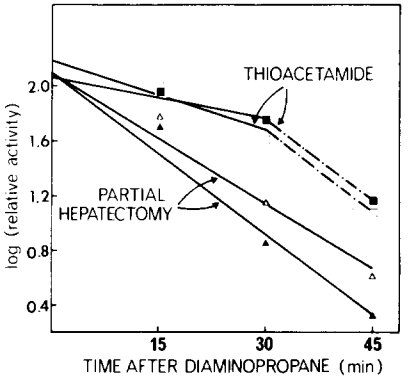


Fig. 1. Half-lives of ornithine (A) and adenosylmethionine (B) decarboxylase activities in rat liver after partial hepatectomy or thioacetamide treatment. The rats were partially hepatectomized or injected with thioacetamide (15 mg/100 g body wt.) 20 h before killing. The animals were injected with cycloheximide (0.8 mg/100 g body wt.) and sacrificed at time points indicated. There were 3 rats in each group. The regression lines have been computed by the least squares method.

Fig. 2. Effect of 1,3-diaminopropane on ornithine decarboxylase activity in rat liver after partial hepatectomy or treatment with thioacetamide. Experimental details as in Fig. 1, except that diaminopropane (100 μmol/s/100 g body wt.) was injected instead of cycloheximide. Two separate experiments with 3 rats in each group are presented. The lines (solid) were computed by the least squares method.

activity was likewise somewhat lengthened (by about 20 min) in the thioacetamide-treated animals (Fig. 1B). The latter finding may indicate that an injection of the drug brought about a more or less general stabilization of liver proteins.

Inhibition of liver ornithine decarboxylase by 1,3-diaminopropane

It was of great interest to determine whether the treatment with thioacetamide would also result in a similar stabilization of liver ornithine decarboxylase against 1,3-diaminopropane, a potent indirect inhibitor of mammalian ornithine decarboxylase [20,21].

As illustrated in Fig. 2, a single injection of diaminopropane resulted in a swift disappearance of ornithine decarboxylase activity in rat liver regenerating after partial hepatectomy. The initial decay of the enzyme activity following diaminopropane was distinctly slower in livers of thioacetamide-treated rats (Fig. 2). However, unlike the regenerating liver where the decay of ornithine decarboxylase activity roughly followed first-order kinetics through the whole period of observation, the enzyme inactivation after diaminopropane in livers

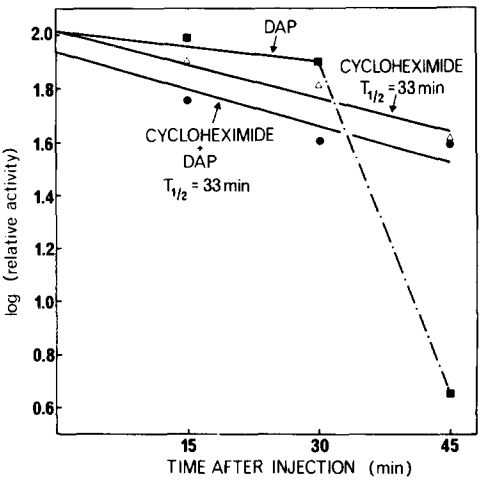


Fig. 3. Effect of 1,3-diaminopropane and cycloheximide on ornithine decarboxylase activity in livers of rats previously treated with thioacetamide. The animals, previously treated with thioacetamide as described in the legend for Fig. 1, received diaminopropane (100 μmol/100 g) or cycloheximide (0.8 mg/100 g) or a combination of them and were killed at time points indicated. There were 3 rats in each group. The lines (solid) were computed by the least squares method. DAP, 1,3-diaminopropane.

of thioacetamide-treated rats appeared to occur in two different phases i.e. between 30 and 45 min after the injection of the amine the enzyme activity suddenly started to decay much more rapidly (Fig. 2). This finding obviously indicates that several mechanisms for inactivation of liver ornithine decarboxylase were involved under these conditions.

We next injected thioacetamide-treated rats with diaminopropane, with cycloheximide or with their combination. As shown in Fig. 3, after the initial slow decay of ornithine decarboxylase activity in response to diaminopropane, the enzyme activity again rapidly disappeared between 30 and 45 min after the injection. When cycloheximide was injected simultaneously with diaminopropane, the rapid phase of the amine-induced inactivation of the enzyme activity totally disappeared and the overall decay rate of the activity after the combined injection was indistinguishable from that observed in response to cycloheximide (Fig. 3). The addition of cycloheximide to the diaminopropane treatment brought about a highly significant increase (8.8-fold; $P < 0.01$) in ornithine decarboxylase activity at 45 min (Fig. 3). This result apparently indicates that the sudden acceleration of the decay rate of the enzyme activity after diaminopropane required undisturbed protein synthesis.

Discussion

The regulation of mammalian ornithine decarboxylase appears to involve control elements at great different levels of gene expression. In contrast to its bacterial counterparts [22,23], mammalian ornithine decarboxylase probably does not have any low molecular weight effectors of physiological occurrence [5], the changes of the enzyme activity being apparently determined by alterations in the rate of the enzyme synthesis and/or degradation. While a stabilization or destabilization of ornithine decarboxylase seems to play an important

role in the regulation of the enzyme activity under some cell culture conditions [10–13] such a mechanism has not been identified in whole animals [1] with the possible exception of the stabilization of the enzyme by amino acids in perfused rat liver [24]. As shown here, some treatments known to enhance ornithine decarboxylase activity in rat liver also resulted in a distinct stabilization of the enzyme. Even though the contribution of this stabilization to the stimulation of the enzyme activity is difficult to assess quantatively, the long lag period before maximum stimulation of ornithine decarboxylase activity after carbon tetrachloride [25] or thioacetamide [26], for instance, suggests that a decreased enzyme degradation may be an important factor in raising the enzyme activity in response to these stimuli. The mechanism of the stabilization of ornithine decarboxylase remains to be determined. The increased stability of ornithine decarboxylase against the action of cycloheximide may be related to the varying degree of liver necrosis produced by these compounds. There appear to be an interesting similarity between the stabilization of ornithine decarboxylase and conversion of exogenous spermidine to liver putrescine in vivo. We have earlier shown that a single injection of carbon tetrachloride or of thioacetamide or partial resection of the liver, in this order, greatly increased the formation of putrescine from radioactive spermidine [25]. The interconversion between polyamines is believed to be catalyzed by a polyamine oxidase residing in the peroxisomal particles of the liver cell [27]. A common reason both for the enhanced polyamine oxidation in vivo and the stabilization of ornithine decarboxylase may be increased release of particulate (lysosomal and peroxisomal) enzymes. If this is the case, it implies that the degrading system for ornithine decarboxylase is more susceptible to changes produced by tissue necrosis than the enzyme itself.

The striking inhibition of mammalian ornithine decarboxylase by various diamines in vivo has recently gained increasing interest. The exact mechanism of this inhibition is not solved yet although indirect evidence indicates that it may occur mainly, if not exclusively, at some posttranscriptional level [9,28, 29]. The important discovery by Canellakis and his coworkers [30—32] showing that the administration of diamines induces a formation of rapidly vanishing macromolecular inhibitor which combines with ornithine decarboxylase resulting in the loss of enzyme activity, has offered a new model for the regulation of ornithine decarboxylase activity. The production of such "antizymes" [31] of ornithine decarboxylase in response to diamine administration has recently been confirmed by others [24,33]. We have earlier shown that a combination of diaminopropane and cycloheximide did not potentiate each other in decreasing liver ornithine decarboxylase activity [29] and diaminopropane-induced inhibition of ornithine decarboxylase activity was intially associated with a decrease in the amount of immunoreactive enzyme protein in regenerating rat liver [9]. However, as shown in the present results the effects of diaminopropane and cycloheximide on ornithine decarboxylase could be clearly dissociated under conditions where the half-life of the enzyme is lengthened.

Although the treatment with thioacetamide produced an initial stabilization of ornithine decarboxylase also against diaminopropane, which would be in accord with a direct control of the enzyme synthesis, the enzyme activity

decreased extremely rapidly after certain lag period following the amine injection. The fact that the second rapid phase of enzyme inactivation could be totally prevented by a simultaneous injection of cycloheximide strongly supports the idea that a protein with a rapid turnover rate [31] is also involved in the acute inactivation of liver ornithine decarboxylase by diamines. Presuming that some of the treatments caused a general stabilization of liver proteins the formation of such inhibitors would even occur at an enhanced rate. This kind of mechanism may also explain the paradoxical stimulation of ornithine decarboxylase activity by puromycin as described by Beck et al. [34]. Whether the formation of macromolecular inhibitors is the primary effect of diamines or whether it is the result of a direct translational control of the synthesis of the enzyme protein remains to be determined.

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