INHIBITION OF ORNITHINE DECARBOXYLASE ACTIVITY AND SPERMIDINE ACCUMULATION IN REGENERATING RAT LIVER

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SUMMARY: Injections of 1,3-diaminopropane, a close structural analogue of putrescine (1,4-diaminobutane), into partially hepatectomized rats powerfully inhibited ornithine decarboxylase (EC 4.1.1.17) activity in the regenerating liver *in vivo*. The compound did not have any effect on the enzyme activity *in vitro* (under assay conditions employed) but appeared to exert an inhibitory influence on the synthesis of ornithine decarboxylase itself.
Repeated injections of diaminopropane into rats after partial hepatectomy, starting at the time of the operation and continued until 33 h postoperatively, markedly diminished the stimulation of ornithine decarboxylase activity in the regenerating liver remnant, and completely prevented the increases in hepatic spermidine concentration normally occurring in response to partial hepatectomy.
Treatment of the rats with diaminopropane did not depress the activity of adenosylmethionine decarboxylase (EC 4.1.1.50) in the regenerating liver. Nor did the compound have any effect, whatsoever, on the activity of spermidine synthase (EC 2.5.1.16) *in vitro*, thus obiviously proving that the increased accumulation of liver spermidine after partial hepatectomy primarily depends upon a stimulation of ornithine decarboxylase activity and a concomitant accumulation of putrescine. The results also showed that 1,3-diaminopropane could not replace putrescine in the synthesis of higher polyamines in rat liver. The inhibition of ornithine decarboxylase by diaminopropane thus appears to represent "gratuitous" repression of polyamine biosynthesis and might conceivably be used for studies devoted to the elucidation of the physiological functions of natural polyamines.

Partial hepatectomy of the rat results in an early and intense stimulation of the synthesis and accumulation of putrescine (1) and spermidine (2,3) in the regenerating liver remnant.

The primary biochemical chance finally leading to the markedly increased accumulation of tissue spermidine in regenerating liver appears to be a dramatic enhancement in the activity of the enzyme ornithine decarboxylase (EC 4.1.1.17) (4,5) resulting in a concomitant increase in the content of liver putrescine (1,5). The elevated levels of putrescine, in turn, activate adenosylmethionine decarboxylase (EC 4.1.1.50) which is the actual rate controlling enzyme in the synthesis of spermidine from adenosylmethionine and putrescine in mammalian tissues (6).

LOCALIZATION OF THE CENTRAL AND PERIPHERAL SH-GROUPS ON THE SAME POLYPEPTIDE CHAIN OF YEAST FATTY ACID SYNTHETASE

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SUMMARY: Purified fatty acid synthetase isolated from wild type yeast cells as well as from two different fas-mutant strains was reacted with (1-^{14}C-)iodoacetamide. Tryptic digests of the ^{14}C-carboxamidomethylated enzymes were fractionated on Sephadex G-50. Hereby, essentially only one radioactively labeled peptide was eluted from the column. From this it is concluded that under the experimental conditions employed only the "peripheral" SH-group of yeast fatty acid synthetase becomes alkylated. By sodium dodecylsulfate-polyacrylamide gel electrophoresis of the ^{14}C-carboxamidomethylated fatty acid synthetase it was shown that in all three enzyme preparations studied the inhibitor is bound to the larger one of the two fatty acid synthetase subunits. These findings indicate that the larger fatty acid synthetase subunit accomodates not only the "central" but also the "peripheral" SH-group of the multienzyme complex.

INTRODUCTION

Acetate, the priming substrate of long chain fatty acid biosynthesis, is covalently bound to several chemically distinct sites of the yeast fatty acid synthetase multienzyme complex (1). These sites have been chemically characterized as a serine OH-group and as two different thiol groups, designated as "central" and as "peripheral" SH-group, respectively (2,3). While the "peripheral" SH-group belongs to a cysteine residue of the enzyme protein (4,15, G.-B. Kresze et al., in preparation), the "central" one is attributed to enzyme-bound 4'-phosphopantetheine (5,6). Both thiol groups are assumed to be involved in the condensation of malonate and acetate to form enzymebound acetoacetate according to the following equation, where S_p indicates the "peripheral" and S_c the "central" SH-group:

$$\text{enzyme}S_c\text{—}COCH_2COO^- + \text{enzyme}S_p\text{-}COCH_3 \rightleftharpoons$$

$$\text{enzyme}S_c\text{—}CO\text{-}CH_2\text{-}CO\text{-}CH_3 + CO_2 + \text{enzyme}S_p^-$$

Inhibition studies performed under carefully controlled conditions indicated that the ß-ketoacyl synthetase component enzyme was inactivated concomitantly with the alkylation of three cysteine SH-groups of the fatty acid synthetase complex (15, G.-B. Kresze et al., in preparation). These results

and S-adenosyl-L-methionine-1-^{14}C (sp.act. 60 mCi/mmole) from the New England Nuclear Corp. (Dreieichenhain,West-Germany). Putrescine,spermidine and spermine (as their hydrochloride salts) were obtained from Calbiochem (San Diego, Calif.,U.S.A.). 1,3-Diaminopropane was the product of Fluka AG (Buchs SG, Switzerland). The amines were neutralized before use.

Partial hepatectomy was performed under light ether anaesthesia as described by Higgins and Anderson (19).

Polyamines were measured after butanol extraction by the method of Raina and Cohen (20).

The activities of ornithine decarboxylase (21),adenosylmethionine decarboxylase (22) and spermidine synthase (23) were assayed by methods routinely used in this laboratory.

Protein was measured by the method of Lowry *et al.* (24).

RESULTS

As shown in Table 1, not only putrescine (16) but also 1,3-diaminopropane injected intraperitoneally (75 μmoles/100 g body wt.) effectively inhibited ornithine decarboxylase activity in 24-h regenerating rat liver 60 min after the injection. In fact, 1,3-diaminopropane appeared to be even more potent than putrescine in depressing ornithine decarboxylase activity (Table 1).

A single injection of 1,3-diaminopropane decreased ornithine decarboxylase activity in regenerating liver almost totally for 2 h, the inhibition gradually disappearing between 2 and 6 h after the injection (results not tabulated) It should be mentioned that neither putrescine nor diaminopropane (up to 10 mM) did influence ornithine decarboxylase activity *in vitro* under standard incubation conditions (the concentration of L-ornithine routinely employed was 2 mM).

When 75 μmoles/100 g of 1,3-diaminopropane was injected at every 3 h after partial hepatectomy (starting at the time of operation),this treatment effectively inhibited (70 to 80%) liver ornithine decarboxylase activity, as shown in Fig. 1.

Unlike ornithine decarboxylase,adenosylmethionine decarboxylase was not inhibited by diaminopropane, except at 3 h postoperatively. In fact, the activity of adenosylmethionine decarboxylase was even enhanced by the amine at 24 and 33 h after partial hepatectomy,as illustrated in Fig. 2. The stimulation of adenosylmethionine decarboxylase activity by diaminopropane might conceivably be due to a stabilization of the enzyme by the amine since the latter compound is known to activate adenosylmethionine decarboxylase *in vitro* in a variety of eukaryotic sources (7,25,26).

Table 2 illustrates that diaminopropane did not have any effect on spermidine synthase activity *in vitro* when assayed under normal incubation conditions.

Table 1. Effect of putrescine and 1,3-diaminopropane on liver ornithine decarboxylase activity in partially hepatectomized rats. The animals, partially hepatectomized 24 h earlier, received 75 μmoles of putrescine or diaminopropane per 100 g body wt. as an intraperitoneal injection 60 min before sacrifice. Four animals in each group.

Treatment	Ornithine decarboxylase activity (pmoles/mg protein ± S.D.)
None	1 070 ±328
Putrescine	355 ±263
1,3-Diaminopropane	100 ± 17

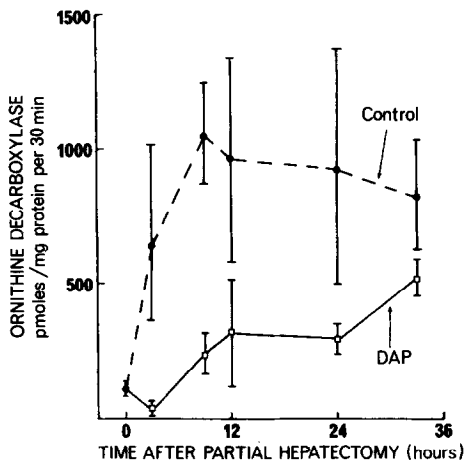

Fig. 1. Effect of injections of 1,3-diaminopropane on ornithine decarboxylase activity in regenerating rat liver. The treated animals received 75 μmoles of 1,3-diaminopropane (DAP) per 100 g of body wt. as an intraperitoneal injection at every 3 h after partial hepatectomy (starting at the time of operation). Three to four rats in each group. The vertical bars represent standard deviations.

As seen in Fig. 3, repeated injections of diaminopropane into partially hepatectomized rats completely prevented the increase in liver spermidine concentration normally beginning during and after the first day of regeneration.

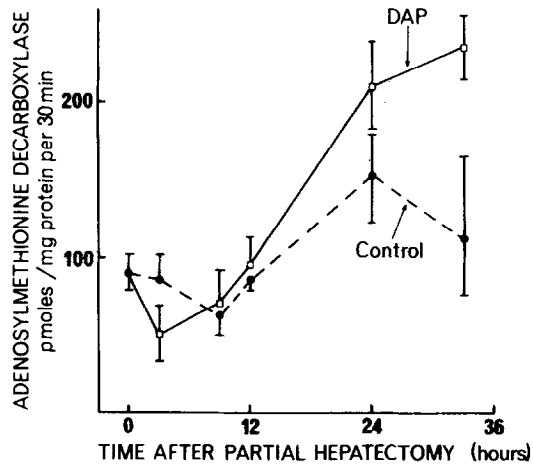

Fig. 2. Effect of injections of 1,3-diaminopropane (DAP) on adenosylmethionine decarboxylase activity in regenerating rat liver. Experimental details as in Fig. 1.

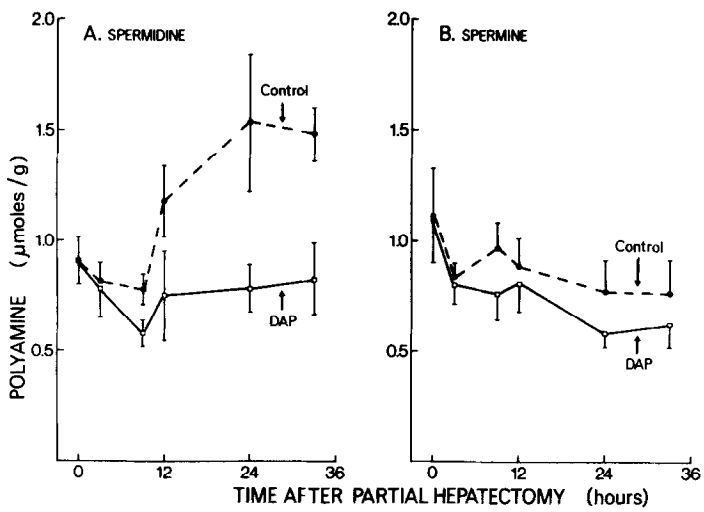

Fig. 3. Effect of injections of 1,3-diaminopropane (DAP) on the accumulation of spermidine (A) and spermine (B) in regenerating rat liver. Experimental details as in Fig. 1.

In fact, the concentration of spermidine increased almost 2-fold at 24 and 33 h after partial hepatectomy in control animals while it hardly reached the level found prior to the operation in those animals receiving diaminopropane (Fig.3A). The concentration of spermine was also clearly lower in the diaminopropane-treated animals than in control animals through the whole period of observation (Fig. 3B).

Unfortunately it was not possible to measure the actual tissue concentration of putrescine in diaminopropane-treated animals because none of the electrophoretic or chromatographic methods commonly used for the quantitation of polyamines can be used for separation and simultaneous quantitation of both putrescine and 1,3-diaminopropane.

DISCUSSION

Several lines of indirect evidence have indicated that the increased synthesis and accumulation of tissue spermidine under a variety of "anabolic" conditions is due to an elevated tissue concentration of putrescine (5,6,27) and hence due to a primary enhancement in the activity of ornithine decarboxylase.

The present investigation appears to be the first direct prove showing that an inhibition of ornithine decarboxylase *in vivo* in partially hepatectomized rats can totally abolish the later increases in the tissue concentration of spermidine in the regenerating liver remnant. It is worth noting that the treatment of the rats with diaminopropane, in fact, increased the activity of adenosylmethionine decarboxylase (Fig. 2) and did not have any effect whatsoever on the activity of spermidine synthase *in vitro* (Table 2). In other words, the prevention of spermidine accumulation was not due to an inhibition of adenosylmethionine decarboxylase or spermidine synthase, which are directly involved in the synthesis of this polyamine.

There exists number of reports suggesting that the amount of ornithine decarboxykase in animal tissues is largely determined by a "coarse" control mechanism (28) involving a repression type regulation of the enzyme by putrescine and spermidine (14-17). As shown in this study, the more unphysiological but structurally closely related 1,3-diaminopropane can also depress the enzyme activity. Whether this regulation occurs at the level of transcription and/or at some post-transcriptional level, as recently suggested (16,17), is not known.

The use of this type inhibition, which might be called as "gratuitous" repression of polyamine synthesis, since diaminopropane is not converted to higher polyamines, apparently offers a specific means to inhibit ornithine decarboxylase and hence to abolish the accumulation of putrescine and spermidine under conditions characterized by marked enhancements in the biosynthetic pathway of polyamines. A compound like diaminopropane is not likely to interfere the neighbouring reactions of polyamines as propably do those compounds structurally resembling ornithine or adenosylmethionine. The speci-

Table 2. Effect of 1,3-diaminopropane on the activity of spermidine synthase from regenerating rat liver *in vitro*. Dialyzed liver cytosol fraction was obtained from animals partially hepatectomized 33 h earlier. Spermidine synthase activity is expressed as nmoles of putrescine incorporated per 30 min. The concentration of 1,3-diaminopropane was 0.5 mM.

Conentration of putrescine (mM)	Spermidine synthase activity	
	-diaminopropane	+diaminopropane
0.05	1.00	0.94
0.1	1.20	1.04
0.5	1.37	1.39

fic inhibition of polyamine synthesis also offers an useful tool for attempts to solve the physiological functions of putrescine, spermidine and spermine in mammalian tissues. A possible disadvantace of employing another amine, albeit unphysiological, is the fact that high intracellular concentrations of this amine might partly take over the cellular functions normally excerted by the natural polyamines

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