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PUTRESCINE AND THE REGULATION OF *S*-ADENOSYL-L-METHIONINE DECARBOXYLASE IN CULTURED MOUSE MAMMARY GLAND

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Summary

The activity of *S*-adenosyl-L-methionine decarboxylase (*S*-adenosyl-L-methionine carboxy-lyase, EC 4.1.1.50) purified from mouse mammary gland can be stimulated by physiological concentrations of putrescine ($K_a = 5 \cdot 10^{-7}$ M). In contrast, the elevation of the intracellular concentrations of putrescine in cultured mouse mammary tissue by exogenous addition of putrescine or by cultivation of the tissue in hypotonic medium results in the decrease in both the activity and the amount of the enzyme in tissue. Conversely, the addition of 1 mM α-methyl ornithine, an inhibitor of ornithine decarboxylase, causes augmentation of the activity and the amount of *S*-adenosyl-L-methionine decarboxylase, despite its inhibitory action on insulin-stimulated increase in the concentration of putrescine. These data suggest that putrescine can act as a 'negative' regulator of the enzyme in this system.

Introduction

S-Adenosyl-L-methionine decarboxylase, a key enzyme in polyamine biosynthesis, catalyzes the formation of decarboxylated *S*-adenosyl-L-methionine, the sole donor of the propylamine moiety for the biosynthesis of spermidine and spermine [1,2]. One of the unique features of mammalian *S*-adenosyl-L-methionine decarboxylase is that the enzyme activity is markedly stimulated by putrescine, a diamine which is formed by ornithine decarboxylase [1—4]. Recent studies of purified *S*-adenosyl-L-methionine decarboxylase from mouse mammary gland have shown that putrescine can stimulate and stabilize the enzyme activity at physiological concentrations [5—7]. In addition, the intracellular concentration of putrescine has been shown to increase prior to the

rise in the activity of *S*-adenosyl-L-methionine decarboxylase during the development of the mammary gland in vivo and in vitro [6—8], as observed in several other tissues undergoing growth and development [9—12]. These observations raise the possibility that putrescine acts as an intracellular regulator of the activity of *S*-adenosyl-L-methionine decarboxylase. On the other hand, there are several reports suggesting that the enzyme has a short half-life and that the enzyme activity is controlled at the level of enzyme turnover [13,14]. The object of the present study was to assess the physiological role of putrescine in the cellular regulation of *S*-adenosyl-L-methionine decarboxylase in mammary tissue using an organ culture system, by comparing the change in the activity and in the amount of the enzyme in culture conditions where the intracellular levels of putrescine were altered in several ways.

Materials and Methods

Putrescine dihydrochloride, α-methyl ornithine, dithiothreitol, and pyridoxal phosphate were purchased from Calbiochem. Medium 199 was obtained from GIBCO. Crystalline porcine zinc insulin was a gift from Eli Lilly and Co. *S*-[*carboxyl*-^{14}C]Adenosyl-L-methionine (54.6 mCi/mmol) was obtained from New England Nuclear.

The activity of *S*-adenosyl-L-methionine decarboxylase was assayed by measuring $^{14}CO_2$ release from carboxyl-labeled substrate as described previously [5]. The enzyme reaction was carried out in a total volume of 2 ml consisting of enzyme samples and 0.25 M sodium phosphate buffer, pH 7.6, containing 0.5 mM putrescine/1 mM dithiothreitol/0.1 mM EDTA/adenosyl-[*carboxy*-^{14}C]methionine (0.05 μCi/ml). Anti-mouse *S*-adenosyl-L-methionine decarboxylase was raised in rabbit with purified enzyme from mouse mammary gland [5].

3-4-month old female C3H/HeN mice were obtained from the Animal Breeding Facility, National Institutes of Health. The abdominal mammary gland was used for preparation of tissue explants which were cultured with Medium 199 containing penicillin G (35 μg/ml) as described previously [15]. Insulin was used at a concentration of 5 μg/ml.

The content of putrescine was determined by a Beckman amino acid analyzer as described previously [16].

Results and Discussion

Previous studies [7] showed that when mammary explants from virgin mice were cultured in the presence of insulin, ornithine decarboxylase activity increased rapidly with resultant elevation of the putrescine level prior to the rise in *S*-adenosyl-L-methionine decarboxylase activity.

In order to assess a possible causal relationship between the increase in the level of putrescine and the activity of *S*-adenosyl-L-methionine decarboxylase, we examined the effect of α-methyl ornithine, an inhibitor of ornithine decarboxylase, which blocks the increase in putrescine [7], on the activity of *S*-adenosyl-L-methionine decarboxylase in cultured mammary explants. The data in Table I show that the addition of α-methyl ornithine at the onset of

TABLE I

EFFECT OF α-METHYL ORNITHINE ON THE ACTIVITY OF *S*-ADENOSYL-L-METHIONINE DECARBOXYLASE IN MOUSE MAMMARY EXPLANTS IN CULTURE

Mammary explants from virgin mice were cultured for 20 h under the indicated conditions, and the activity of *S*-adenosyl-L-methionine decarboxylase in tissue explants was determined as described in Materials and Methods. α-Methyl ornithine was used at a concentration of 1 mM.

Culture condition	*S*-Adenosyl-L-methionine decarboxylase (pmol/mg tissue per h)	Putrescine concentration (nmol/mg tissue)
Uncultured control	0.037	0.005
Insulin	0.133	0.025
Insulin + α-methyl ornithine	0.606	0.005

culture prevented the insulin-induced increase in the amount of putrescine but elicited a marked stimulation of the activity of *S*-adenosyl-L-methionine decarboxylase. The drug, however, did not cause any direct activation of the enzyme when added at concentrations of 0.1 μM—10 mM to the enzyme assay reaction (data not shown).

Incubation of mammary explants in a hypotonic medium resulted in a rapid, striking increase in ornithine decarboxylase activity with an over-production of putrescine in cells [17]. Accordingly, we examined the change in the activity of *S*-adenosyl-L-methionine decarboxylase in mammary explants cultured in hypotonic medium containing insulin. The data in Table II show that in tissue explaints cultured in the hypotonic medium for 6 h the intracellular level of putrescine was elevated 10-fold over that in the isotonic medium, but the activity of *S*-adenosyl-L-methionine decarboxylase remained at the basal level of uncultured tissue. In contrast, in isotonic medium both the concentration of putrescine and the enzyme activity increased 50% above the respective initial levels. Addition of exogenous putrescine to the isotonic medium resulted in a marked elevation of the putrescine level in cells and similarly inhibited the emergence of the activity of *S*-adenosyl-L-methionine decarboxylase.

The isolation of pure *S*-adenosyl-L-methionine decarboxylase from mammary tissues permitted the preparation of the enzyme-specific antibody

TABLE II

EFFECT OF HYPOTONIC CULTURE MEDIUM ON THE *S*-ADENOSYL-L-METHIONINE DECARBOXYLASE ACTIVITY AND THE PUTRESCINE CONTENT IN MOUSE MAMMARY EXPLANTS IN CULTURE

Mammary explants from midpregnant mice were cultured in isotonic or hypotonic (0.53 isotonicity) medium for 6 h and the activity of *S*-adenosyl-L-methionine decarboxylase and the content of putrescine were determined as described in Materials and Methods.

Culture condition	*S*-Adenosyl-L-methionine decarboxylase (pmol/mg tissue per h)	Putrescine concentration (nmol/mg tissue)
Uncultured control	0.08	0.030
Isotonic medium	0.12	0.046
Hypotonic medium	0.08	0.326
Isotonic medium + 0.1 mM putrescine	0.08	0.312

which was capable of inactivating the enzyme activity in the tissue extracts and thus allowed us to assess the amount of antigen in a given enzyme preparation. Accordingly, the Ab_{50}, defined as the amount of antiserum required to inactivate 50% of the enzyme activity, has been determined with samples containing various amounts of the decarboxylase activity (Fig. 1). The data in Table III demonstrate that α-methyl ornithine, an ornithine decarboxylase inhibitor, caused a 7-fold increase in the Ab_{50} value which corresponds to the degree of change in the enzyme activity. A hypotonic treatment which caused no increase in the enzyme activity also did not increase the Ab_{50} value, whereas in isotonic medium the Ab_{50} value was increased 1.5-fold. These results indicate that the observed changes in enzyme activity are due to an alteration in the amount of enzyme molecules.

The present studies show that the activity of *S*-adenosyl-L-methionine decarboxylase is increased in the presence of α-methyl ornithine, which prevents the increase in the intracellular level of putrescine, whereas the augmentation of the putrescine level by hypotonic culture or by exogenous addition of putrescine blocks the subsequent increase in the enzyme activity. The observed apparent inverse relationship between the intracellular concentration of putrescine and the activity of *S*-adenosyl-L-methionine decarboxylase in cultured mammary tissue is quite unexpected: previous studies [1—12] showed that the activity of mammalian *S*-adenosyl-L-methionine decarboxylase is almost totally dependent on the presence of putrescine in the assay system and that the increase in the activity in cells often occurs subsequent to the elevation of the intracellular concentration of putrescine. Recently Mamont et al. [18] also reported that the ornithine decarboxylase inhibitor increases the activity of *S*-adenosyl-L-methionine decarboxylase in HTC cells in culture. It is also noteworthy that the activity of *S*-adenosyl-L-methionine decarboxylase in

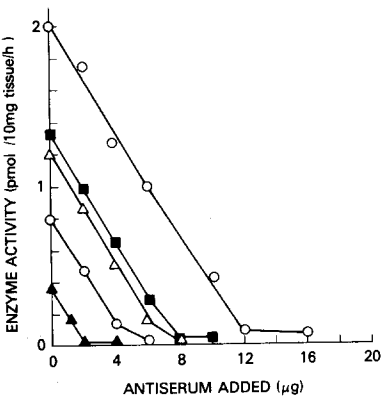

Fig. 1. Determination of Ab_{50} for *S*-adenosyl-L-methionine decarboxylase in mammary explants. *S*-Adenosyl-L-methionine decarboxylase preparations containing various amounts of enzyme activities (see Table I and II) were incubated with varying amounts of anti-*S*-adenosyl-L-methionine decarboxylase serum in a final volume of 0.2 ml. The mixtures were incubated at 27° C for 30 min and then at 4° C overnight. The mixtures were centrifuged and the supernatant fractions were assayed for remaining enzyme activity. Ab_{50} is determined graphically. Samples from Table I. (▲———▲), uncultured control; (■———■), insulin system; (○———○), insulin plus α-methyl ornithine system (3-times diluted). Samples from Table II. (△———△), isotonic system; (●———●), hypotonic system.

TABLE III

COMPARISON OF THE ACTIVITY AND Ab_{50} OF *S*-ADENOSYL-L-METHIONINE DECARBOXYLASE IN MAMMARY EXPLANTS IN CULTURE

Expts. I and II refer to the experiments described in Tables I and II, respectively. Ab_{50} values were tabulated from Fig. 1.

Culture condition	*S*-Adenosyl-L-methionine decarboxylase	
	Activity (pmol/10 mg tissue per h)	Ab_{50} (μg)
Expt. I		
Uncultured control	0.38	1.2
Insulin	1.33	3.8
Insulin + α-methyl ornithine	6.06	18
Expt. II		
Uncultured control	0.80	2.2
Isotonic culture 6 h	1.2	3.6
Hypotonic culture 6 h	0.80	2.2

Ehrlich ascites cells inoculated into mice increases in response to the acute treatment with putrescine, but decreases after the chronic treatment [19]. More recently it was suggested by Hopkins and Manchester [20] that in muscle *S*-adenosyl-L-methionine decarboxylase may be regulated by spermidine.

At present, the apparent disparity regarding the actions of putrescine on *S*-adenosyl-L-methionine decarboxylase in the assay system and in cells cannot be readily explained. It is possible, however, that putrescine has dual regulatory functions such that it acts as an activator of the decarboxylase at low concentrations ($<10^{-7}$ M) but can exert a negative influence at higher concentrations. In this respect, it is noteworthy that putrescine has been shown to exert negative control on ornithine decarboxylase activity via the induction of 'anti-enzyme' which apparently inhibits synthesis of ornithine decarboxylase [21]. Alternatively, it may be that putrescine in cells is compartmentalized or exists in bound forms because of its cationic character; hence the total cellular content of the diamine could not be directly related to the changes in the activity of *S*-adenosyl-L-methionine decarboxylase. Further delineation of the function of putrescine in cellular regulation of *S*-adenosyl-L-methionine decarboxylase should facilitate a better understanding of the overall regulatory mechanism of polyamine biosynthesis.

α-Methyl ornithine, an inhibitor of ornithine decarboxylase, has been used to assess the role of putrescine in cell growth and function. However, the observed stimulatory effect of the drug on *S*-adenosyl-L-methionine decarboxylase may complicate the interpretation of experimental results obtained with the drug and points out the need to evaluate carefully its side effects.

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