DICYCLOHEXYLAMINE, A POTENT INHIBITOR OF SPERMIDINE SYNTHASE IN MAMMALIAN CELLS

H. HIBASAMI, M. TANAKA, J. NAGAI and T. IKEDA
Department of Biochemistry, Mie University School of Medicine, Tsu, Mie 514, Japan

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1. Introduction

Much effort has been made to develop specific inhibitors of polyamine synthesis which may help in elucidating the role of polyamines in cellular metabolism and in cell proliferation in particular [1–7]. Furthermore, inhibitors of polyamine synthesis may find applications as antiproliferative agents. Four enzymes are involved in the synthesis of polyamines in eukaryotic cells, i.e., ornithine decarboxylase, *S*-adenosylmethionine(SAM) decarboxylase and two aminopropyltransferases, one catalyzing the synthesis of spermidine, the other producing spermine [8–12]. Most inhibitors used are inhibitors of the two decarboxylases, whereas several inhibitors are reported at the aminopropyltransferases [13–15]. The relative activities of these enzymes must determine which of the polyamines accumulate in the cells. Marked differences in the spermidine to spermine ratio have been observed in comparisons of various mammalian cells [8,16] and spermidine levels are usually elevated in response to growth-promoting stimuli [8,16,17]. Here we demonstrate that partially purified spermidine synthase from rat ventral prostate was strongly inhibited by dicyclohexylamine and also by cyclohexylamine, and that administration of dicyclohexylamine caused a decrease in the concentration of spermidine but not spermine in the liver of partially hepatectomized rats. These inhibitors may be useful as experimental tools for elucidating the physiological function of the polyamines.

2. Materials and methods

2.1. *Chemicals*

Dicyclohexylamine sulfate(dicyclohexylammonium sulfate) was purchased from Sigma Chemical Co. Cyclohexylamine hydrochloride and aniline sulfate were products of Wako Pure Chemical Indust. *S*-Adenosyl-L-[*methyl*-^{14}C]methionine (spec. act. 50–60 mCi/mmol) was obtained from New England Nuclear Corp. Decarboxylated SAM, both unlabeled and labeled in the methyl group was prepared by the action of SAM decarboxylase from *Escherichia coli* and purified by chromatography on Dowex-50-H$^+$ and high-voltage paper electrophoresis [18]. All other chemicals were products of Nakarai Chemicals Ltd.

2.2. *Animals*

Male Sprague-Dawley rats (160 g) were used for all experiments. Partial hepatectomy was performed under light ether anaesthesia as in [19]. Dicyclohexylamine sulfate was dissolved in 0.14 M NaCl before administration and injected intraperitoneally at every 24 h after partial hepatectomy (starting at the time of operation) until animals were sacrificed by decapitation.

2.3. *Preparation of spermidine and spermine synthases*

Spermidine and spermine synthases (aminopropyltransferases) were purified from rat ventral prostate as in [20,21]. Extracts containing both enzyme activities were fractionated into spermidine synthase and spermine synthase preparations by the treatment with ammonium sulfate followed by DEAE-cellulose and Ultrogel ACA 34 column chromatography. The enzyme preparations used here were ~250-fold (spermidine synthase) and 160-fold (spermine synthase) of purification over the specific activity present in the crude ultracentrifugal extracts.

2.4. *Assay of aminopropyltransferase activities*

The aminopropyltransferase activity was deter-

mined by measuring the production of [*methyl*-^{14}C]-methylthioadenosine from decarboxylated *S*-adenosyl-[*methyl*-^{14}C]methionine in the presence of putrescine (spermidine synthase) or of spermidine (spermine synthase) as in [12]. The assay medium contained 100 mM sodium phosphate buffer (pH 7.5), 5 mM dithiothreitol, 40 μM decarboxylated *S*-adenosyl-[*methyl*-^{14}C] methionine (2 μCi/μmol), putrescine or spermidine (concentrations indicated in the legends) and the enzyme preparation in 0.2 ml total vol. Assays were incubated at 37°C for 30 min. The production of methylthioadenosine was entirely dependent on putrescine and spermidine.

2.5. *Determination of polyamines*

The amounts of spermidine and spermine present in the liver were determined as in [22]; homogenization of the tissue in 0.1 N HCl, extraction of amines into *n*-butanol at alkaline pH and separation by paper electrophoresis followed by staining with ninhydrin.

3. Results and discussion

Fig.1 shows the dose–response curve for dicyclohexylamine on the inhibition of spermidine synthase and spermine synthase activities. Spermidine synthase was much more sensitive to the inhibitor than spermine synthase. The effect of the concentration of putrescine on the inhibition of spermidine synthase by dicyclohexylamine is shown in fig.2. This inhibition was competitive and the calculated K_i of dicyclohexylamine was 0.2 μM; K_m for putrescine was 29 μM.

Removal of dicyclohexylamine from the active form of spermidine synthase by dialysis restored the activity to that found in preparations treated similarly except for exposure to the inhibitor.

Dicyclohexylamine, when given to animals, was expected to lower the cellular spermidine level without affecting the spermine level. Fig.3 shows effects of administration of dicyclohexylamine on polyamine contents in the remnant livers of partially hepatec-

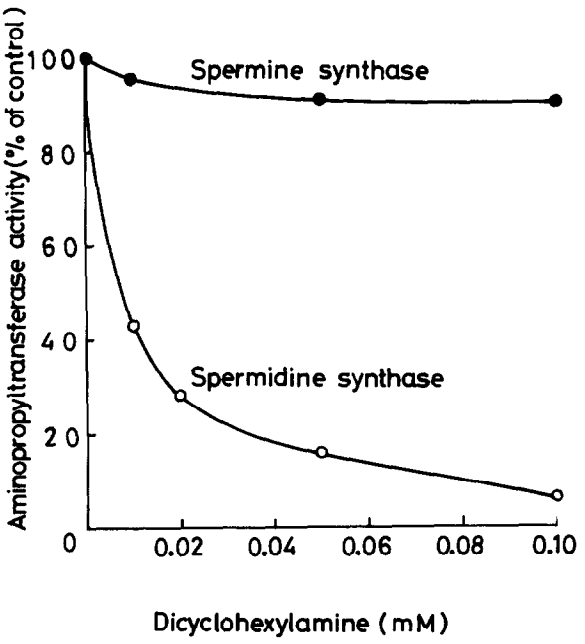

Fig.1. Inhibition of spermidine synthase and spermine synthase by dicyclohexylamine. The results are expressed as percentages of the control activity measured in the absence of dicyclohexylamine. Putrescine and spermidine were 0.5 mM. Experimental details are given in the text.

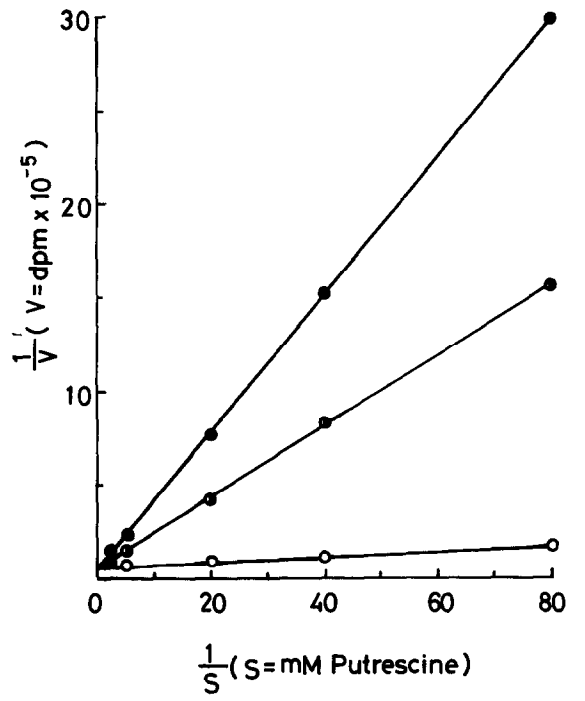

Fig.2. Competitive inhibition of spermidine synthase by dicyclohexylamine with putrescine as the variable substrate. Spermidine synthase activity was assayed in the absence (○) or presence of 5 μM (◑) or 10 μM (●) dicyclohexylamine, 40 μM decarboxylated *S*-adenosylmethionine and 0.0125–0.5 mM putrescine and an enzyme preparation.

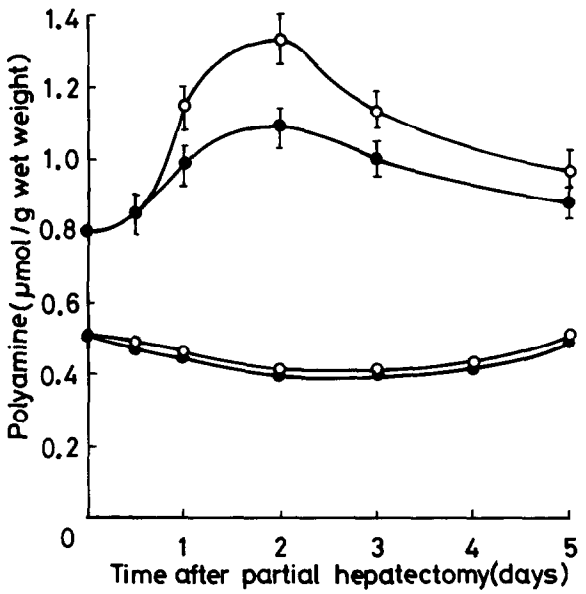

Fig.3. Effect of administration of dicyclohexylamine on polyamine concentrations in the liver after partial hepatectomy. Groups of 3 rats were partially hepatectomized and injected intraperitoneally with dicyclohexylamine (200 mg/kg) (•) or saline (○) every 24 h after partial hepatectomy (starting at the time of operation). Polyamine levels were determined as described in text. The upper two lines refer to spermidine, the lower two lines to spermine.

tomized rats. A dose of 200 mg/kg caused a decrease in the concentration of spermidine with little effect on the spermine concentration, suggesting a decrease in spermidine synthase activity.

As shown in table 1, spermidine synthase activity was inhibited also by cyclohexylamine to the same extent as by dicyclohexylamine. A cyclohexane ring having either a primary- or secondary-amino group might be essential for the inhibition of spermidine synthase activity. In this connection it is of interest that the benzene ring having an amino group had no inhibitory effect.

Table 1
A comparison of the inhibitory effect of dicyclohexylamine on spermidine synthase activity with those of related compounds

Compound	Concn. (mM)	Inhibn. (%)
Dicyclohexylamine	0.1	93.6
Cyclohexylamine	0.1	88.7
Aniline	0.1	2.7
Tris-(hydroxymethyl)aminomethane	0.1	0.0

Spermidine synthase activity was measured with the addition of the compound shown. The pH value of the reaction mixture was not changed by the addition of the compounds shown to the assay. Details of the experimental conditions were as described in the text

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