Inhibition of Ornithine Decarboxylase of HeLa Cells by Diamines and Polyamines

EFFECT ON CELL PROLIFERATION

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(Received 12 September 1979)

1. Ornithine decarboxylase activity is stimulated in high-density HeLa-cell cultures by dilution of or replacement of spent culture medium with fresh medium containing 10% (v/v) horse serum. 2. After stimulation, ornithine decarboxylase activity reaches a peak at 4–6 h, then rapidly declines to the low enzyme activity characteristic of quiescent cultures, where it remains during the remainder of the cell cycle. 3. The stimulation of ornithine decarboxylase is eliminated by the addition of 0.5 μ M-spermine or -spermidine or 10 μ M-putrescine to the HeLa-cell cultures at the time of re-feeding with fresh medium. Much higher concentrations (1 mM) of the non-physiological diamines, 1,3-diaminopropane or 1,3-diamino-2-hydroxypropane, are required to eliminate the stimulation of ornithine decarboxylase in re-fed HeLa-cell cultures. 4. A heat-labile, non-diffusible inhibitor, comparable with the inhibitory protein ornithine decarboxylase antizyme, is induced in HeLa cells by the addition of exogenous diamines or polyamines. 5. Intracellular putrescine is eliminated, intracellular spermidine and spermine are severely decreased and proliferation of HeLa cells is inhibited when cultures are maintained for 48 h in the presence of the non-physiological inducer of ornithine decarboxylase antizyme, 1,3-diamino-2-hydroxypropane. Exogenous putrescine, a physiological inducer of the antizyme, does not decrease intracellular polyamines or interfere with proliferation of HeLa cells.

Ornithine decarboxylase (L-ornithine carboxylase, EC 4.1.1.17) produces putrescine, which is the precursor for the biosynthesis of the polyamines, spermidine and spermine. The appearance of an ornithine decarboxylase-inhibitory protein on the addition of putrescine to H-35 hepatoma-cell cultures was reported by Fong *et al.* (1976). The protein inhibitor was also induced by putrescine in L1210 and neuroblastoma cells as well as in rat liver, and spermidine or spermine induced the synthesis of a similar inhibitor in H-35 cells (Heller *et al.*, 1976). The inhibitor derived from the animal cell cultures or rat liver was eluted from Sephadex G-75 columns in a protein fraction with an apparent mol.wt of 26 500 and formed enzymically inactive complexes with ornithine decarboxylase (Heller *et al.*, 1976). The name 'antizyme' was proposed for these proteins, and similar ornithine decarboxylase antizymes were identified as normal components, without the necessity of induction by exogenous polyamines, in H-35 cells and rat liver (Heller *et al.*, 1977). The induction of apparently comparable orni-

thine decarboxylase antizymes by putrescine or other diamines was confirmed in rat liver by Kallio *et al.* (1979) and Pegg *et al.* (1978), in rat thyroid by Friedman *et al.* (1977) and in rat hepatoma (HTC) cells by McCann *et al.* (1977). A lower-mol.wt. ($15\,000 \pm 2000$) ornithine decarboxylase antizyme was induced by the addition of polyamines to *Escherichia coli* strains MA255 and MA197 (Kyriakidis *et al.*, 1978).

It has been proposed that the antizyme proteins of eukaryotic cells (Heller *et al.*, 1977) and of prokaryotic cells (Kyriakidis *et al.*, 1978) have a role in the modulation of ornithine decarboxylase activity and the intracellular concentrations of putrescine, spermidine and spermine. There is evidence that the induction of ornithine decarboxylase antizyme by diamines that cannot serve as precursors of the naturally occurring polyamines decreases the intracellular concentrations of the polyamines. For example, injections of 1,3-diaminopropane or 1,3-diamino-2-hydroxypropane into partially hepatectomized rats completely prevented the stimulation of

ornithine decarboxylase and the accumulation of spermidine in regenerating liver (Pösö & Jänne, 1976; Piik *et al.*, 1978), and the administration of cadaverine to mice carrying peritoneal Ehrlich ascites cells decreased the ornithine decarboxylase activity and spermidine and putrescine concentrations of the tumour cells (Kallio *et al.*, 1977). Furthermore, the induction of the inhibitor and the decrease in cellular polyamines decreased DNA synthesis in regenerating liver (Pösö & Jänne, 1976) and in CHO cells (Sunkara *et al.*, 1977) and blocked the growth of goitrogen-stimulated rat thyroid glands (Friedman *et al.*, 1977).

Prouty (1976) established the rapid induction by glutamine of ornithine decarboxylase in HeLa cells, and Munro *et al.* (1975) reported the rapid inactivation of ornithine decarboxylase by exogenous putrescine in HeLa cultures. In the present work we have demonstrated the induction of ornithine decarboxylase antizyme in HeLa cells grown in suspension culture. Ornithine decarboxylase activity was eliminated, intracellular polyamines were decreased and proliferation of HeLa cells was blocked in cultures in which ornithine decarboxylase antizyme was induced by 1,3-diamino-2-hydroxypropane.

Experimental

Chemicals

Putrescine, spermidine and spermine were obtained from Calbiochem-Behring Corp. (LaJolla, CA, U.S.A.) as the hydrochlorides. 1,3-Diaminopropane dihydrochloride and 1,3-diamino-2-hydroxypropane were purchased from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). The free base was neutralized with HCl and all solutions added to the cultures were sterilized by filtration through 0.22 μ m-pore-size membrane filters.

Cell cultures

HeLa-cell stocks were obtained from Professor G. C. Mueller, McArdle Laboratory for Cancer Research (Madison, WI, U.S.A.) and the culture media and conditions for maintenance of cell stocks were based on established culture procedures of that laboratory (Mueller *et al.*, 1962). Stock cultures were grown in silicone-treated spinner flasks at 37°C in Ca²⁺- and Mg²⁺-free BME medium (GIBCO, Grand Island, NY, U.S.A.) supplemented further with 0.1 mM-glycine, 0.1 mM-serine, 0.01 mM-inositol, 0.1% (w/v) of the surfactant Pluronic F-68 (Wyandotte Chemical Co., Wyandotte, MI, U.S.A.) and 10% (v/v) horse serum (GIBCO). Experimental suspension cultures were diluted from high-density spinner stocks (6 $\times$ 10⁵ cells/ml) with fresh BME medium, supplemented as above, to an initial density of 3 $\times$ 10⁵ cells/ml, and were incubated in a

rotary shaker-incubator (150 rev./min at 37°C) in silicone-treated round-bottomed flasks gassed with CO₂/air (1:19). The generation time under these conditions averaged about 23 h. Cell counts were determined in a haemocytometer and viability was estimated by counting cells excluding Trypan Blue. Cultures were harvested by centrifugation in the cold at 1200g for 3 min, cell pellets were washed with cold 0.9% NaCl and cell extracts were prepared by freezing and thawing cell suspensions four or five times in water (for polyamine analysis) or in Tris Assay Buffer (for ornithine decarboxylase assays).

Ornithine decarboxylase assay

Frozen-and-thawed cell suspensions in Tris Assay Buffer (50 mM-Tris/HCl, pH 7.2, 0.1 mM-EDTA, 0.05 mM-pyridoxal phosphate, 5 mM-dithiothreitol) were centrifuged at 3000g for 15 min in the cold. The supernatant was removed and either assayed immediately or dialysed against Tris Assay Buffer before assay for ornithine decarboxylase activity. The enzyme activity was determined by measuring the release of ¹⁴CO₂ from DL-[1-¹⁴C]ornithine. Assays were performed in 15 ml Corex centrifuge tubes (17 mm $\times$ 100 mm) equipped with a rubber septum and polyethylene centre-well assembly (Kontes Glass Co., Vineland, NJ, U.S.A.) containing 50 μ l of NCS solubilizer (Amersham/Searle Corp., Arlington Heights, IL, U.S.A.) on a 1 cm-square folded filter paper as a CO₂-trapping agent. Assays contained Tris Assay Buffer, 0.2 mM-L-ornithine monohydrochloride (Calbiochem-Behring Corp.) containing 0.25 μ Ci of DL-[1-¹⁴C]ornithine monohydrochloride (30 mCi/mmol; New England Nuclear Corp., Boston, MA, U.S.A.) and cell supernatant in a total volume of 0.25 ml. Incubations were carried out with gentle shaking for 1 h at 37°C and the reaction was terminated and CO₂ was released from the assay medium by the injection of 0.25 ml of 0.2 M-H₂SO₄ through the rubber septum; the incubation was continued for a further 30 min to ensure complete absorption of the ¹⁴CO₂ by the NCS trapping agent on the filter paper. The filter paper was transferred to scintillation vials containing 5 ml of toluene/Omnifluor (New England Nuclear Corp.) and radioactivity was measured in a Packard Tri-Carb scintillation counter at 70% efficiency. All enzyme activities were corrected against a blank heated at 100°C for 10 min and assayed as described above.

Polyamine determinations

Frozen-and-thawed cell extracts in water were centrifuged at 3000g for 15 min in the cold, the supernatants were removed and an equal volume of cold 0.4 M-HClO₄ was added. After precipitation in the cold overnight, the precipitated protein was removed by centrifugation at 2000g for 10 min and

the supernatant was removed and frozen until analyses were carried out. The analysis of polyamines was performed by the modification of Seiler's dansyl (5-dimethylaminonaphthalene-1-sulphonyl) procedure previously described (Herbst & Dion, 1970).

Ornithine decarboxylase antizyme assays

Ornithine decarboxylase antizyme was assayed in HeLa-cell cultures supplemented with diamines or polyamines by the procedure developed by Fong *et al.* (1976) and Heller *et al.* (1976) for the detection of the antizyme in cell cultures. The diamine or polyamine inducer was added to quiescent high-density (6×10^5 cells/ml) suspension cultures or to cultures in which ornithine decarboxylase was stimulated by dilution of high-density cultures with an equal volume of fresh BME medium. (Induction of the antizyme was not dependent on the prior or simultaneous stimulation of ornithine decarboxylase.) Cultures were harvested after 3–4 h incubation in the presence of the amine inducer, and washed cell pellets and broken-cell supernatants were prepared as described above for the assay of ornithine decarboxylase. Each assay contained 0.25–0.5 unit of HeLa-cell ornithine decarboxylase, increasing amounts of broken-cell supernatant (usually from 25 to 150 μ l) that had been dialysed overnight against 2×100 vol. of 'dialysis buffer' (50 mM-Tris/HCl, pH 7.2, 0.1 mM-EDTA, 0.04 mM-pyridoxal phosphate and 10 mM-mercaptoethanol), substrate (L-ornithine containing DL-[1- 14 C]ornithine) and Tris Assay Buffer in a total volume of 0.25 ml under conditions of incubation, 14 CO $_2$ trapping and liquid-scintillation counting employed in the ornithine decarboxylase assay. The ornithine decarboxylase antizyme activity was estimated from a titration curve derived from the decrease in ornithine decarboxylase activity plotted against volume of antizyme-containing dialysed broken-cell supernatant. One unit of ornithine decarboxylase is defined as the enzyme activity that releases 1 nmol of CO $_2$ /h and 1 unit of ornithine decarboxylase antizyme is defined as the activity required to inhibit 1 unit of enzyme activity.

Preparation of ornithine decarboxylase

HeLa-cell ornithine decarboxylase was induced by the dilution of high-density (6×10^5 cells/ml) cultures with an equal volume of fresh BME medium and, after 4 h of incubation with shaking at 37°C, the cells were harvested by centrifugation, washed with saline, and broken by freezing and thawing in Tris Assay Buffer as described above. Ornithine decarboxylase was partially purified by precipitation of inactive proteins in broken-cell supernatants with 20%-satd. (NH $_4$) $_2$ SO $_4$ followed by precipitation of the enzyme by increasing the (NH $_4$) $_2$ SO $_4$ to 50% saturation. The proteins precipitated by 50%-satd.

(NH $_4$) $_2$ SO $_4$ were collected by centrifugation at 10000g for 15 min, dissolved in dialysis buffer (50 mM-Tris/HCl, pH 7.2, 0.1 mM-EDTA, 0.05 mM-pyridoxal phosphate and 10 mM-mercaptoethanol) and dialysed overnight in the cold against 2×100 vol. of dialysis buffer. Dialysed preparations of the (NH $_4$) $_2$ SO $_4$ -fractionated enzyme were stored at -70° C with little loss of activity in storage.

Results

Ornithine decarboxylase activity of HeLa-cell cultures

The ornithine decarboxylase activity of HeLa cells in high-density cultures is low and is not increased when such quiescent cultures are incubated in the 'spent' BME medium (Fig. 1). When high-density cultures are 're-fed' by dilution with fresh BME medium to a cell density of 3×10^5 cells/ml, the ornithine decarboxylase activity increases sharply after 4–6 h of incubation at 37°C, but the enzyme activity is transient and falls to a low value characteristic of the quiescent high-density cultures during the remainder of the cell cycle (Fig. 1).

Fig. 1. Time course of ornithine decarboxylase activation in HeLa-cell cultures

Ornithine decarboxylase activity was stimulated in a high-density HeLa-cell culture (6×10^5 cells/ml) by dilution with fresh BME medium to an initial cell density of 3×10^5 cells/ml. An initial sample of culture was removed, and at 2 h intervals thereafter, during incubation with shaking at 37°C, further samples of culture were removed, cell extracts were prepared and ornithine decarboxylase activity was assayed (●—●) as described in the Experimental section. The high-density culture (6×10^5 cells/ml) was also incubated without dilution and samples of culture were removed at the indicated intervals and ornithine decarboxylase activity was assayed on cell extracts of this unstimulated control culture (●---●).

Decrease of ornithine decarboxylase activity and induction of ornithine decarboxylase antizyme by diamines and polyamines

As reported previously in studies with H-35 (Fong *et al.*, 1976) and HTC (McCann *et al.*, 1977) rat hepatoma-cell cultures and with Ehrlich ascites cells (Kallio *et al.*, 1977), ornithine decarboxylase activity of HeLa cells is decreased by the addition of polyamines or diamines to the culture medium (Table 1). Furthermore, the presence of an inhibitor activity, entirely analogous to the ornithine decarboxylase antizyme demonstrated in the studies with cell cultures cited above and in rat liver (Heller *et al.*, 1976; Pegg *et al.*, 1978; Kallio *et al.*, 1979), was detected by the antizyme titration assay (Table 1). In sharp contrast with all prior studies with cell cultures, in which high concentrations (1–10 mM) of diamines or polyamines were required to induce

demonstrable titres of free ornithine decarboxylase antizyme, we have induced the antizyme in HeLa cells with low concentrations of the diamines and polyamines in the range of the dose response for the inhibition of endogenous ornithine decarboxylase by these compounds.

A close similarity between the ornithine decarboxylase antizyme of HeLa cells and the inhibitory proteins previously characterized (Heller *et al.*, 1976; Fong *et al.*, 1976) is indicated. The inhibitory activity was not decreased by exhaustive dialysis, and it was completely destroyed by boiling the dialysed cell extract for 10 min (results not shown). Furthermore, the inhibitory activity cannot be attributed to diamine or polyamine 'toxicity', in view of its heat lability, and since only traces of the amines added to the culture medium can be detected in the dialysed cell extracts.

Table 1. *Decrease of ornithine decarboxylase activity and induction of ornithine decarboxylase antizyme by diamines and polyamines*

For experimental details see the text and the footnotes.

Inhibitor (inducer)	Inhibition of ornithine decarboxylase activity (%)*	Free antizyme (unit/10 ⁶ cells)†
1,3-Diamino-2-hydroxypropane (mM)		
0.1	1.1	0.007
0.2	43.4	0.013
0.5	91.8	0.019
1.0	97.1	0.022
1,3-Diaminopropane (mM)		
0.1	40.9	0.008
0.2	68.6	0.011
0.5	93.2	0.020
1.0	97.0	0.022
Putrescine (μM)		
1	45.9	0
5	81.7	0.014
10	88.0	0.017
50	93.5	0.020
Spermidine (μM)		
0.1	15.7	0
0.5	91.0	0.013
1.0	93.1	0.017
5.0	94.1	0.021
Spermine (μM)		
0.1	48.8	0
0.5	89.1	0.016
1.0	93.0	0.020
5.0	93.3	0.022

* Ornithine decarboxylase activity was induced by the addition of an equal volume of fresh BME medium to high-density (6×10^5 cells/ml) HeLa-cell cultures. Diamines or polyamines were added at the concentrations indicated and the inhibition of ornithine decarboxylase activity was calculated with reference to unsupplemented control cultures.

† High-density (6×10^5 cells/ml) HeLa-cell cultures were incubated in the 'spent' medium for 24 h to decrease the endogenous ornithine decarboxylase activity (0.053 unit/10⁶ cells). Diamines or polyamines were added at the concentrations indicated and, after 3 h incubation, cell supernatants were prepared and ornithine decarboxylase antizyme assays were performed by using 0.25 unit of purified HeLa-cell ornithine decarboxylase as described in the Experimental section.

Table 2. *Effect of inducers of ornithine decarboxylase antizyme on proliferation of HeLa cells*

HeLa-cell suspension cultures at comparable low cell density in BME medium or in BME medium containing antizyme inducer were prepared at zero time. After 24 h incubation (1 'cell cycle') viable cell counts were determined, medium was removed by centrifugation (50 g/10 min) at 25°C and cultures were adjusted to a comparable low cell density with fresh BME medium or with BME medium containing antizyme inducer. At 48 h (2 'cell cycles') viable cell counts were determined, medium was removed by centrifugation and all cell cultures were adjusted to a comparable low cell density with fresh BME medium ('None'). After 72 h (3 'cell cycles') viable cell counts were determined on all cultures. Cell counts are the means of triplicate cultures $\pm$ S.E.M. Numbers in parentheses are the cell density to which the culture was adjusted after the change of medium.

Antizyme inducer	$10^{-3} \times$ Cell density (cells/ml) at:			
	0 h	24 h	48 h	72 h
Expt. 1				
None	266	533 $\pm$ 9 (266)	534 $\pm$ 19 (267)	562 $\pm$ 9
1,3-Diamino-2-hydroxypropane (1 mM)	266	411 $\pm$ 7 (257)	263 $\pm$ 11 (263)	429 $\pm$ 9
Expt. 2				
None	300	570 $\pm$ 19 (285)	580 $\pm$ 12 (290)	570 $\pm$ 36
Putrescine (1 mM)	300	584 $\pm$ 23 (292)	484 $\pm$ 27 (242)	424 $\pm$ 33

Effect of ornithine decarboxylase antizyme on proliferation of HeLa cells

The inhibition of ornithine decarboxylase by the competitive inhibitors α -methylornithine or α -difluoromethylornithine blocks the proliferation of rat hepatoma (HTC) cells and mouse leukaemia (L1210) cells in culture (Mamont *et al.*, 1978). The anti-proliferative properties of the inhibitors can be reversed by putrescine, spermidine or spermine. Similar anti-proliferative effects on cell cultures of the inhibition of ornithine decarboxylase by inducers of ornithine decarboxylase antizyme have not been clearly delineated.

Evidence, summarized in Table 2, for the complete inhibition of HeLa-cell proliferation by the induction of ornithine decarboxylase antizyme by 1,3-diamino-2-hydroxypropane has been obtained. Maintenance of HeLa-cell cultures in the presence of 1 mM-1,3-diamino-2-hydroxypropane, a concentration of non-physiological diamine that promotes the formation of ornithine decarboxylase antizyme (see Table 1), partially prevented HeLa-cell proliferation during the first cell cycle (24 h) and completely blocked all proliferation at 48 h (Table 2). The anti-proliferative effect of the ornithine decarboxylase antizyme inducer does not destroy the viability of the arrested culture, since cell replication occurs after removal of the antizyme inducer from the media.

In contrast with the results obtained with the non-physiological diamine (1,3-diamino-2-hydroxypropane), a physiological inducer of ornithine decarboxylase antizyme, putrescine, does not block the proliferation of HeLa cells, although there is a slightly decreased viable cell count at 48 h and after removal of putrescine from the culture medium (Table 2).

The interpretation of the differential effect of the two antizyme inducers on HeLa-cell proliferation is clarified by the data (Table 3) on the diamine and polyamine contents of HeLa cells grown in the absence or presence of the non-physiological (1,3-diamino-2-hydroxypropane) or physiological (putrescine) diamines. Growth of HeLa cells in medium containing 1 mM-1,3-diamino-2-hydroxypropane seriously depletes the intracellular putrescine and lowers the polyamine content of the cells to about 20% of the spermidine concentration and 30% of the spermine concentration of HeLa cells grown in the absence of the antizyme inducer. On the other hand, the normal physiological polyamine content of HeLa cells is not seriously depleted by the high intracellular concentration of putrescine, although there is a slight decrease in both spermidine (15%) and spermine (25%) as compared with control cells grown in the absence of putrescine.

Discussion

Ornithine decarboxylase activity is increased by the dilution of high-density HeLa-cell cultures with fresh culture medium. The time course of the induction of the enzyme resembles the pattern previously established in other cell cultures (Fong *et al.*, 1976; Heller *et al.*, 1976), and the early activity peak (4–6 h) and rapid inactivation of ornithine decarboxylase is identical with the pattern of activation and inactivation of the enzyme in HeLa-cell cultures supplemented with 10 mM-glutamine (Prouty, 1976). Activation of the enzyme occurs only once during a cell cycle (Fig. 1), and the peak of the enzyme activity at 4–6 h is far in advance of cell proliferation, which can be detected at 20–24 h under these culture conditions.

Table 3. *Effect of ornithine decarboxylase antizyme on polyamine content of HeLa cells*

HeLa-cell suspension cultures (3×10^5 cells/ml) in BME medium or in BME medium containing antizyme inducer were prepared at zero time and, after 24 h incubation (0–24 h), samples of each culture were removed and cell extracts were prepared and analysed for polyamines as described in the Experimental section. Viable cell counts were made on the 24 h cultures, medium was removed by centrifugation (50 g/10 min) at 25°C and cultures were again adjusted to 3×10^5 cells/ml with fresh BME medium or with BME medium containing antizyme inducer. After a second 24 h period of incubation (24–48 h), samples were again removed for polyamine analysis, viable cell counts were made, medium was removed by centrifugation and all cultures were adjusted to 3×10^5 cells/ml with fresh BME medium ('None'). After an additional 24 h period of incubation (48–72 h), samples were removed for polyamine analysis and viable cell counts were determined. Polyamine data are the means of the concentration in cell extracts from triplicate cultures $\pm$ S.E.M.

Antizyme inducer	Incubation (h)	Diamine or polyamine (nmol/10 ⁶ cells)			
		1,3-Diamino-2-hydroxypropane	Putrescine	Spermidine	Spermine
Expt. 1					
None	0–24	Absent	1.58 $\pm$ 0.10	3.09 $\pm$ 0.07	3.50 $\pm$ 0.21
None	24–48	Absent	1.78 $\pm$ 0.07	3.83 $\pm$ 0.12	5.25 $\pm$ 0.26
None	48–72	Absent	1.81 $\pm$ 0.01	3.53 $\pm$ 0.12	4.78 $\pm$ 0.30
1,3-Diamino-2-hydroxypropane (1 mM)	0–24	9.84 $\pm$ 0.36	0.34 $\pm$ 0.03	0.85 $\pm$ 0.12	2.83 $\pm$ 0.11
1,3-Diamino-2-hydroxypropane (1 mM)	24–48	16.25 $\pm$ 2.34	Absent	0.55 $\pm$ 0.04	1.65 $\pm$ 0.04
None	48–72	3.14 $\pm$ 0.41	0.53 $\pm$ 0.07	1.59 $\pm$ 0.17	7.25 $\pm$ 0.84
Expt. 2					
None	0–24	Absent	1.28 $\pm$ 0.17	2.31 $\pm$ 0.25	2.84 $\pm$ 0.18
None	24–48	Absent	1.10 $\pm$ 0.13	2.56 $\pm$ 0.39	3.27 $\pm$ 0.59
None	48–72	Absent	0.96 $\pm$ 0.09	2.51 $\pm$ 0.21	3.50 $\pm$ 0.17
Putrescine (1 mM)	0–24	Absent	17.89 $\pm$ 1.39	1.87 $\pm$ 0.07	2.00 $\pm$ 0.07
Putrescine (1 mM)	24–48	Absent	23.77 $\pm$ 1.13	2.11 $\pm$ 0.19	2.40 $\pm$ 0.22
None	48–72	Absent	2.35 $\pm$ 0.14	3.24 $\pm$ 0.30	3.53 $\pm$ 0.40

The enzyme-activity peak is completely eliminated by the addition of low (1–10 μ M) concentrations of the naturally occurring 'polyamines' of HeLa cells to the culture medium. Exogenous spermine and spermidine are the most active inducers of this inhibitory response, and putrescine is about one-tenth as effective (Table 1). Addition of diamines structurally related to putrescine, but absent from HeLa cells, e.g. 1,3-diaminopropane or the 2-hydroxy derivative, also inhibits the ornithine decarboxylase activity, but only at concentrations that are 100 times greater than the concentration of putrescine that induces comparable inhibition.

The inhibition of endogenous ornithine decarboxylase activity of HeLa cells by diamines and polyamines and the induction of the inhibitor, ornithine decarboxylase antizyme, appear to be identical phenomena. The dose responses for the inhibition of ornithine decarboxylase by both physiological polyamines and non-physiological diamines and for the induction of ornithine decarboxylase antizyme are comparable in HeLa cells. In contrast with these results, McCann *et al.* (1979) were unable to demonstrate the induction of ornithine decarboxylase antizyme in a clone of HTC cells with greatly

stabilized ornithine decarboxylase (HMO_A cells) by 10 μ M-putrescine, a concentration of diamine which inhibited the endogenous enzyme activity in these cultures. A high concentration of putrescine (10 mM), on the other hand, induced the formation of ornithine decarboxylase antizyme in HMO_A cells. The apparent dependence of the induction of ornithine decarboxylase antizyme on high concentrations of polyamines led these investigators (McCann *et al.*, 1979) to postulate the existence of two distinct mechanisms for the regulation of ornithine decarboxylase: a 'blocking' of ornithine decarboxylase synthesis by low concentrations of polyamines, and the inhibition of endogenous enzyme activity by the induction of ornithine decarboxylase antizyme by high concentrations of polyamines.

The comparable dose responses for the inhibition of endogenous ornithine decarboxylase and for the induction of ornithine decarboxylase antizyme of HeLa cells by diamines and polyamines suggest that, in this culture system, there is no need to invoke the existence of separate and distinct mechanisms for the regulation of ornithine decarboxylase activity.

The anti-proliferative effect of 1,3-diamino-2-hydroxypropane is of special interest because of the

low toxicity of this inducer of the ornithine decarboxylase antizyme. Viability of HeLa cells is not seriously decreased by exposure of the growth-arrested cultures to the diamine for at least 48 h, as is evident from the essentially normal growth of cultures after removal of the inducer (Table 2). Especially remarkable is the decrease of the entire physiological polyamine pool; putrescine is eliminated, spermidine is severely decreased to only 15% of that in the control culture, and spermine is decreased by almost 70% (Table 3). The lowering of the cellular spermine content has been difficult or impossible to achieve by the inhibition of ornithine decarboxylase with either reversible or irreversible inhibitors (Mamont *et al.*, 1978). Although the inhibitors α -methylornithine or α -difluoromethylornithine effectively lower putrescine and spermidine concentrations in cultured cells, spermine concentrations are unaffected, even during prolonged exposure (10 days) to these inhibitors (Mamont *et al.*, 1978).

The depletion of polyamines in HeLa cells demonstrated in the present study as a result of the induction of ornithine decarboxylase antizyme should provide an alternative to the use of competitive inhibitors to investigate polyamine-dependent metabolic pathways in these cells. The applicability to other cell-culture systems remains to be demonstrated, but it is likely that 1,3-diamino-2-hydroxypropane and structurally related inducers of ornithine decarboxylase antizyme will be useful in polyamine research.

This is Scientific Contribution 984 from the New Hampshire Agricultural Experiment Station. This investigation was supported by Hatch Project 252, New Hampshire Agricultural Experiment Station, and technical training and assistance, cell cultures and supplies were gene-

rously provided by Professor R. K. Boutwell and Professor G. C. Mueller of the McArdle Laboratory for Cancer Research, University of Wisconsin.

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