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POLYAMINE DEPLETION INDUCES ENHANCED SYNTHESIS AND ACCUMULATION OF CADAVERINE IN CULTURED EHRLICH ASCITES CARCINOMA CELLS

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SUMMARY: An exposure of cultured Ehrlich ascites carcinoma cells to DL-α-difluoromethyl ornithine, an irreversible inhibitor of ornithine decarboxylase (EC 4.1.1.17), rapidly depleted the tumor cells of putrescine and spermidine. The decrease in the cellular concentrations of these two natural polyamines, however, was accompanied by a striking appearance of two new major amines: cadaverine and a compound tentatively identified as *N*-3-aminopropyl-1,5-diaminopentane (aminopropylcadaverine). When the cultures were grown in the presence of uniformly labeled [^{14}C]lysine, tumor cells exposed to difluoromethyl ornithine converted lysine to cadaverine and aminopropylcadaverine at strikingly enhanced rate. The difluoromethyl ornithine-induced accumulation and synthesis of cadaverine and aminopropylcadaverine were totally prevented by the presence of micromolar concentrations of spermidine (or spermine) in the culture media.

The recent introduction of a number of specific inhibitors of polyamine biosynthesis has greatly improved the possibilities to elucidate the physiological roles of these widely distributed organic cations. Drug-induced polyamine deprivation mostly results in a marked antiproliferative effect in various cells (1), although the sensitivity of cells towards polyamine antimetabolites may vary. An apparent resistance to inhibitors of polyamine synthesis is commonly attributable to the fact that cells exposed to these antimetabolites activate a series of compensatory mechanisms that effectively prevent the level of at least one of the polyamines from decreasing. The latter mechanisms include a stabilization of ornithine and adenosylmethionine decarboxylases (the key enzymes of polyamine biosynthesis) by their competitive inhibitors (2-4), a secondary induction of adenosylmethionine decarboxylase during spermidine depletion (5) and an activation of preexisting adenosylmethionine decarboxylase by diamine inhibitors of ornithine decarboxylase (6).

We now report a novel mechanism by which eukaryotic cells attempt to compensate putrescine and spermidine depletion.

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MATERIALS AND METHODS

Ehrlich ascites carcinoma cells were maintained in the peritoneal cavity of female albino mice by weekly inoculations. The cells were adapted to growth in suspension cultures in RPMI 1640 medium (Orion Diagnostica, Helsinki, Finland), supplemented with 10% (v/v) pooled human serum (Finnish Red Cross Transfusion Service), 2 mM glutamine and 50 µg of both penicillin and streptomycin, as described in (6). After the period of adaptation the dubling time of the tumor cells was 13 h.

The cells were harvested, disintegrated ultrasonically and the extracts for enzyme assays and polyamine measurements were prepared as described earlier (6).

S-Adenosyl-L-[1-^{14}C]methionine was prepared enzymically (7). DL-1-[^{14}C]-Ornithine (spec. radioactivity 59 Ci/mol) and L-[U-^{14}C]lysine (spec. radioactivity 341 Ci/mol) were purchased from the Radiochemical Centre (Amersham, Ergland). 1,5-[^{14}C]Cadaverine (spec. radioactivity 3.27 Ci/mol) was obtained from the New England Nuclear Corporation (Dreieich, West-Germany). Unlabeled polyamines were obtained from Calbiochem (San Diego,CA,U.S.A.).

The activities of ornithine (8) and adenosylmethionine (9) decarboxylases were measured by published methods. Polyamines were measured after dansylation by the method of Seiler (10) with the modification introduced by Dreyfuss *et al.* (11). DNA was measured by the method of Giles and Myers (12) and protein by the method of Lowry *et al.* (13). Cell densities were measured in an electronic particle counter (Coulter Electronics Ltd, Harpenden, England).

RESULTS

Difluoromethylornithine, a "suicide" inhibitor of mammalian ornithine decarboxylase (14), is one of the most potent inhibitors of putrescine and spermidine formation. An exposure of cultured Ehrlich ascites carcinoma cells to the drug for 48 h reduced the cellular concentrations of putrescine and spermidine to 16 and 26% of that found in control cells (Table 1).The fact that the level of spermine remained unchanged was, at least partly, attributable to the almost 10-fold stimulation of adenosylmethionine decarboxylase activity (Table 1), which, in the absence of putrescine, could supply decarboxylated adenosylmethionine solely for the synthesis of spermine. The disappearance of putrescine and spermidine in the difluoromethyl ornithine-treated cultures was associated with the appearance of two new major amines in the tumor cells (Fig. 1). The more slowly migrating compound shown in the chromatogram (Fig. 1), was identified as dansyl cadaverine by its chromatographic behavior in chloroform-*n*-butanol-dioxan (48:1:1) and in ethyl acetate-cyclohexane (30:60). The other fraction, migrating between spermidine and spermine, was tentatively identified as dansyl *N*-3-aminopropyl-1,5-diaminopentane, i.e. a spermidine analogue in which putrescine was replaced by cadaverine.

Table 1. Effect of difluoromethyl ornithine on ornithine decarboxylase and adenosylmethionine decarboxylase activities and on polyamine concentrations in Ehrlich ascites carcinoma cells grown in culture. The cells (0.1×10^6 cells per ml) were grown in the absence or presence of 6 mM difluoromethyl ornithine (DFMO) with or without 10 μM spermidine and spermine for 48 h.

Treatment	Ornithine decarboxylase activity	Adenosyl-methionine decarboxylase activity	Putrescine	Cadaverine	Spermidine	Amino-propyl-cadaverine	Spermine
	(pmol/mg protein)		(nmol/mg DNA)				
None	471	529	148	91	355	23	126
DFMO	42	4 600	24	564	94	173	126
DFMO + spermidine	51	539	22	62	644	24	152
DFMO + spermine	32	0	34	62	159	32	351

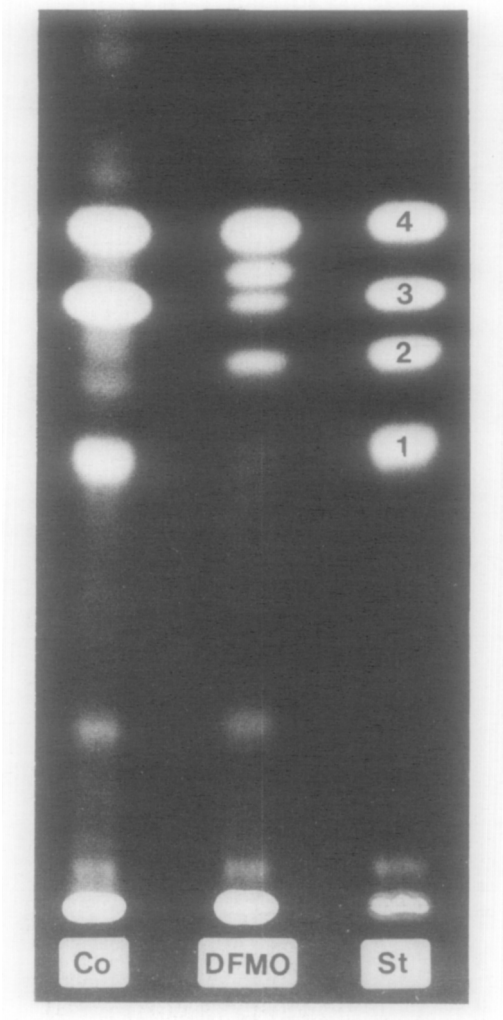

Fig. 1. Polyamine pattern in Ehrlich ascites carcinoma cells grown in the absence (Co) or presence of 6 mM difluoromethyl ornithine (DFMO) for 48 h. Polyamines were dansylated and chromatographed on thin layer plates in chloroform-n-butanol-dioxan (48:1:1). Authentic standards (St) were: 1, putrescine; 2, cadaverine; 3, spermidine; 4, spermine.

Even though some cadaverine (or diffuse material migrating close to the cadaverine fraction, see also Fig. 1) was found in the control cells, the concentration of this diamine increased many-fold in tumor cells exposed to difluoromethyl ornithine for 48 h (Table 1). Similarly, the concentration of the supposed aminopropylcadaverine in the inhibitor-treated cells was more than 7 times that found in the control cells (Table 1). It is noteworthy, that the antiproliferative effect of difluoromethyl ornithine appeared to diminish parallelly with the enhanced production of cadaverine and its aminopropyl derivative (results not shown).

Table 2. Incorporation of radioactivity from [U-^{14}C]lysine into various polyamines in ascites tumor cells grown in the absence or presence of difluoromethyl ornithine (DFMO). The cells were grown (0.4×10^6 cells per ml) for 24 h as described in Table 1, except that each culture was supplemented with 4 µCi of uniformly labeled [^{14}C]lysine. Polyamines were dansylated and the radioactivity of each fraction was measured.

Treatment	Distribution of radioactivity (cpm/culture)				
	Putrescine	Cadaverine	Spermidine	Aminopropylcadaverine	Spermine
None	8	57	90	2	3
DFMO	23	1 263	71	290	17
DFMO + spermidine	0	24	83	23	6

When the cultures grown in the presence of difluoromethyl ornithine were supplemented with 10 μM spermidine or spermine, not only was the dramatic stimulation of adenosylmethionine decarboxylase abolished, but the enhanced formation of cadaverine and aminopropylcadaverine was likewise prevented (Table 1).

When tumor cells grown in the presence of uniformly labeled [^{14}C]lysine were exposed to difluoromethyl ornithine for 24 h, the radioactivity of the cadaverine and aminopropylcadaverine fractions was strikingly increased (Table 2). The enhanced incorporation of radioactivity from labeled lysine into the new amines was totally prevented in cells exposed to the anti-metabolite in the presence of 10 μM spermidine (Table 2). Although the enhanced conversion of lysine to cadaverine and its aminopropyl derivative definitely occurred in the cells (and not in the medium), lysine decarboxylase activity (forming cadaverine) has so far detected only in washed whole cells and not in cell free extracts (results not shown).

Not only was the formation of cadaverine and aminopropylcadaverine from lysine enhanced, but as shown in Table 3, the polyamine-depleted cells likewise converted exogenous [^{14}C]cadaverine to aminopropylcadaverine at strikingly increased rate. Some radioactivity was also found in a fraction, which corresponded spermine analogue containing cadaverine instead of putrescine [bis(aminopropyl)cadaverine, Table 3]. Difluoromethyl ornithine also stimulated the uptake of radioactive cadaverine by a factor of 4 (Table 3). It is not known whether the stimulation of aminopropylcadaverins was only based on increased availability of decarboxylated adenosylmethionine (due to the stimulation of adenosylmethionine decarboxylase) or whether difluoromethyl ornithine induced also the propylamine transferases.

DISCUSSION

Cadaverine has been occasionally found in animal tissues (15-17) and enhanced decarboxylation of lysine has been reported to occur in response to androgen administration in mouse kidney (17). Some evidence likewise exists indicating that the decarboxylation of lysine in animal tissues is catalyzed by ornithine decarboxylase (17,18), although the affinity of mouse or rat ornithine decarboxylase for L-lysine is only about 1% of that for L-ornithine (17,18).

Our present results support the idea, although not prove, that the formation of cadaverine from lysine is catalyzed by a separate lysine decarboxylase, since enhanced cadaverine production occurred virtually in total absence of ornithine decarboxylase activity (Table 1). It likewise appears that

Table 3. Distribution of radioactivity derived from 1,5-[^{14}C]cadaverine between cadaverine, aminopropylcadaverine and bis(aminopropyl)cadaverine in Ehrlich ascites cells. Cultures of ascites cells (0.10×10^6 cells per ml) were supplemented with 2.5 µCi of 1,5-[^{14}C]cadaverine and grown in the absence or presence of 6 mM difluoromethyl ornithine (DFMO) for 3 days. Polyamines were dansylated and the radioactivity of each fraction was measured.

Treatment	Distribution of radioactivity (cpm/culture)		
	Cadaverine	Aminopropylcadaverine	Bis(aminopropyl)cadaverine
None	10 260	1 159	48
DFMO	42 811	40 993	1 541

the formation of cadaverine is induced by spermidine depletion in the ascites carcinoma cells, and again repressed by increased levels of spermidine or spermine. The situation thus closely resembles that described for a polyamine auxotroph of *Escherichia coli* in which polyamine starvation induces formation of cadaverine and aminopropylcadaverine (19). Interestingly, although the concentration of cadaverine and its aminopropyl derivative was elevated in the polyamine-starved bacteria (19), the levels of lysine decarboxylase were not raised suggesting that other control mechanisms than enzyme induction were involved (20).

Polyamine depletion in Ehrlich ascites carcinoma cells thus induces an intense stimulation of adenosylmethionine decarboxylase activity which is associated with greatly enhanced production of cadaverine. Under these conditions cadaverine, which itself is a feeble substrate of spermidine synthase (21), appears to be converted to its aminopropyl derivative, i.e. to a close analogue of spermidine.

The view that the stimulation of adenosylmethionine decarboxylase activity and the induction of increased cadaverine production are co-ordinately regulated is supported by the repression of both processes by exogenous spermidine or spermine. Whether the stimulation of cadaverine production in polyamine-depleted cells is a specific feature for Ehrlich ascites carcinoma cells, or whether it is a general regulatory mechanism in all eukaryotic cells, is not known at the present.

In any event, even the potential existence of an inducible cadaverine pathway in animal cells makes it more difficult to achieve total polyamine depletion by chemical intervention of a single reaction of polyamine synthesis.

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