Polyamine starvation causes accumulation of cadaverine and its derivatives in a polyamine-dependent strain of Chinese-hamster ovary cells

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(Received 22 November 1982/Accepted 11 January 1983)

Starvation of the polyamine-dependent Chinese-hamster ovary cells for ornithine or ornithine-derived polyamines in serum-free culture resulted in the formation of cadaverine and its aminopropyl derivatives, N-(3-aminopropyl)cadaverine and NN'-bis(3-aminopropyl)cadaverine. The synthesis of these unusual amines was inhibited by treatment of the cells with DL-2-difluoromethylornithine, a specific inhibitor of ornithine decarboxylase (EC 4.1.1.17). In the absence of ornithine (the normal substrate), ornithine decarboxylase thus appeared to catalyse the decarboxylation of lysine to cadaverine. Cell proliferation was markedly inhibited by ornithine deprivation of the cells, and further depressed by exposure of the cultures to difluoromethylornithine.

The polyamines putrescine, spermidine and spermine are organic cations which are required for normal proliferation of both prokaryotic and eukaryotic cells (see Tabor & Tabor, 1976; Jänne et al., 1978; Morris & Harada, 1980; Heby, 1981). Studies with the recently introduced polyamine-dependent-strains of Chinese-hamster ovary (CHO) cells (Pohjanpelto et al., 1981; Steglich & Scheffler, 1982) suggest that these compounds are vital for animal cell replication. The importance of polyamines is not hard to understand in the light of our observations that the polyamine-deprived CHO cells, A7, show major chromosome aberrations (Pohjanpelto & Knuttila, 1982) and disappearance of actin filaments and microtubules (Pohjanpelto et al., 1981). However, certain unusual amines, particularly cadaverine and its aminopropyl derivatives, can partly replace polyamines in supporting growth, as demonstrated in cells depleted of polyamines by difluoromethylornithine (Mamont et al., 1978; Hölttä et al., 1979; Alhonen-Hongisto et al., 1982a).

There are only few reports (see Rosengren et al., 1981; Persson, 1981) on the accumulation of cadaverine in animal tissues. Biosynthesis of cadaverine from lysine in mammalian tissues was originally reported in mouse kidney stimulated to grow by an anabolic steroid (Henningson et al., 1976). For cultured animal cells there is no firm evidence for the accumulation of cadaverine, except for the mycoplasma-infected cultures (Alhonen-Hongisto et al., 1982b).

In the present paper we report the formation of cadaverine and its aminopropyl derivatives in the ornithine-starved A7 cells, which are unable to synthesize enough ornithine and polyamines, owing to insufficient arginase activity (Hölttä & Pohjanpelto, 1982). We show further that cadaverine is formed from lysine most probably through decarboxylation by ornithine decarboxylase.

Materials and methods

Chemicals

L-[U-14C]Lysine (sp. radioactivity 341 Ci/mol) was obtained from The Radiochemical Centre (Amersham, Bucks., U.K.). L-Ornithine was purchased from Fluka A.G. (Buchs, Switzerland), and putrescine, cadaverine, spermidine and spermine were from Calbiochem (San Diego, CA, U.S.A.). N-(3-Aminopropyl)cadaverine was synthesized from cadaverine by the spermidine synthase reaction. DL-2-Difluoromethylornithine was generously given by the Centre de Recherche Merrell International (Strasbourg, France).

Cell culture

The A7 cells were cultured in serum-free medium on plastic Petri dishes coated with gelatin (Pohjanpelto et al., 1981). The medium was a 1:1 (v/v) mixture of Eagle's minimal essential medium and Ham's nutrient mixture F12 (Gibco, Paisley, Scotland, U.K.), supplemented with 0.1% bovine serum albumin.
The cells were regularly checked for mycoplasmal contamination both by autoradiography with \(^3\)Hthymidine and by fluorescence staining with the Hoechst 33258 dye (Russell et al., 1975). All the tests were negative.

**Analytical methods**

Polyamines were determined by the dansylation method (Seiler, 1970) as described previously (Hölttä et al., 1979). The dansylated amine fractions were eluted with ethanol, and their radioactivity was measured in a liquid-scintillation spectrometer. DNA was determined by the method of Giles & Myers (1965). Cells were counted in an electric cell counter (Coulter Counter).

All the results were confirmed by repeating the experiments at least twice. Despite some variation (up to 25%) of the basal values from experiment to experiment, the pattern of the changes under particular conditions was quite consistent, with less than 10% variation in them. In each experiment analysis of the different parameters from duplicate dishes gave values that agreed within 10%.

**Results**

Starvation of the A7 cells for ornithine and polyamines in serum-free medium caused appearance of three new amines in the cells. One of them migrated like authentic cadaverine and the other like authentic \(N\)-(3-aminopropyl)cadaverine on t.l.c. in our solvent systems (Hölttä et al., 1979), containing

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**Table 1. Incorporation of radioactivity from \([U-^{14}C]lysine into cadaverine with its derivatives and protein in the A7 cells grown in the absence and presence of ornithine and difluoromethylornithine**

<table>
<thead>
<tr>
<th>Additions</th>
<th>Acid-soluble fraction</th>
<th>Protein</th>
<th>Cadaverine</th>
<th>Aminopropylcadaverine</th>
<th>Bis(aminopropyl)cadaverine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13 949</td>
<td>71 167</td>
<td>60</td>
<td>215</td>
<td>25</td>
</tr>
<tr>
<td>Ornithine</td>
<td>13 836</td>
<td>92 983</td>
<td>15</td>
<td>&lt;3</td>
<td>&lt;3</td>
</tr>
<tr>
<td>Difluoromethylornithine</td>
<td>17 584</td>
<td>69 844</td>
<td>10</td>
<td>70</td>
<td>7</td>
</tr>
</tbody>
</table>

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**Table 2. Polyamine contents in the A7 cells grown in the absence and presence of ornithine and difluoromethylornithine**

<table>
<thead>
<tr>
<th>Additions</th>
<th>Time of incubation (days)</th>
<th>Putrescine</th>
<th>Cadaverine</th>
<th>Spermidine</th>
<th>Aminopropylcadaverine</th>
<th>Spermine</th>
<th>DNA ((\mu)g/plate)</th>
</tr>
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<tr>
<td></td>
<td>1</td>
<td>1.1</td>
<td>2.8</td>
<td>3.8</td>
<td>17</td>
<td>82</td>
<td>15.6</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>21</td>
<td>1.9</td>
<td>85</td>
<td>12</td>
<td>120</td>
<td>16.4</td>
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<tr>
<td></td>
<td>1</td>
<td>0.6</td>
<td>1.3</td>
<td>3.7</td>
<td>11</td>
<td>45</td>
<td>14.0</td>
</tr>
<tr>
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<td>2</td>
<td>1.2</td>
<td>4.8</td>
<td>2.9</td>
<td>27</td>
<td>80</td>
<td>22.8</td>
</tr>
<tr>
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<td>2</td>
<td>34</td>
<td>3.2</td>
<td>180</td>
<td>9.0</td>
<td>190</td>
<td>51.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.8</td>
<td>1.3</td>
<td>2.9</td>
<td>12</td>
<td>65</td>
<td>19.4</td>
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<tr>
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<td>5.6</td>
<td>2.8</td>
<td>40</td>
<td>89</td>
<td>27.9</td>
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<tr>
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<td>10</td>
<td>3.0</td>
<td>140</td>
<td>9.0</td>
<td>230</td>
<td>59.7</td>
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<tr>
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<td>0.6</td>
<td>1.4</td>
<td>2.6</td>
<td>11</td>
<td>68</td>
<td>24.4</td>
</tr>
</tbody>
</table>

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chloroform/dioxan/butan-1-ol (48:1:1, by vol.) or chloroform/butan-1-ol (49:1, v/v). The third, minor, fraction migrated slightly ahead of spermine, and presumably is \( NN' \)-bis(3-aminopropyl)cadaverine. The identity of the new amines was further confirmed by labelling the cells with radioactive lysine, which is decarboxylated to cadaverine, and this in turn is transformed into its aminopropyl derivatives through the subsequent actions of spermidine synthase and spermine synthase (Käpyaho, 1980; Pegg et al., 1981). Table 1 shows that in the ornithine-starved cells the radioactivity from \([U-^{14}C]\)-lysine was incorporated into the fractions representing cadaverine, \( N-(3\text{-aminopropyl})\text{cadaverine} \) and \( NN'\)-bis(3-aminopropyl)cadaverine. Formation of cadaverine and its congeners was inhibited by the addition of 0.1 mM-ornithine, 5 mM-difluoromethylornithine (Table 1) or 1 \( \mu \text{M}\)-putrescine (results not shown) to the cultures. Also, there was a slight decrease in the incorporation of the label from lysine into protein in the ornithine-starved cells, in both the absence and the presence of difluoromethylornithine, as compared with the ornithine-containing control cultures.

Table 2 illustrates the amounts of the normal and unusual polyamines in the cells cultured for different periods of time in the absence or presence of ornithine and difluoromethylornithine. In the ornithine-deprived cultures the concentrations of cadaverine and aminopropylcadaverine increased with time. The cells supplemented with ornithine had high contents of putrescine, spermidine and spermine, but the formation of cadaverine derivatives was inhibited. Some cadaverine was still accumulated in the presence of 0.2 mM exogenous ornithine, but the content of aminopropylcadaverine was decreased. Addition of 5 mM-difluoromethylornithine, an irreversible inhibitor of ornithine decarboxylase (Metcalf et al., 1978), to the ornithine-starved cultures inhibited not only the synthesis of polyamines but also the formation of cadaverine and aminopropylcadaverine. There was a distinct decrease in the concentrations of putrescine and spermine in the cultures exposed to difluoromethylornithine as compared with the cells starved only for ornithine (Table 2).

Fig. 1 depicts the effects of ornithine starvation and difluoromethylornithine on the proliferation of the A7 cells. Ornithine deprivation of the cells caused a marked decrease in the proliferation rate, which was further depressed by difluoromethylornithine. Conceivably, inhibition of the synthesis of both the residual polyamines and the cadaverine-based amines (Table 2) contributes to the additional growth inhibition caused by difluoromethylornithine.

**Discussion**

In the present paper we show that starvation of the A7 cells for ornithine and putrescine resulted in the accumulation of the unusual polyamines cadaverine and aminopropylcadaverine. Polyamine starvation of a polyamine-auxotrophic strain of *Escherichia coli* is also known to cause the formation of cadaverine and aminopropylcadaverine (Dion & Cohen, 1972). Accumulation of these amines is further reported by mycoplasmas (Alhonen-Hongisto et al., 1982b). However, the mechanisms involved in the formation of these...
lysine-descended amines in the A7 cells are different from those in bacteria and mycoplasma-infected animals cells. In *E. coli* cadaverine is synthesized from lysine by lysine decarboxylase (Leifer & Maas, 1973), and mycoplasmas appear also to have lysine decarboxylase activity, which is responsible for the formation of cadaverine in the cells harbouring mycoplasmas (Alhonen-Hongisto et al., 1982b). In the A7 cells, on the other hand, the production of cadaverine and its aminopropyl derivatives was inhibited by difluoromethylornithine, a highly specific inhibitor of ornithine decarboxylase (Metcalf et al., 1978), indicating that ornithine decarboxylase catalysed the formation of cadaverine from lysine. Purified ornithine decarboxylase from rat liver (Pegg & McGill, 1979) and mouse kidney (Persson, 1981) is known to catalyse the decarboxylation of both ornithine and lysine, although the affinity of the enzyme for ornithine is about 100-fold higher than for lysine (Pegg & McGill, 1979). Separate ornithine decarboxylase and lysine decarboxylase activities have not been found in mammalian cells. Our results with the A7 cells also support the view that a single enzyme, ornithine decarboxylase, is responsible for the decarboxylation of both ornithine and lysine.

Since ornithine decarboxylase favours ornithine as a substrate, lysine concentration must be relatively high in comparison with that of ornithine for cadaverine formation to occur. As an essential amino acid, lysine is always present in the tissue-culture media, but ornithine is not included in the commonly used media. However, most cells are cultured in the presence of serum, which contains arginase, which catalyses the formation of ornithine from arginine (Hölttä & Pohjanpelto, 1982). This explains why cadaverine is not normally found in the serum-containing cultures.

The role of cadaverine and its higher polyamine analogues in animal cells is not known, but apparently they can partly substitute for the normal polyamines in the maintenance of cellular growth (Mamont et al., 1978; Hölttä et al., 1979; Alhonen-Hongisto et al., 1982a). The ornithine decarboxylase-catalysed formation of cadaverine in the absence of ornithine and polyamines may be considered as a defence mechanism which helps to keep the cells alive during the polyamine-deficient condition.

Noted added in proof (received 4 January 1983)

A reprint paper (Paulus et al., 1982), which appeared after submission of the present paper, shows that ornithine deprivation causes formation of cadaverine and aminopropylcadaverine in an arginase-deficient *Neurospora crassa* mutant as well.

We thank Ms. Irma Lantinen and Kristina Tuunen for their skilful technical assistance. This research was supported by grants from the Sigrid Jusélius Foundation, The Paulo Foundation and from the Medical Research Council of the Academy of Finland.

References


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