1-Aminooxy-3-aminopropane Reversibly Prevents the Proliferation of Cultured Baby Hamster Kidney Cells by Interfering with Polyamine Synthesis*

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Several polyamine antimetabolites affecting the biosynthesis or interconversions of polyamines have been tested as inhibitors of the proliferation of development of cells and of the production of viruses in both cell cultures and clinical situations (for references see Heby, 1981; Jänne et al., 1983; Pegg, 1986; Williamson and Tynns, 1984). Although some of these compounds, either singly or in combination with other drugs, are likely to find valuable applications (Jänne et al., 1983; Pegg and McCann, 1982), their effects may not be strictly polyamine-specific. Even the growth-inhibitory effects of the most widely used and very target-specific 2-difluoro-1-aminooxy-3-aminopropane, both on its own and in combination with spermidine synthase inhibitors, could partly be due to the enormous intracellular build-up of decarboxylated S-adenosylmethionine (Mamont et al., 1982, Pegg, 1984; Pegg et al., 1982a), which might interfere with adenine metabolism, methionine metabolism, histone acetylation, and membrane function. In general, the efficacy of the available antimetabolites in the prevention of polyamine synthesis is limited by a compensatory stabilization of ornithine and S-adenosyl-L-methionine (AdoMet) decarboxylases and an increased accumulation of spermine. A significantly reduced spermine level and the largest total reduction of cellular polyamine levels have been achieved by the combination of difluoromethylornithine with spermidine synthase inhibitors (Pegg et al., 1982b) or with polyamine analogues (Pegg, 1984; Casero et al., 1984).

We have recently shown that 1-aminooxy-3-aminopropane is a potent competitive inhibitor of mammalian (Khomutov et al., 1985, a and b) and bacterial (Paulin, 1986) ornithine decarboxylase (EC 4.1.1.17), the inhibition being due to a direct and very tight, but reversible, binding of the drug to the enzyme (Lapinjoki et al., 1987). The drug is also a strong competitive inhibitor of mammalian spermidine synthase (EC 2.5.1.16) and a potent inactivator of liver adenosylmethionine decarboxylase (EC 4.1.1.50), but it does not affect spermine synthase (Khomutov et al., 1985a). Of these mammalian enzymes, AdoMet decarboxylase is also known to be affected by nanomolar concentrations of some aminooxy derivatives of the reaction product, decarboxylated AdoMet (Khomutov et al., 1983; Artamonova et al., 1986).

Aminooxy compounds, most notably aminooxyacetate, are known to be carbonyl reagents (Karpeisky et al., 1963) and their reactivity toward enzymes dependent on carbonyl functions such as pyridoxal phosphate is well recognized (John et al., 1978). The reactivity, however, strongly depends on the structure of the aminooxy derivative. 1-Aminooxy-3-aminopropane is a putrescine analog that inhibits ornithine decarboxylase much more strongly than other pyridoxal phosphate-dependent enzymes and shows specificity toward enzymes using putrescine (Khomutov et al., 1985a). This is further supported by the finding that bacterial AdoMet decarboxylase, which unlike the mammalian enzyme does not require putrescine as an allosteric effector, is only weakly inhibited by the drug (see Khomutov et al., 1985b). These properties and some preliminary data of the in vivo effects (Khomutov et al., 1985b) of 1-aminooxy-3-aminopropane prompted us to

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1 The abbreviations used are: AdoMet, S-adenosyl-L-methionine; PBS, phosphate-buffered saline.
clarify its effects on the polyamine metabolism of cultured mammalian cells. The results show that the metabolic effects of this drug are similar to, but more potent than, those of difluoromethylornithine. They further indicate that the accumulation of decarboxylated AdoMet is not harmful to the cells, but that this compound can only be removed through polyamine synthesis and catabolism. The new drug is likely to become a valuable tool in unraveling the physiology of spermidine.

EXPERIMENTAL PROCEDURES

Chemicals—S-Adenosyl-L-[2-14C]methionine was synthesized from DL-[2-14C]methionine (48.8 mCi/mmol), obtained from Du Pont-New England Nuclear, as described by Pegg and Williams-Ashman (1969). Unlabeled and propylamine-14C-labeled decarboxylated adenosylmethionine were prepared by decarboxylation with bacterial adenosylmethionine descarboxylase (Pösö et al., 1976; Pajula et al., 1979), and further purified by Cee-P chromatography (Eloranta et al., 1976).

L-[U-14C]Cysteine (346 mCi/mmol) was obtained from Du Pont-New England Nuclear and [6-3H]thymidine (22 Ci/mmol), L-[1-14C]ornithine, and [1-14C]acetyl coenzyme A (54 mCi/mmol) from the Radiocladic Centre (Amersham). Putrescine, spermidine, and spermine (Behring Diagnostics) were recrystallized from hot 6 M HCl/ethanol solution, and spermine was further purified on a Dowex 50 (H+ form) column (Pajula et al., 1979). Acetylated polyaniline (1-aminooxy-3-aminopropane, 1-methyl-5-methylthio-1-aminooxy-3-aminopropane) was synthesized from a hot HClO4 extract as described by Giles and Myers (1965).

The inhibitor was removed from the supernatant fraction by dialysis using the sonication buffer. For the ornithine decarboxylase assays the dialysis or desalting buffer was supplemented with 0.06% Brij 35 and 50 µM pyridoxal 5'-phosphate (Lapinjoki et al., 1987). The aminopropyltransferase assays (Raina et al., 1983) were modified by reducing the incubation volume and all the subsequent additions by 50%, by increasing the specific activity of 28.5 µM decarboxylated propylamine-[14C]-adenosylmethionine to 1.16 mCi/mmol, by increasing the putrescine concentration to 2.5 mM (spermidine synthase assay), and by increasing the incubation time to 30 min (spermidine synthase) or 60 min (spermine synthase).

A 5-ml sample of the growth medium pooled from the dishes was supplemented with 500-2000 pmol of sym-nor-spermidine, as an internal standard, and 2 ml of [15N]-labeled NHS (346 mCi/mmol) to a final concentration of 8% and centrifuged for 10 min at 1000 x g at 4 °C. The supernatant was extracted four times with an equal volume of ether. After adding 2 g of anhydrous Na2SO4, the remaining ether was carefully removed by evaporation. Then, 0.3 ml of 10 M NaOH was added, and the solution was thoroughly extracted (20 min at room temperature) with 5 ml of 1-butanol. The butanol extract was acidified with three drops of concentrated HCl, evaporated to dryness at room temperature, dissolved in 0.5-1.0 ml of loading buffer (LKB, Stockholm, Sweden; 0.2 M trisodium citrate, 2% (v/v) thioglycol, and 0.12% (v/v) phenol adjusted to pH 2.2 by distilled concentrated HCl, Tris-Glycine, 2% (v/v) phenol, and 0.1 M NaAc (pH 4.0). Millipore), 

Polyamines were analyzed from 100-150-µl samples by an LKB 4151 Alpha Plus analyzer using an 80 × 4.6-mm column filled with Ultrapac 8 (± 0.5 µm) cation-exchange resin (LKB Biochrom Ltd., Cambridge, United Kingdom). Elution conditions were similar to those described by Mach et al. (1981) and fluorescence detection was by the orthophthalaldehyde method (Andrews and Baldar, 1983). The flow rates were 25 ml/h for the buffers and 15 ml/h for the fluorescein reagent.

For the assay of decarboxylated AdoMet, cells from individual Petri dishes (total DNA content 20-40 µg) were extracted with 0.15 M NaCl, 10 mM phosphate buffer (pH 7.4), 10% (v/v) glycerol, 208.5 mM NaHPO4, 29 mM KH2PO4, detached into PBS by scraping with a rubber "police"-man, collected by centrifugation, and stored at −80 °C until analyzed.

DNA and protein synthesis were measured by incubating 5 × 10⁵ cells with 1 µCi of [6-3H]thymidine and 1 µCi of L-[U-14C]cysteine for 6 h at 37 °C in 5% CO₂ atmosphere. The cells were washed with PBS, trypsinized with 0.25% trypsin, precipitated with 8% cold trichloroacetic acid, and centrifuged for 5 min at 12,000 rpm (8,500 × g) using an Eppendorf 5414 microcentrifuge (Eppendorf Gerate, Hamburg, Germany). The pellet was dissolved in 0.5 ml of 0.1 M NaOH, passed by filtration, precipitated (0.25% trypsin, 1 ml), and collected by centrifugation at 1000 × g and intact AdoMet, and 5'-deoxy-5'-methylthioadenosine were calculated from the peak areas, with standards.

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The addition of 0.1 mM 1-aminooxy-3-aminopropane to the culture medium rapidly reduced spermine levels in baby hamster kidney cells, even though spermidine synthase activity was slightly increased but this did not affect spermine levels nor the activity of spermine synthase (Fig. 1). During the first 2 days, putrescine concentrations decreased to levels...
that could not be accurately determined, both in the control cultures and in the cultures containing the drug (data not shown). Only in the control cultures, however, was this accompanied by significant extracellular putrescine accumulation (Table I). At 0.5 mM the drug prevented both proliferation (data not shown) and further accumulation of DNA and polyamines from day 2 onwards (Table I). The intracellular spermine concentration remained fairly unchanged throughout the experiment, but due to the steadily decreased spermidine concentration the cellular polyamine level was reduced to 44% of the initial 4-h value during the 96-h culture period with the drug (Table I). Unlike in the control cultures, no initial increase in the intracellular spermidine concentration and no release of polyamines into the culture medium were seen in the presence of the drug. As shown in Table I, the control cells had excreted significant amounts of putrescine, spermidine, and \textit{N'}-acetyl spermidine even by day 2 (when cell densities were similar in the presence and absence of the drug), and by day 4 the extracellular accumulation of polyamines already amounted to over 4-fold the intracellular level. Adding together the intracellular and the extracellular polyamine amounts, it is evident that cellular spermidine production was increased in the control cultures throughout the experiment, although the cellular spermidine concentration started to decrease with increasing cell density (and decreasing proliferation rate) from day 2 on. Thus, the spermidine levels of the drug-treated cells should be compared with those of the control cells either at identical cell density or taking into account also the excreted amount. It can be calculated that at day 4, the spermidine level of the drug-treated cells was only 8.7% of that of the control cells at day 2 (i.e. at identical cell density), or only 3.0% of the total spermidine amount found in the control cultures at day 4. Judged by the total accumulation of polyamines in the culture (nanomoles per dish), the drug eventually prevented the net synthesis of spermidine but allowed its continued catabolism (Table I).

As shown in Table II, the activities of ornithine decarboxylase and \textit{AdoMet} decarboxylase, but not the activity of spermidine \textit{N'}-acetylsynthase, greatly increased in baby hamster kidney cells cultured in the presence of 0.5 mM 1-aminooxy-3-aminopropane. The increased activity of ornithine decarboxylase appeared only after dialysis, whereas the other two enzyme assays were not affected by the drug residues in the cell extracts. The substrate concentrations in vivo were equal to or less than those employed in the assays, whereas (even after the 90-fold dilution in the assay mixtures) the 1-aminooxy-3-aminopropane from the cell extracts still caused a 90% inhibition of ornithine decarboxylase (data not shown). The drug is a potent competitive inhibitor of kidney ornithine decarboxylase and an irreversible inactivator of hepatic \textit{AdoMet} decarboxylase (Khonumov et al., 1985a), but it had no effect on the activity of hepatic spermidine \textit{N'}-acyltransferase \textit{in vitro} (data not shown). In the cultured cells, more than a 40-fold increase in the activities of ornithine decarboxylase and \textit{AdoMet} decarboxylase was progressively caused by the drug within 96 h, whereas the activity of spermidine \textit{N'}-acyltransferase was not significantly affected. The activity of spermidine synthase was only slightly increased and that of spermidine synthase was not affected by the presence in the culture medium of either 0.1 mM drug (Fig. 1) or 0.5 mM drug (data not shown).

To check whether 1-aminooxy-3-aminopropane competed with the natural polyamines for the same transport system, baby hamster kidney cells were cultured for 4 days with and without 0.5 mM drug. The cells were then treated for 30 min with fresh medium containing 0–1.2 mM drug and either 0.12

![Fig. 1. Effect of 0.1 mM 1-aminooxy-3-aminopropane on the proliferation and polyamine synthesis of cultured baby hamster kidney cells. Closed symbols and solid lines indicate control cultures, and open symbols and broken lines indicate cultures including the drug. Each point represents the mean of two (A, B, C) or the mean ± S.D. (D) of three pooled samples obtained from four to six cultures. When not shown the bars fall within the symbols.](image_url)
The effect of 1-aminooxy-3-aminopropane (APA) on the enzyme activities of ornithine and AdoMet decarboxylases and spermidine N\(^\text{1}\)-acetyltransferase was investigated in baby hamster kidney cells. All the values are means of two to three pooled samples, each obtained by combining the cells from three to five Petri dishes and dialyzing the enzyme preparations as described under "Experimental Procedures." APA indicates the presence of 0.5 mM 1-aminooxy-3-aminopropane or 5 mM spermidine, and extracted with 10% trichloroacetic acid. The radioactivity in the extracts indicated the presence of 0.5 mM l-aminooxy-3-aminopropane or 5 mM spermidine was in fact increased by the simultaneous presence of 1-aminooxy-3-aminopropane, the values being, respectively, 21\(\times\) and 30\(\times\) for control cells (cultured without the drug) and 40\(\times\) and 17\(\times\) for cells cultured with the drug at concentrations varied from 0.1 to 1.2 mM.

Cells grown in the presence of 0.5 mM 1-aminooxy-3-aminopropane for 4 days accumulated added putrescine (0.5 mM) or spermidine (0.1 mM) within 12 h to intracellular levels exceeding those found in the control cultures (data not shown). These changes were accompanied by the restoration of normal rates of DNA and protein synthesis (Fig. 2) and DNA accumulation (Fig. 3). Removing 1-aminooxy-3-aminopropane from the culture medium also restored normal rates of macromolecule synthesis, but only after a lag period (Fig. 2). A similar lag period was also found in the intracellular accumulation of putrescine and spermidine (data not shown). The spermidine concentration (picomoles per \(\mu\)g of DNA), however, remained fairly constant in the cells under all the conditions studied. The delay in DNA accumulation after the removal of the drug could be shortened by supplementing the cultures with 1 mM L-ornithine (Fig. 3).

The clonogenic potential of the cells treated with 0.5 mM 1-aminooxy-3-aminopropane for 1, 2, 3, or 4 days varied from 75 to 94% of the control value, showing no decrease with prolonged treatment. In separate experiments, the mean value of 86% of the control was obtained after 4-day treatment as well as after shorter treatment periods. The colonies formed by the drug-treated cells tended to be smaller than those formed by the control cells, probably due to the lag period needed by these cells to recover from growth arrest (see also Fig. 3). The addition of 0.5 mM putrescine to the culture media did not significantly change the clonogenic potential of the control cells, but slightly improved that of the drug-treated cells. If putrescine was included in the colony formation assay media, the clonogenic potential of the drug-treated cells varied from 87 to 99% of the controls. Neither control cells nor the drug-treated cells were able to form visible colonies in the presence of 0.5 mM 1-aminooxy-3-aminopropane during the 7-day colony formation assay.

Although 1-aminooxy-3-aminopropane is a potent inactivator of AdoMet decarboxylase in vitro (Khomutov et al., 1985a), the concentration of decarboxylated AdoMet in cultured cells was greatly increased by the drug (Table III). Presumably the large increase in enzyme activity (Table II) was able to compensate for inactivation by the drug and for the lack of putrescine, which is an allosteric activator of the enzyme (Pegg and Williams-Ashman, 1969). There was also a clear, although less marked accumulation of AdoMet (Table III). The addition of 0.5 mM putrescine to the culture medium resulted in a steady but very slow decrease in the pool of decarboxylated AdoMet (Table III). This change is a clear, although less marked accumulation of AdoMet (Table III). The addition of 0.5 mM putrescine to the culture medium resulted in a steady but very slow decrease in the pool of decarboxylated AdoMet (Table III). This change is a clear, although less marked accumulation of AdoMet (Table III). The addition of 0.5 mM putrescine to the culture medium resulted in a steady but very slow decrease in the pool of decarboxylated AdoMet (Table III). This change is a clear, although less marked accumulation of AdoMet (Table III).
1-Aminoxy-3-aminopropane and Polyamine Metabolism

Fig. 3. Reversal of the growth-inhibitory action of 1-aminoxy-3-aminopropane on baby hamster kidney cells. The cells were cultured for 4 days either in the absence (○) or presence (□, ■, ▲, △) of 0.5 mM 1-aminoxy-3-aminopropane, as explained under “Experimental Procedures.” The media were then changed at 24-h intervals to fresh ones including no additions (○), 1 mM L-ornithine (□) or 0.5 mM 1-aminoxy-3-aminopropane either alone (□) or in combination with 0.5 mM putrescine (■) or 1 mM L-ornithine (▲). Each point represents the mean ± S.D. of three cultures.

TABLE III
Changes in cellular pools of AdoMet and decarboxylated AdoMet in response to treatment with 1-aminoxy-3-aminopropane

<table>
<thead>
<tr>
<th>Time in culture</th>
<th>APA Compound added</th>
<th>DNA Decarboxylated AdoMet</th>
<th>AdoMet</th>
</tr>
</thead>
<tbody>
<tr>
<td>days</td>
<td>µg/dish</td>
<td>pmol/µg DNA</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>–</td>
<td>24.5 ± 2.5 &lt;0.35</td>
<td>11.3 ± 5.0</td>
</tr>
<tr>
<td>6</td>
<td>–</td>
<td>80.3 ± 7.5 &lt;0.25</td>
<td>8.0 ± 4.2</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>9.6 ± 0.5 24.2 ± 0.8</td>
<td>13.8 ± 3.4</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>15.5 ± 2.3 103 ± 16.2</td>
<td>26.8 ± 7.5</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>22.4 ± 1.0 116 ± 10.2</td>
<td>37.6 ± 9.6</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>25.2 ± 0.6 118 ± 12.4</td>
<td>20.8 ± 12.7</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>22.9 ± 0.6 137 ± 2.9</td>
<td>31.2 ± 2.5</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>18.5 ± 0.2 177 ± 3.0</td>
<td>20.6 ± 1.0</td>
</tr>
<tr>
<td>4.5</td>
<td>+</td>
<td>21.1 ± 1.8 50.5 ± 5.1</td>
<td>21.4 ± 3.5</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>27.9 ± 1.4 15.8 ± 1.8</td>
<td>19.4 ± 1.6</td>
</tr>
<tr>
<td>5.5</td>
<td>+</td>
<td>27.9 ± 3.5 7.5 ± 6.8</td>
<td>16.3 ± 6.4</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>20.1 ± 0.1 202 ± 30.6</td>
<td>15.7 ± 6.2</td>
</tr>
<tr>
<td>Cultures grown for 4 days in the presence of APA before removal of the inhibitor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>–</td>
<td>23.0 ± 0.9 154 ± 27.9</td>
<td>13.2 ± 4.6</td>
</tr>
<tr>
<td>5</td>
<td>–</td>
<td>19.8 ± 1.5 208 ± 14.6</td>
<td>38.5 ± 8.2</td>
</tr>
<tr>
<td>5.5</td>
<td>–</td>
<td>18.2 ± 0.9 327 ± 14.9</td>
<td>47.3 ± 5.2</td>
</tr>
<tr>
<td>6</td>
<td>–</td>
<td>20.9 ± 0.6 272 ± 26.2</td>
<td>23.0 ± 8.9</td>
</tr>
<tr>
<td>4.5</td>
<td>–</td>
<td>21.3 ± 0.8 197 ± 12.4</td>
<td>28.5 ± 11.9</td>
</tr>
<tr>
<td>5</td>
<td>–</td>
<td>22.5 ± 1.6 201 ± 21.0</td>
<td>15.2 ± 9.5</td>
</tr>
<tr>
<td>5.5</td>
<td>–</td>
<td>20.3 ± 0.7 152 ± 12.0</td>
<td>7.8 ± 1.5</td>
</tr>
<tr>
<td>6</td>
<td>–</td>
<td>30.4 ± 4.5 52 ± 8.3</td>
<td>12.9 ± 4.8</td>
</tr>
</tbody>
</table>

The effects of 1-aminoxy-3-aminopropane on cultured baby hamster kidney cells closely resemble those of difluoromethylornithine on a variety of mammalian cell lines (for references see Heby, 1981; Janne et al., 1983; Pegg, 1986). Both drugs cause marked decreases in putrescine and spermidine levels, followed by growth inhibition and drastically elevated activities of ornithine and AdoMet decarboxylases and levels of decarboxylated AdoMet. The effects of both drugs are reversible by exogenous putrescine. Unlike difluoromethylornithine, however, 1-aminoxy-3-aminopropane did not cause a compensatory accumulation of spermine (Fig. 1, Table I) and is thus the more potent restrictor of cellular polyamine levels.

Provided that intracellular 1-aminoxy-3-aminopropane is not sequestered from ornithine and AdoMet decarboxylases (which are cytosolic enzymes), then the activities of these enzymes in the undialyzed cell extracts provide maximum estimates of their intracellular activities. Thus, synthesis of putrescine from ornithine should have been markedly impaired in the cells by day 2 after drug addition, in spite of the greatly increased ornithine decarboxylase activity (Table II). Net synthesis of spermidine should only be possible under such circumstances from pre-existing putrescine pools, including the serum added to the culture media. Judged by the total accumulation of polyamines (Table I), the drug eventually prevented cellular putrescine synthesis and polyamine formation, although significant putrescine synthesis must have occurred during the first 2 days. From day 2 on, the major cause of the rapid loss of spermidine was probably its catabolism to putrescine and further catabolites, which has been shown to occur, for example, during vitamin D-induced intestinal putrescine formation (Shinki et al., 1986). The methods used in this study were not suitable for detecting acetylpurines or other putrescine catabolites.

The growth inhibition of baby hamster kidney cells by serum depletion is known to be accompanied by a leakage of spermidine into the culture medium (Melvin and Keir, 1978). This was not the case during 1-aminoxy-3-aminopropane treatment (Table I). Only control cells excrated significant amounts of putrescine, spermidine, and N-acetylspermidine into the culture medium. The rate of excretion increased and the cellular spermidine concentration decreased with increasing cell density, in accordance with a previous report (Wallace and Keir, 1982). On the other hand, the much smaller amounts of spermine found in the culture medium probably result from lysis of dead cells rather than leakage from vital cells. The accelerating escape of N-acetylspermidine from the cells with increasing growth rate is consistent with the exceptionally low polyamine oxidase activity of baby hamster kidney cells (Wallace and Keir, 1986), and indicates the inducibility of the acetylation/oxidation pathway responsible for polyamine catabolism and interconversion in mammals (Pegg and Erwin, 1985). The catabolism and excretion of spermidine, in addition to the excretion of putrescine, may be an important mechanism for the regulation of intracellular polyamine concentrations by proliferating baby hamster kidney cells. The inability of 1-aminoxy-3-aminopropane-treated cells to excrete N-acetylspermidine, spermidine, or putrescine into the medium either before or after the cessation of proliferation (Table I), and in spite of a normal spermidine N-acetyltransferase activity (Table II), suggests that the drug effectively
eliminates the pool of free polyamines by allowing a normal rate of catabolism, while preventing synthesis. Spermidine seems not to be as tightly bound to cellular structures as spermine, and can be used up by the catabolic enzymes (Table I).

Judged by the polyamine-uptake experiments, 1-aminoxy-3-aminopropane was not an inhibitor of polyamine transport, and within 4 days nearly doubled the capacity of the transport system. This resembles the effects of difluoromethylornithine (Alhonen-Hongisto et al., 1980). The lag between the removal of the inhibitor from the culture medium and the restoration of macromolecule synthesis, like the one needed by rat hepatoma cells to recover after difluoromethylornithine treatment (Gerner and Mamont, 1986), probably represents the time taken to clear the drug from the cells and then to synthesize adequate amounts of polyamines. The ability of ornithine to shorten this lag period both in the presence and after removal of the drug (Fig. 3) suggests that the primary reason for the delay was the competitive inhibition of ornithine decarboxylase by the intracellular drug. The alternative hypothesis, viz., that intracellular polyamines are needed only to displace the drug from some unknown binding sites which mediate its effects, cannot, however, be excluded. In any case, the excellent clonogenic potential of the cells even after 4-day treatment with the drug indicates no irreversible, detrimental effects on cellular physiology.

Decarboxylated AdoMet has been previously shown to accumulate when polyamine synthesis is inhibited by drugs in proliferating cells (Pegg, 1984). However, unlike the situation during inhibition by difluoromethylornithine (for references see Pegg, 1984), the present 1000-fold accumulation of decarboxylated AdoMet did not result in an increased spermine level (Table I), presumably because no putrescine and spermidine were available to supply spermine for new cells once the existing stores had been consumed. From day 2 on, there is probably no free spermidine and no net synthesis of spermine, although some interconversion between spermine and spermidine may occur. The restoration of growth by the addition of putrescine or ornithine (Fig. 3) occurred while the level of decarboxylated AdoMet was still greatly elevated (Table III), showing that the increased decarboxylated AdoMet was not itself responsible for the antiproliferative effects of 1-aminoxy-3-aminopropane. The accumulation suggests that the synthesis of polyamines is the only way of removing this nucleoside.

The cause and significance of the relatively small (Table III) increase in the level of AdoMet itself during treatment with 1-aminoxy-3-aminopropane are obscure. Probably the rate of AdoMet consumption via decarboxylation and further polyamine catabolism and excretion declines when the synthesis of polyamines is blocked, and the AdoMet pool attains a new steady-state level. Since spermidine triggers a rapid loss of AdoMet decarboxylase protein (Shirahata et al., 1985; Póso and Pegg, 1981), the supply of exogenous putrescine or spermidine may prevent the decarboxylation of AdoMet and maintain its new level until the stores of decarboxylated AdoMet are used up. Although 1-aminoxy-3-aminopropane is a good competitive inhibitor of spermidine synthase in vitro (Khomutov et al., 1985a), the reversal of its effects by putrescine suggests that it could not block spermidine synthesis in vivo when high concentrations of both of the substrates were available. The increased AdoMet level and constant S-adenosylhomocysteine concentration favors essential methylation reactions and renders any defects in transmethylation unlikely.

Taken together, the present results indicate that 1-aminoxy-3-aminopropane does not interfere with the transsulfuration pathway, polyamine catabolism, or cell viability, but that it blocks the proliferation of baby hamster kidney cells by preventing polyamine synthesis. Although baby hamster kidney cells, like other fibroblasts, are apparently over-producers of putrescine and spermidine, they probably have very little extra spermine during logarithmic growth and do not start to catabolize it until they are at the stationary phase (McCormick, 1978; Wallace and Keir, 1982). Since 1-aminoxy-3-aminopropane was unable to deplete the growing cells of spermine, a significant portion of spermidine depletion during rapid growth (see Fig. 1) must have resulted from conversion to spermine. The spermine level has also been found to be the primary polyamine regulator of proliferation in some other cultured mammalian cell lines (Pegg, 1984; Casero et al., 1984; Gerner and Mamont, 1986). The combination of 1-aminoxy-3-aminopropane with potent inhibitors of polyamine oxidase (Bolkenius et al., 1985) or of spermine synthase (Pegg and Coward, 1985) might offer specific means to unravel the physiology of putrescine and spermidine separately from that of spermine or total polyamines.

REFERENCES

1-Aminooxy-3-aminopropane and Polyamine Metabolism

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