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Factors affecting polyamine excretion from mammalian cells in culture

Inhibitors of polyamine biosynthesis

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Canavanine, diaminopropane, α-methylornithine and methylglyoxal bis(guanylhydrazone) decreased the intracellular polyamine concentrations in growing baby hamster kidney cells. Each of the inhibitors also prevented polyamine efflux into the extracellular medium. Concomitant with the decrease in polyamine excretion was a change in the distribution of polyamines in the extracellular medium. In each case there was a decrease in the amount of radioactivity present as free spermidine and an increase in that found as acetyl polyamines. The magnitude of this shift correlated with the degree of inhibition of excretion. It may be that acetyl polyamines play a role in the regulation of polyamine excretion.

Polyamine synthesis *Polyamine excretion* *(BHK-21/C13 cell)*

1. INTRODUCTION

There are three routes of catabolism for polyamines in mammalian cells. The first is the direct oxidation of the polyamines to aldehydes via polyamine oxidase [1]. The second is the 'so-called' retroconversion pathway which converts spermine to spermidine and putrescine by a combination of acetylation and oxidation reactions [2,3]. The final route is elimination of polyamines from the cell either as free spermidine or as an acetylated derivative [4,5]. The last two pathways are related since each appears to be mediated via an acetyl intermediate [2,5].

This paper is dedicated to Professor S.P. Datta

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Abbreviations: ODC, ornithine decarboxylase; MGBG, methylglyoxal bis(guanylhydrazone)

BHK-21/C13 cells, a non-transformed fibroblast cell line, have very low levels of polyamine oxidase activity (Wallace and MacGowan, unpublished), and consequently utilise the acetylation/excretion pathway to regulate their intracellular polyamine concentrations [4,6]. A number of factors alter the excretion of polyamines from BHK-21/C13 cells. For example, increasing the rate of cell growth completely stops polyamine excretion [4]; transformation of the cells by an oncogenic virus, polyoma virus, has a similar effect [5]. Infection of the cells with herpes simplex virus type 1 also decreases the total amount of polyamine excreted [7].

In this paper we have continued these studies by examining the effects of four compounds on the excretion and acetylation of polyamines in confluent cultures of BHK-21/C13 cells. Three of these compounds are known to be inhibitors of polyamine biosynthesis and the fourth is an analogue of arginine known to affect protein metabolism [8]. Some of this work has been published as a preliminary communication [9].

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2. MATERIALS AND METHODS

BHK-21/C13 cells were grown to confluence in Dulbecco's medium supplemented with 10% (v/v) horse serum as described previously [5]. Cells for excretion experiments were labeled with [^{3}H]putrescine (1 μCi/ml) for 16–20 h and then transferred to medium containing no radioactivity. The medium was changed again at confluence and samples were taken at various times thereafter [4].

Polyamines were extracted from cells and medium in 0.2 M $HClO_4$ and were analysed by fluorescence or by liquid scintillation spectrometry after dansylation and separation of the dansyl derivatives by TLC [10]. DNA, RNA and protein were extracted from BHK cells as described previously and analysis performed by standard assay procedures [5]. All drugs were dissolved in 0.9% (w/v) NaCl and filter-sterilized before use.

3. RESULTS

All of the four compounds tested inhibited polyamine synthesis in BHK-21/C13 cells in culture (table 1). MGBG was the most effective drug depleting both spermidine and spermine by 45 and 63%, respectively. Diaminopropane was also effective in decreasing the cellular content of both polyamines. Canavanine decreased the spermidine content but produced a small increase in the concentration of spermine (table 1). α-Methylornithine was the least effective drug as an inhibitor of polyamine biosynthesis. As was found with canavanine, the spermidine content was decreased

Table 1

The effect of polyamine inhibitors on the cellular content of DNA, RNA, protein and polyamines

Inhibitor	Concentration (mM)	Protein (mg)	DNA (μg/mg protein)	RNA (μg/mg protein)	Put (nmol/mg protein)	Spd (nmol/mg protein)	Spm (nmol/mg protein)	Total (nmol/mg protein)
None	–	2.28	91.7	126.1	0.37	9.64	5.64	15.28
α-Methylornithine	5.00	2.48	66.9	120.8	nd	4.62	8.68	13.30
Diaminopropane	1.50	2.03	98.3	142.1	nd	5.25	5.33	10.58
Canavanine	1.00	1.84	71.6	120.2	0.02	3.94	6.03	9.97
Methylglyoxal bis(guanylhydrazone)	0.01	1.99	70.3	108.5	2.34	5.27	2.10	7.37

Cells were grown and all parameters were measured as described in section 2. Values are average of duplicates. Putrescine (Put) could not be measured in cells treated with α-methylornithine or diaminopropane due to interference of the drugs with the dansyl putrescine spot. nd, not determined; Spd, spermidine; Spm, spermine

Table 2

The effect of inhibitors of polyamine biosynthesis on the release of polyamines from BHK-21/C13 cells

Inhibitor	Concentration (mM)	Total radioactivity in medium (cpm $\times 10^{-4}$/plate)	% of control value
None	–	2.72 ± 0.08	100.0
α-Methylornithine	5.00	0.67 ± 0.14	24.6
Diaminopropane	1.50	2.04 ± 0.05	75.0
Canavanine	1.00	2.35 ± 0.09	86.4
Methylglyoxal bis(guanylhydrazone)	0.01	0.76 ± 0.07	27.9

Intracellular polyamines were labelled as described in section 2. Inhibitors were dissolved in 0.9% (w/v) NaCl and were added to the cultures 24 h before sampling. Medium, plus inhibitors, was replaced 12 h before harvesting. Values are mean ± SD (n = 3)

Table 3

Distribution of radioactivity in the extracellular medium of BHK-21/C13 cells in the presence of inhibitors

Inhibitor	Concentration (mM)	Radioactivity (% of total)	
		Acetyl polyamines	Spermidine
None	–	64	26
α-Methylornithine	5.00	22	70
Diaminopropane	1.50	52	41
Canavanine	1.00	57	37
Methylglyoxal bis(guanylhydrazone)	0.01	22	60

Acid extracts of extracellular medium from the experiment described in the legend to table 2 were dansylated and the dansyl polyamines were separated by TLC [10]. The radioactivity in each polyamine was determined by liquid scintillation spectrometry. Values are average of duplicates and are expressed as a percentage of the total radioactivity in the medium, since the medium contains different amounts of radioactivity (table 2). Spermine and putrescine were also present in the medium. These amines each accounted for less than 10% of the total radioactivity

and the spermine content increased. The increase was more marked, in this case being about 54%. All of the inhibitors except α-methylornithine inhibited cell growth (table 1). Diaminopropane had little effect on the DNA content of the cells, but α-methylornithine, canavanine and MGBG all decreased the amount of DNA present by about 25% suggesting that these drugs block cell growth prior to S phase. None of the drugs had a significant effect on the RNA content of the cells.

All of the drugs decreased the excretion of polyamines from confluent cell cultures (table 2). α-Methylornithine and MGBG were most effective producing about a 75% decrease in the amount of radioactivity found in the extracellular medium. The other compounds produced much smaller decreases of the order of 14–25%.

Analysis of the distribution of radioactivity in the medium revealed that spermidine and acetylpolyamines accounted for more than 80% of the total radioactivity, with spermidine being the major product (table 3). The presence of the drugs altered the distribution of the radioactivity between these polyamines. In each case, the amount of radioactivity in spermidine decreased while that in the acetylpolyamine fraction increased. The most marked changes were seen in cells treated with α-methylornithine and MGBG, the two drugs which most affected polyamine excretion (tables 2,3). Hydrolysis of the medium [16] released mostly free spermidine and some free spermine (not shown).

4. DISCUSSION

In every case, depletion of intracellular polyamine concentrations resulted in a decrease in the total excretion of polyamines (tables 1,2). This suggests that there is a mechanism for the conservation of existing polyamines. A similar observation was seen in BHK cells induced to grow by the addition of fresh serum [4]. In this case, polyamines were required for cell growth and therefore excretion was stopped. At the same time, the uptake of polyamines by these cells was increased [4] indicating a complete switch from the catabolic to anabolic mode of metabolism. It may be that uptake and excretion of polyamines are linked in an inverse manner such that when uptake is low, excretion is high and vice versa. Indeed, BHK cells treated with α-methylornithine [15] or with canavanine (H.M. Wallace, unpublished) showed enhanced uptake of exogenous polyamines.

The degree of inhibition of polyamine biosynthesis was not related to the amount of inhibition of excretion. Neither did the mechanism of inhibition correlate with excretion. α-Methylornithine and diaminopropane are recognised inhibitors of ODC, the first enzyme in the polyamine biosyn-

thetic pathway. How canavanine influences polyamine metabolism is not known but the results in table 1 indicate that it is an inhibitor of ODC, having similar effects to α-methylornithine. α-Methylornithine and diaminopropane act by two completely separate mechanisms. The former inactivates the enzyme by binding to the active site [11], while the latter inhibits ODC via the production of an antizyme [12]. MGBG inhibits biosynthesis at yet another site, *S*-adenosylmethionine decarboxylase [14], an effect which produces a different pattern of decreased polyamine concentrations (table 1). MGBG had a similar effect on excretion of polyamines from serum-deprived cells [13].

Whenever total polyamine excretion was decreased there was an alteration in the distribution of radioactivity in the extracellular medium (table 3), and a shift in radioactivity from spermidine to acetyl polyamines. The magnitude of this shift correlated with the degree of inhibition of excretion (tables 2,3). α-Methylornithine and MGBG, the most effective inhibitors of excretion, produced the greatest increase in the proportion of acetylpolyamines. The results suggest that the inhibitors directly affect polyamine acetylation either by increasing the activity of polyamine acetyltransferase or by decreasing the activity of polyamine deacetylase. In BHK-21/C13 cells, the former seems to be the case, since MGBG has been found to increase the acetylase activity by 4–5-fold (H.M. Wallace, unpublished).

The highest concentrations of acetylpolyamines were found in the medium where excretion was most decreased; it may be therefore that the increase in the amount of acetylpolyamines in the extracellular medium, either directly or indirectly, regulates polyamine excretion (tables 2,3).

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