1,3-DIAMINOPROPANE RAPIDLY INHIBITS PROTEIN SYNTHESIS AND VIRUS PRODUCTION IN BKT-1 CELLS

K. TUOMI, A. RAINA* and R. MÄNTYJÄRVI

**Department of Biochemistry* and *Department of Clinical Microbiology, University of Kuopio, PO Box 138, SF-70101 Kuopio 10, Finland*

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1. Introduction

Selective depletion of cellular polyamines by using inhibitors of polyamine synthesis has become an important tool in elucidating the physiological function of polyamines in eukaryotic organisms [1,2]. This presumes that the inhibitor used does not have other effects on cellular metabolism and is metabolically resistant. Most of the inhibitors presently available depress the activity of ornithine decarboxylase or *S*-adenosylmethionine decarboxylase, but compounds acting at the propylamine transferase step have also been described [2,3].

Repeated treatment of partially hepatectomized [4–6] or normal rats [7] with large doses of 1,3-diaminopropane markedly represses liver ornithine decarboxylase activity, resulting in inhibition of normal postoperative or nutritionally-induced accumulation of putrescine and spermidine and inhibition of DNA synthesis, without having any effect on the synthesis of RNA and protein. Diaminopropane at 1–13 mM also effectively lowered polyamine content and DNA synthesis in various types of cells in culture [8–10]. That diaminopropane reduced cellular spermidine and spermine concentrations and both RNA and DNA accumulation in BKT-1 cell cultures [11] led us to study virus production in polyamine-depleted cells. As shown here, diaminopropane also had an acute inhibitory effect on virus production and cellular protein synthesis. Therefore, the specificity of diaminopropane as an inhibitor of polyamine synthesis can be questioned.

*To whom correspondence should be addressed

2. Material and methods

L-[U-^{14}C]Leucine (spec. act. 311 mCi/mmol), [6-^{14}C]orotic acid (59 mCi/mmol) and [2-^{14}C]thymidine (57 mCi/mmol) were supplied by the Radiochemical Centre (Amersham). 1,3-Diaminopropane (puriss.) was purchased from Fluka AG (Buchs, SG) and was recrystallized twice from hot 6 N HCl–ethanol solution. Aminoguanidine sulphate was a product of BDH (Poole). Stock cultures of a hamster cell line (BKT-1B) derived from BK virus-induced tumours [12] were maintained in Eagle's minimal essential medium (MEM; Gibco, Grand Island, NY) supplemented with 10% foetal calf serum (FCS), penicillin (200 U/ml) and streptomycin (100 μg/ml), and passaged by trypsinization (0.25% trypsin, Difco). In the experiments the cells (1×10^6 cells/dish) were seeded on plastic Petri dishes (diam. 50 mm) in MEM supplemented with 10% FCS and grown for various times before addition of diaminopropane and infection with virus.

Semliki Forest virus (SFV), a rapidly replicating RNA virus, was obtained from Dr L. Kääriäinen (Department of Virology, University of Helsinki). Virus stock was grown in baby hamster kidney cells (BHK-21) and the infectivity (PFU) determined as in [13]. Before infection the control and diaminopropane-treated cells were washed with Hanks' balanced salt solution to remove serum. The cells were infected with SFV, 50 p.f.u./cell in MEM. After adsorption for 1 h, virus inoculum was removed and the cells were washed twice with Hanks' solution. The maintenance medium (MEM + 0.2% bovine serum albumin or 2% horse serum ± 5 mM diaminopropane) was then added and samples of the medium for virus

titrations [13] were taken at 8 h after infection.

Nucleic acid synthesis was determined by labelling the cells with [^{14}C]thymidine (0.05 μCi/dish) for 1 h or with [^{14}C]orotic acid (2 μCi/dish) for 2 h. Growth medium was then removed and the cells were washed with phosphate-buffered saline, detached by scraping with a rubber 'policeman' and collected by centrifugation. Acid-soluble radioactivity was determined from a cold perchloric acid (PCA) and nucleic acid synthesis from a hot PCA extract of the cell pellet. RNA was determined as in [14] and DNA according to [15].

Protein synthesis was measured by treating the cultures with [^{14}C]leucine (0.2 μCi/dish) for 1 h. Acid-soluble radioactivity was determined as above. The acid-insoluble fraction was dissolved in 0.1 N NaOH and counted for radioactivity in Insta-Gel (Packard Instrument Co., IL). Protein was measured as in [16].

3. Results and discussion

3.1. *Replication of Semliki Forest virus in diaminopropane-treated cells*

As reported in [11], 1,3-diaminopropane at 5 mM rapidly decreased cellular spermidine and spermine, and after a 24 h incubation also inhibited the accumulation of RNA and DNA in BKT-1 cells. It was therefore of interest to see whether the replication of a rapidly growing RNA virus (SFV) would be inhibited by polyamine depletion caused by diaminopropane treatment. We discovered that not only a long-term pretreatment for 2–3 days but also a treatment of cells for 24 h with 5 mM diaminopropane markedly depressed virus production (table 1). Table 2 demonstrates that preincubation of cells for 8 h in the presence of 5 mM diaminopropane clearly inhibited virus formation. The inhibition was less if diaminopropane was present in the culture medium only during the preincubation period, the diamine probably leaking out of cells during adsorption and virus growth (table 2). We further noticed that the addition of 5 mM diaminopropane only at 1 h or 3 h after infection, i.e., without a previous preincubation, was sufficient to reduce the virus yield by 40% (results not tabulated).

Although diamine oxidase activity is low in ruminant sera [1,17], it appeared possible that not diaminopropane itself but its oxidation products inhibited virus production. To exclude this possibility the above experiments were repeated replacing foetal calf serum by horse serum during diaminopropane treatment. As seen in table 2(B), the results were similar to those obtained with calf serum.

Table 1
SF virus production in BKT-1 cells pretreated with 1,3-diaminopropane

Time of pre-treatment (h)	Virus production (%)
0	100.0
24	28.8
48	27.1
72	0.1

The cells were grown for 24–72 h in the presence of 5 mM purified diaminopropane in MEM supplemented with 10% FCS before infection with SF virus, 50 p.f.u./cell. Diaminopropane was also present during virus growth except in the control culture. PFU was determined 8 h after infection. Virus production in the control culture, 4.14×10^7 p.f.u./μg cellular DNA, was taken as 100%. All values are means of 2 parallel cultures

Taken together, our results suggested that diaminopropane treatment of cells had also effects unrelated to the inhibition of polyamine biosynthesis.

3.2. *Inhibition of protein synthesis in diaminopropane-treated cells*

To see the acute effect of diaminopropane on the

Table 2
SF-virus production in BKT-1 cells after short-term treatment with 1,3-diaminopropane

	Treatment		Virus production (%)
	Before infection	After adsorption	
A	None	None	100.0
	DAP	None	53.7
	DAP	DAP	16.4
B	None	None	100.0
	DAP	None	39.6
	DAP	DAP	30.2

(A) Almost confluent cultures were treated with 5 mM purified diaminopropane (DAP) in MEM supplemented with 10% FCS for 8 h before infection, 50 p.f.u./cell. At 1 h after adsorption the cells were washed and maintenance medium (MEM + 0.2% bovine serum albumin, BSA) with or without 5 mM DAP was added. PFU was determined 8 h after infection.

(B) The experiment was performed as in (A) except that FCS was replaced by 10% horse serum (HS) and 0.2% BSA by 2% HS

macromolecular synthesis of BKT-1 cells the cells were incubated for 8 h in the presence of 5 mM diaminopropane before labelling with [^{14}C]thymidine, [^{14}C]orotic acid or [^{14}C]leucine as in section 2. The results (not shown) indicated that only protein synthesis was significantly inhibited by the diamine. The mode of inhibition of protein synthesis was therefore studied in greater detail.

As shown in table 3, diaminopropane at 1 mM significantly inhibited [^{14}C]leucine incorporation into protein, without having any effect on the uptake of the amino acid by the cells (the latter data not tabulated). The inhibition of protein synthesis was observed after 1 h preincubation with the diamine and increased progressively with time. As in the case of virus production (see table 2) diaminopropane also inhibited protein synthesis if calf serum was replaced by horse serum (table 4). Aminoguanidine which effectively blocks serum amino-oxidase activity [18] did not significantly change the inhibitory effect produced by diaminopropane. This is in accordance with the view (see above) that possible oxidation products of diaminopropane formed by serum enzymes are not responsible for the inhibition.

Additional experiments not detailed here demonstrated that commercial diaminopropane, used in most studies without recrystallization, was at least as effective as the purified diamine in inhibiting virus production and [^{14}C]leucine incorporation into protein in BKT-1 cells. We also noticed that inhibition of protein synthesis was more pronounced in actively growing cells as compared to cells reaching confluency. It should be noted that the effect of diaminopropane on ornithine decarboxylase activity and the accumulation of polyamines had in most cases been studied during the rapid phase of cell growth [2]. It further appears that the tissue concentration of diaminopropane after repeated injections of the diamine [4–7] must reach several mM [6,19,20], which is in the same range as that used in the present experiments. Of course, the cellular concentration of diaminopropane may somewhat vary depending on the cell type.

Table 3
Effect of short-term treatment with 1,3-diaminopropane on protein synthesis in BKT-1 cells

Time (h) of treatment	DAP (mM)	Protein synthesis (%)
1	1	88.9
	2	86.9
	5	79.1
4	1	88.2
	2	80.6
	5	62.8
8	1	82.3
	2	75.2
	5	55.1

Almost confluent cultures of BKT-1 cells in MEM supplemented with 5% FCS were treated with diaminopropane (DAP) as indicated before the addition of [^{14}C]leucine (0.2 μCi/dish) for 1 h. Protein synthesis in the control cultures was 84.4 pmol [^{14}C]Leu/mg protein in 60 min

Table 4
Inhibition of protein synthesis in BKT-1 cells by 1,3-diaminopropane

Additions	Protein synthesis (pmol/mg protein)	Acid-soluble pool (pmol/mg protein)
None	79.6 (100)	5.48
DAP	31.4 (39)	7.91
DAP+AG	37.9 (48)	5.89
AG	83.1 (104)	5.48

The cells (0.5 × 10^6/dish) were grown for 48 h in MEM added with 10% horse serum. At 48 h the medium was changed, and 5 mM purified diaminopropane (DAP) and/or 1 mM aminoguanidine (AG) were added. After 8 h incubation [^{14}C]leucine (0.2 μCi/dish) was added for 60 min. The % of the control value is shown in parentheses

Our results indicate that a general inhibition of protein synthesis by diaminopropane contributes to though may not be the major mechanism (see [19]) in the depression of ornithine decarboxylase activity observed in animals and in tissue culture cells [2,7,18]. Some investigators [6,19] have also found a decrease in the activity of *S*-adenosylmethionine decarboxylase, another enzyme having a short biological half-life. As discussed in [1,2], inhibition of protein synthesis would first be reflected in the activities of enzymes turning over rapidly.

In conclusion, our results have demonstrated a rapid inhibition by diaminopropane of virus production and protein synthesis in tissue culture cells, an effect which cannot be regarded as a result of polyamine depletion. Therefore results showing an inhibition of nucleic acid synthesis and cell proliferation by this diamine should be interpreted with caution as regards the biological function of polyamines. The mechanism by which diaminopropane produces a general inhibition of protein synthesis is not known

at present. Diaminopropane may have effects unrelated to the function of polyamines or it may replace polyamines at some essential step(s) of polypeptide synthesis.

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