Biochemical Pharmacology, Vol. 38, No. 7, pp. 1083–1089, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

SWELLING OF THE GOLGI APPARATUS AND DECREASE OF GALACTOSYLTRANSFERASE IN POLYAMINE-DEFICIENT BOVINE LYMPHOCYTES AND EPITHELIUM OF MOUSE SMALL INTESTINE

YOSHIHARU SAKAMAKI,* KIYOSHI TERAO,† EMIKO ITO,† KEIKO KASHIWAGI* and KAZUEI IGARASHI*‡

*Faculty of Pharmaceutical Sciences, Inohana Campus, and †Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, 1-8-1, Inohana, Chiba 280, Japan

(*Received* 11 *July* 1988; *accepted* 18 *October* 1988)

Abstract—When bovine small lymphocytes stimulated by concanavalin A were treated with inhibitors [α-difluoromethylornithine (5 mM) and ethylglyoxal bis(guanylhydrazone) (100 μM)] of polyamine biosynthesis, swelling of the Golgi apparatus was observed. This was accompanied by decreases in the amount of the Golgi apparatus and of the specific activity of galactosyltransferase. Both spermidine and spermine, at physiological concentrations, stimulated galactosyltransferase activity 2–3-fold. When mice were treated with these inhibitors, the following changes were observed in the epithelial cells of small intestine: swelling of the Golgi apparatus; decrease in the amount of the Golgi apparatus; and decrease of galactosyltransferase activity.

It is well known that polyamines are important for cell growth [1]. In animal cells, the physiological functions of polyamines usually have been studied with cells made deficient by inhibitors of polyamine biosynthesis. Recently it has been reported that the combination of DFMO,§ an inhibitor of ornithine decarboxylase, and EGBG, an inhibitor of *S*-adenosylmethionine decarboxylase, is useful for studying the physiological function of polyamines [2, 3]. Through the use of these inhibitors, we introduced the possibility that polyamines may regulate the synthesis of specific proteins through polyamine interaction with ribonucleic acids [4]. Since polyamines bind to not only nucleic acids but also phospholipids [5], we carefully investigated how the membrane structure of animal cells is changed under polyamine-deficient conditions. In this communication, we report that swelling of the Golgi apparatus is induced by polyamine deficiency in bovine lymphocytes stimulated by Con A. This swelling is accompanied by a decrease of galactosyltransferase activity. Similar results were obtained with epithelial cells of mouse small intestine when mice were treated with DFMO and EGBG.

MATERIALS AND METHODS

Culture of lymphocytes. Bovine suprapharyngeal lymphocytes were prepared, purified and cultured at 37° in a humid incubator in RPMI 1640 medium (Nissui Pharmaceutical Co., Tokyo, Japan) with 10% new-born calf serum (Nacalai Tesque, Inc., Kyoto, Japan) [6]. The lymphocytes (3×10^6 cells/ml) were activated by addition of Con A (Type III, Sigma Chemical Co., St Louis, MO) at a final concentration of 10 μg/ml. The pH of the medium was adjusted to 7.4 with 24 mM $NaHCO_3$ at an atmosphere of 5% CO_2. The inhibitors of polyamine biosynthesis were added to the medium simultaneously with Con A. The concentrations of EGBG and DFMO used were 100 μM and 5 mM, respectively [2]. EGBG and DFMO were kindly supplied by Nippon Carbide Industries, Inc. (Tokyo, Japan) and Merrell Dow Pharmaceutical, Inc. (Cincinnati, OH), respectively. The cells were harvested 36 hr after addition of Con A.

Treatment of mice with the inhibitors of polyamine biosynthesis. Male BALB/c mice (7 weeks old) were maintained on polyamine-deficient chow (B, Oriental Yeast Co., Tokyo, Japan) [7] and tap water. The inhibitors were suspended in saline and given daily (i.p.) in two divided doses at 9 a.m. and 4 p.m. for 4 days at the following doses: DFMO, 2 g/kg/day and EGBG, 100 mg/kg/day. Mice were sacrificed 3 hr after the last injection of the inhibitors.

Electron microscopy. Bovine lymphocytes (9×10^8 cells) and small pieces of epithelium of mouse small intestine were fixed in 2.5% glutaraldehyde solution in 0.1 M sodium cacodylate buffer (pH 7.4) for 12 hr at 4° and then postfixed in 1% OsO_4 for 2 hr at room temperature. After dehydration with a graded series of ethanol solutions, the pieces were embedded in Epon 812. Ultra-thin sections were stained with uranyl acetate and lead citrate solutions. The stained specimens were examined with a Hitachi H700H electron microscope.

Purification of the Golgi apparatus by sucrose gradient centrifugation. The Golgi apparatus was

‡ To whom correspondence should be addressed.

§ Abbreviations used: EGBG, ethylglyoxal bis(guanylhydrazone); MGBG, methylglyoxal bis(guanylhydrazone); DFMO, α-difluoromethylornithine; Con A, concanavalin A; PBS, phosphate-buffered saline (0.8% NaCl: 0.02% KH_2PO_4: 0.216% $Na_2HPO_4 \cdot 7H_2O$: 0.02% KCl: 0.01% $MgCl_2 \cdot 6H_2O$: 0.01% $CaCl_2$, pH 7.5); PUT, putrescine; SPD, spermidine; SP, spermine.

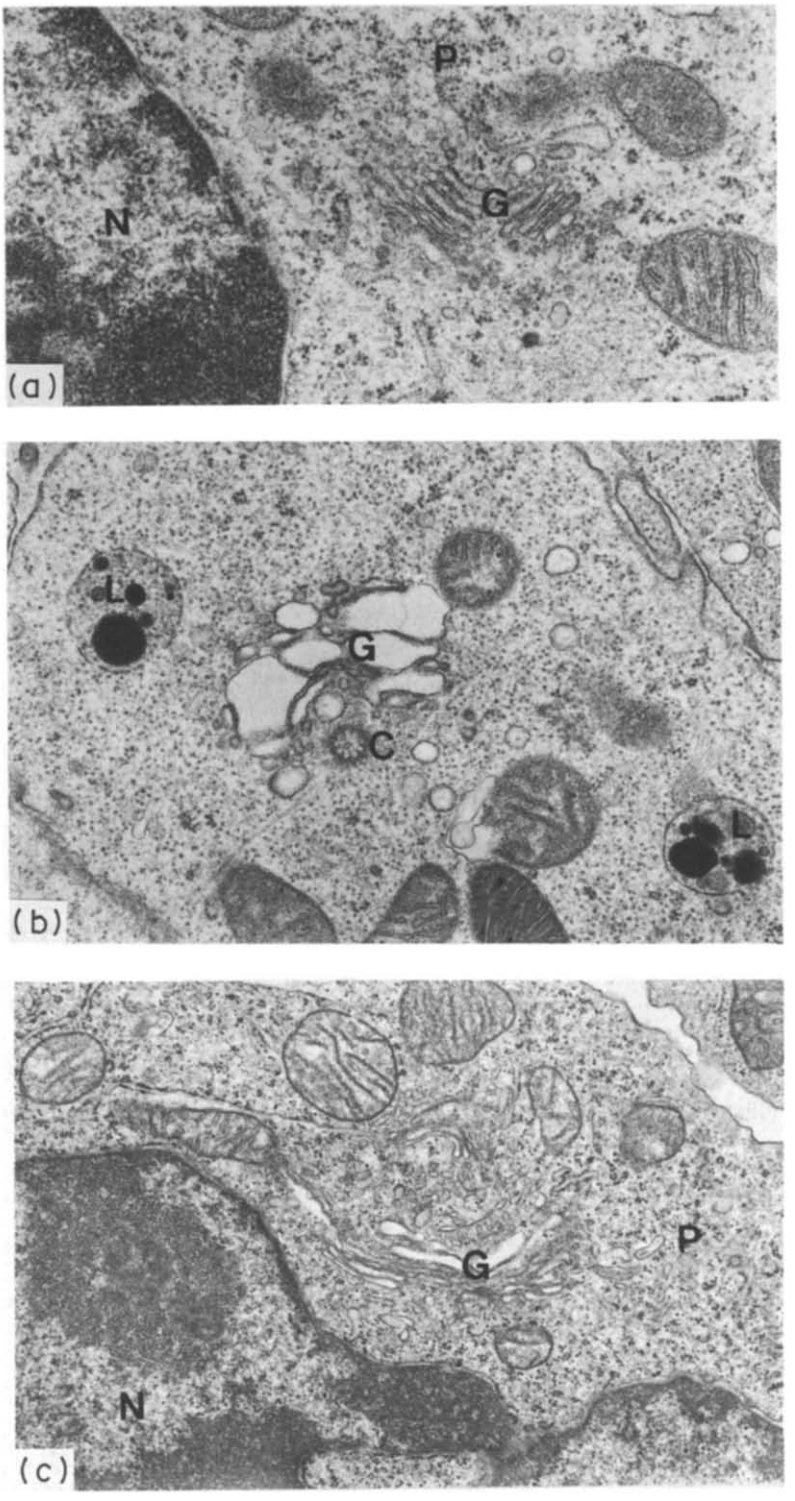

Fig. 1. Electron micrographs of untreated Con A-stimulated lymphocytes (A), lymphocytes treated with 5 mM DFMO and 100 μM EGBG (B) and lymphocytes treated with 5 mM DFMO, 100 μM EGBG and 50 μM spermidine (C). Spermidine was added at 18 hr after Con A. N, nucleus; G, Golgi apparatus; P, polysome; L, lysosome; C, centrosome; ×26,000.

purified from bovine lymphocytes or epithelium of mouse small intestine according to the method of Balch *et al.* [8] with some modifications. A crude homogenate (30 mg protein) was fractionated through the use of discontinuous sucrose density gradient centrifugation. All sucrose solutions were prepared with 10 mM Tris–HCl, pH 7.4. The gradient consisted of layers of 2 M sucrose (2 ml), 1.6 M sucrose (2 ml), the homogenate adjusted to 1.4 M sucrose (4.5 ml), 1.2 M sucrose (12 ml), and 0.8 M sucrose (8 ml). After the gradient was centrifuged in a Hitachi RPS 25 rotor for 2.5 hr at 25,000 rpm, 35 drop fractions were collected from the top of the tube. Then 100 μl of each fraction was assayed for galactosyltransferase activity. Protein was measured by the method of Bradford [9].

Assay for galactosyltransferase. The assay was performed with some modifications of the method of Morré [10]. The reaction mixture (0.2 ml) contained 50 mM Tris–HCl, pH 7.5, 0.2 mM $MnCl_2$, 0.5% Triton X-100, 2.5 mM *N*-acetylglucosamine, 0.35 mM UDP-[^{14}C]galactose (0.9 Ci/mol), enzyme, and polyamine or $MgCl_2$ at the specified concentrations. After incubation at 37° for 30 min, the reactions were terminated by cooling in ice and adding 100 μl of 0.25 M EDTA. The solutions were transferred quantitatively to columns of Dowex-1 anionic resin (1 ml, Cl^- form) in cut-off Pasteur pipettes and the neutral sugars were eluted into vials with water, the total volume being 0.8 ml. The radioactivity was measured with a liquid scintillation counter after addition of 10 ml of Triton X-100-containing scintillation fluid. Purified bovine milk galactosyltransferase was obtained from Sigma Chemical Co.

Measurement of polyamines, EGBG and phospholipids. Polyamine content was determined by high-performance liquid chromatography as described previously [11]. The EGBG content was determined by the inhibition of *S*-adenosylmethionine decarboxylase according to the method of Sëppanen *et al.* [12]. Phospholipids were extracted with chloroform–methanol (2:1, v/v) from the Golgi apparatus and their concentration was calculated by the method of Morrison [13].

Assays for globin mRNA-directed protein synthesis and the binding of [^{14}C]*spermine to submitochondrial particles.* Reticulocyte nuclease-treated lysate was prepared from the blood of rabbits made anemic by acetylphenylhydrazone injection according to the method of Jackson and Hunt [14]. Globin mRNA-directed protein synthesis was determined as described previously [15].

Gel filtration of rat liver submitochondrial particles in the presence of 10 μM [^{14}C]spermine was performed as described previously [5] through the use of a column (0.5 × 11 cm) of Bio-Gel P-10, equilibrated with a buffer containing 10 mM Tris–HCl (pH 7.5), 0.5 mM magnesium acetate, 30 mM KCl and 10 μM [^{14}C]spermine. Where indicated, 10 or 30 μM EGBG or MGBG was added to the buffer. Submitochondrial particles (500 μg protein) [16] in 0.3 ml of the above buffer were applied to the column. The column was eluted at 4° with the same buffer at a flow rate of 1 ml/10 min, and 5-drop fractions were collected. A 0.2 ml sample of each fraction was counted with a liquid scintillation spectrometer. Since the area of the radioactive peak should be equal to that of the radioactive trough, the average of the two independent measurements was used to estimate the amount of spermine bound.

RESULTS

Morphological change of the Golgi apparatus in polyamine-deficient cells

Bovine lymphocytes stimulated by Con A were made polyamine-deficient by DFMO and EGBG. The polyamine and EGBG contents were almost the same as those found in previous experiments [17]. The cellular contents of putrescine, spermidine and spermine in the polyamine-deficient cells were decreased by 80, 65 and 50%, respectively, from levels in control cultures, when determined 36 hr after Con A addition (Table 1). The cellular level of EGBG was approximately 250 μM, on the basis of 3 μl cell volume per mg protein [18]. Under these experimental conditions, the Golgi apparatus in polyamine-deficient lymphocytes was swollen as shown in Fig. 1. In addition, a breakdown of polysomes was observed, although no significant morphological change in mitochondria was observed in polyamine-deficient cells. Addition of spermidine to the deficient cells restored normal Golgi structure (Fig. 1C). When the cells were treated with DFMO only, putrescine and spermidine levels were definitely decreased and some portions of the Golgi apparatus were swollen (data not shown).

To investigate whether similar phenomena occur *in vivo* or not, mice were treated with DFMO and EGBG. As shown in Fig. 2, the Golgi apparatus in the epithelial cells of small intestine was clearly swollen. The cellular contents of putrescine, spermidine, and spermine in the epithelial cells of small intestine treated with DFMO and EGBG were decreased by 45, 66 and 60%, respectively, from levels in normal epithelial cells of small intestine (Table 2). The cellular level of EGBG was approxi-

Table 1. Contents of polyamines and EGBG in Con A-activated bovine lymphocytes

Inhibitor added	Cellular levels (nmol/mg protein)			
	PUT	SPD	SP	EGBG
None	2.14 ± 0.18	4.73 ± 0.21	5.12 ± 0.25	—
DFMO + EGBG	0.14 ± 0.05	1.64 ± 0.16	2.53 ± 0.19	0.86 ± 0.19

Values are expressed as means ± SD of three experiments.

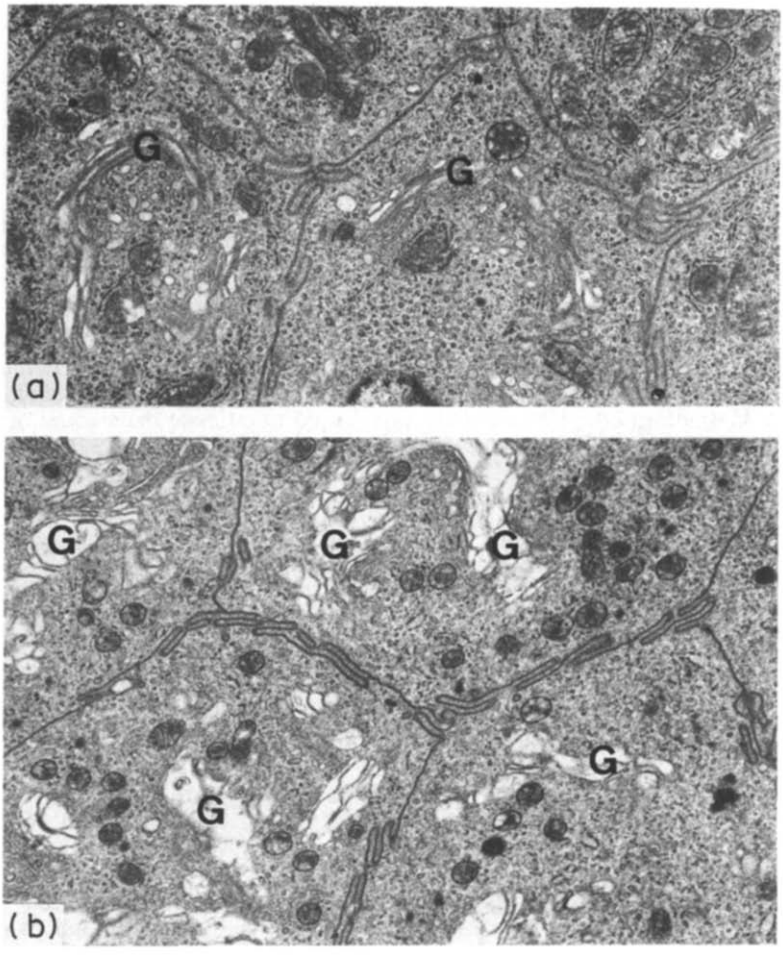

Fig. 2. Electron micrographs of the epithelial cells of small intestine from normal mice (A) and from mice treated with DFMO and EGBG (B). G, Golgi apparatus; ×10,000.

mately 130 μM. When mice were treated with DFMO only, the cellular contents of putrescine, spermidine and spermine in the epithelial cells of small intestine were decreased by 87, 49 and 7%, respectively, and the Golgi apparatus was partially swollen.

Decrease of galactosyltransferase in polyamine-deficient cells

To learn how the swelling of the Golgi apparatus might influence its enzymatic activities, the Golgi apparatus in bovine lymphocytes was purified by fractionation of cell homogenates through sucrose density gradient centrifugation. Since galactosyltransferase is recognized as an enzymatic marker of the Golgi apparatus [19], this enzyme was used as an enzymic monitor. As shown in Fig. 3, galactosyltransferase activity of the Golgi apparatus in DFMO and EGBG treated lymphocytes stimulated by Con A was approximately 55% of that in normal stimulated lymphocytes when the assay was performed in the presence of optimal Mn^{2+} concentration (2.5 mM). These calculations are based on the 30% decrease in the amount of Golgi apparatus (Fig. 3) and the 25% decrease of specific activity

Table 2. Effect of DFMO and EGBG on polyamine and EGBG contents in the epithelial cells of mouse small intestine

Mouse	Cellular levels (nmol/mg protein ± SD)			
	PUT	SPD	SP	EGBG
Normal	0.70 ± 0.09	1.96 ± 0.27	1.62 ± 0.16	—
DFMO + EGBG	0.28 ± 0.11*	0.70 ± 0.10*	0.67 ± 0.13*	0.42 ± 0.11

Five mice were used in each group.
* $P < 0.01$, calculated according to Student's *t*-test.

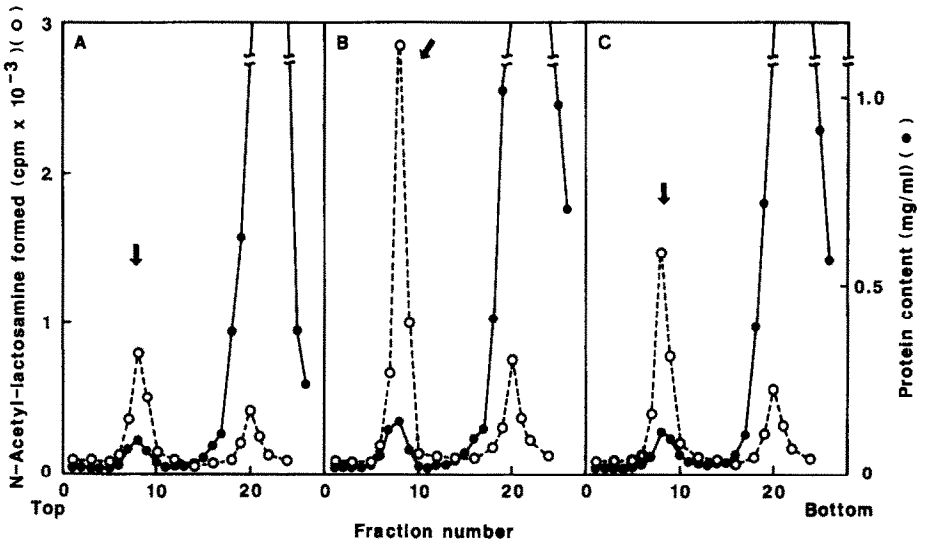

Fig. 3. Decrease of galactosyltransferase activity in polyamine-deficient lymphocytes. The Golgi apparatus was fractionated as described in Materials and Methods, and galactosyltransferase activity (○) was measured with 0.1 ml aliquots in the presence of 2.5 mM $MnCl_2$. (A) unstimulated lymphocytes; (B) Con A stimulated lymphocytes; (C) Con A stimulated lymphocytes treated with 5 mM DFMO and 100 μM EGBG. Protein (●) was measured as described in Materials and Methods. Arrow, Golgi apparatus. Standard error is within the range of 10% for each point. When Con A stimulated lymphocytes were homogenized with sucrose solution containing 5 mM DFMO And 100 μM EGBG, the behavior of the Golgi apparatus on sucrose density gradient centrifugation did not change significantly.

of galactosyltransferase (Table 3). Since the ratio of phospholipid and protein in the Golgi apparatus of polyamine-deficient cells was nearly equal to that of normal cells (Table 3), the relative amount of the Golgi apparatus in DFMO and EGBG treated cells was calculated from the protein content of Fig. 3. The polyamine contents were much less in DFMO and EGBG treated cells, although some portion of polyamines of the Golgi apparatus were probably removed during fractionation (Table 3). When lymphocytes were treated with DFMO only, a slight decrease of galactosyltransferase activity was observed consistently (data not shown). Similar results were obtained with the Golgi apparatus in the epithelial cells of mouse small intestine when mice were treated with DFMO and EGBG.

Polyamine activation of galactosyltransferase activity

The effect of polyamines on galactosyltransferase activity in the Golgi apparatus from polyamine-deficient lymphocytes was examined next in the presence of a limiting amount of Mn^{2+} (0.2 mM). As shown in Fig. 4, either spermine or spermidine, at physiological concentrations, stimulated galactosyltransferase 2–3-fold. Similar results were obtained with the Golgi apparatus from normal lymphocytes (data not shown). When purified galactosyltransferase from bovine milk was used, the degree of polyamine stimulation was 4–5-fold (Fig. 4). Mg^{2+} (0.1–10 mM) did not stimulate the enzymatic activity. Polyamine stimulation of this enzyme from rat mammary Golgi membranes has been reported recently [20]. The degree of polyamine stimulation decreased gradually

Table 3. Effect of DFMO and EGBG on galactosyltransferase activity and polyamine and phospholipid contents in the Golgi apparatus of bovine lymphocytes

Golgi apparatus	N-Acetyl-lactosamine formed (cpm/ μg protein)	Polyamine (nmol/mg protein)			Phospholipid (μg/ mg protein)
		PUT	SPD	SP	
Con A activated	114 ± 13	<0.01	0.22 ± 0.05	0.38 ± 0.07	518 ± 34
Con A activated and DFMO, EGBG treated	84.2 ± 8.7	<0.01	0.04 ± 0.02	0.13 ± 0.04	485 ± 29

For analysis of polyamine and phospholipid, 3×10^9 cells were used. The specific activity of galactosyltransferase was calculated from the data of Fig. 3 and of other two independent experiments. Values are expressed as means ± SD of three experiments.

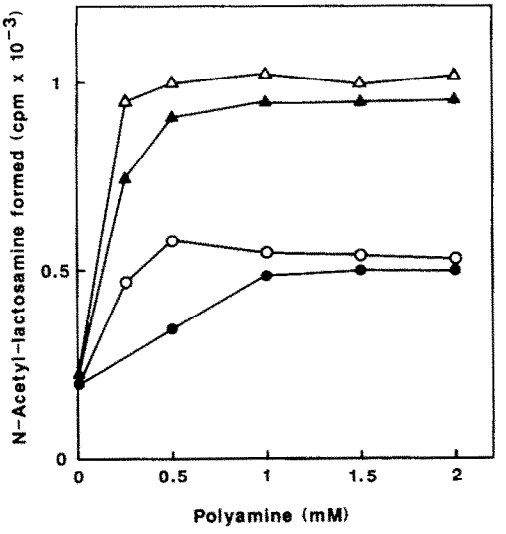

Fig. 4. Activation of galactosyltransferase by polyamines. In the presence of various concentrations of polyamines, galactosyltransferase assays were performed with Golgi membrane from polyamine-deficient lymphocytes (5 μg protein, ● and ○) or with purified bovine milk galactosyltransferase (0.4 μg, ▲ and △). ○ and △, spermine; ● and ▲, spermidine. Standard error is within the range of 10% for each point.

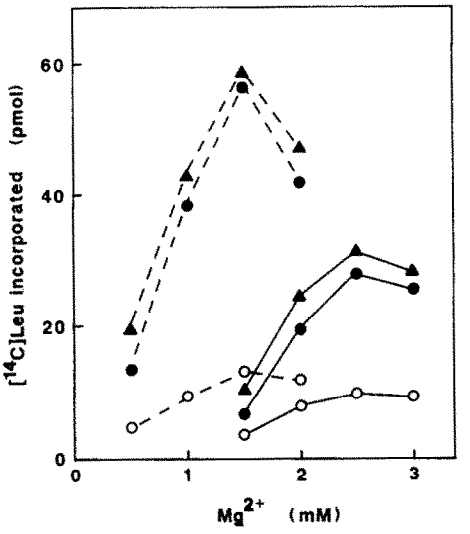

Fig. 5. Effect of EGBG and MGBG on globin mRNA-directed protein synthesis in a rabbit reticulocyte cell-free system. ——, without spermidine, – – –, with 0.3 mM spermidine; ●, no inhibitor; ▲, with 0.3 mM EGBG; ○, with 0.3 mM MGBG. Standard error is within the range of 10% for each point.

as the concentration of Mn^{2+} in the reaction mixture was increased (data not shown).

Effect of EGBG on protein synthesis and spermine binding to membrane

As shown in Fig. 5, EGBG did not inhibit protein synthesis although MGBG, another inhibitor of *S*-adenosylmethionine decarboxylase, inhibited protein synthesis markedly. The latter finding confirms previous results, which showed that MGBG inhibits the binding of polyamines to ribosomal RNA [21], but EGBG does not influence this binding significantly (data not shown). The effect of EGBG on spermine binding to membrane was examined next. Since the amount of Golgi membrane was small, submitochondrial particles of rat liver were used as the membrane fraction. As shown in Table 4, EGBG inhibited [^{14}C]spermine binding to the membrane slightly, but MGBG inhibited the binding significantly. Thus, the swelling of the Golgi apparatus is likely to be due to polyamine deficiency rather than a side effect of EGBG.

DISCUSSION

Since polyamines bind not only to nucleic acids but also to phospholipids [5], it might be that membrane structure is changed by polyamine deficiency. The first report of membrane alteration related to polyamine content was that of Mikles-Robertson *et al.* [22], who observed swelling of mitochondria in cells treated with MGBG. It was suggested that swelling of the mitochondria might occur through the binding of MGBG to membrane phospholipids normally occupied by polyamines since the cellular concentration of MGBG was high (1–2 mM). A later paper showed that DFMO causes ultrastructural damage of mitochondria in 9L rat brain tumor cells [23]. These findings were interpreted to result from polyamine deficiency since DFMO cannot bind to membranes.

Recently, we have demonstrated that the combined use of DFMO and EGBG is useful for studying the physiological functions of polyamines, as EGBG and DFMO exhibit no direct effects on macromolecular synthesis and mitochondrial function

Table 4. Effect of EGBG and MGBG on spermine binding to rat liver submitochondrial particles

Addition	[^{14}C]SP bound (nmol/mg phospholipid)
10 μM [^{14}C]SP	9.42
10 μM [^{14}C]SP + 10 μM EGBG	9.51
10 μM [^{14}C]SP + 30 μM EGBG	8.03
10 μM [^{14}C]SP + 10 μM MGBG	9.34
10 μM [^{14}C]SP + 30 μM MGBG	6.12

Each value was the average of duplicate determinations.

[2, 3]. This is supported by the findings that the cellular concentration of EGBG is low and the binding of EGBG to nucleic acids and phospholipids is weak. Under these conditions, the Golgi apparatus definitely was swollen but swelling of mitochondria was not observed. Swelling of the Golgi apparatus was observed also in cells treated with DFMO only. Our results suggest that the Golgi apparatus is the most susceptible of various membrane structures to polyamine deficiency.

Although 2.5 mM Mn^{2+} is required for the maximal activity of galactosyltransferase, only trace amounts of Mn^{2+} exist in cells. Mg^{2+} does not stimulate the enzymatic activity. Since polyamines can stimulate the activity of galactosyltransferase greatly, the amines probably are very important regulators for this enzyme. Our results show that the amount of galactosyltransferase also was diminished when cells were made polyamine-deficient. Therefore, swelling of the Golgi apparatus may be one of the compensatory effects of decreased galactosyltransferase function. Other enzymatic activities in Golgi membranes may also be influenced by polyamine levels.

Previously, we have reported that polyamines, through their interaction with phospholipids, influence lipid peroxidation and prostaglandin synthesis on the endoplasmic reticulum [24, 25]. The interaction of polyamines with membranes, together with their interaction with nucleic acids, may play important regulatory roles in cell proliferation.

Acknowledgements—We express our thanks to Dr. B. K. Joyce for her help in preparing this manuscript. We are also grateful to Nippon Carbide Industries, Inc. and Merrell Dow Pharmaceutical, Inc. for their kind gifts of EGBG and DFMO, respectively.

REFERENCES

1. Tabor CW and Tabor H, Polyamines. *Annu Rev Biochem* **53**: 749–790, 1984.
2. Igarashi K, Porter CW and Morris DR, Comparison of specificity of inhibition of polyamine synthesis in bovine lymphocytes by ethylglyoxal bis(guanylhydrazone) and methylglyoxal bis(guanylhydrazone). *Cancer Res* **44**: 5326–5331, 1984.
3. Sëppanen P, Ruohola H and Jänne J, Ethylglyoxal bis(guanylhydrazone) as an inhibitor of polyamine biosynthesis in L1210 leukemia cells. *Biochim Biophys Acta* **803**: 331–337, 1984.
4. Igarashi K and Morris D, Physiological effects in bovine lymphocytes of inhibiting polyamine synthesis with ethyiglyoxal bis(guanylhydrazone). *Cancer Res* **44**: 5332–5337, 1984.
5. Igarashi K, Sakamoto I, Goto N, Kashiwagi K, Honma R and Hirose S, Interaction between polyamines and nucleic acids or phospholipids. *Arch Biochem Biophys* **219**: 438–443, 1982.
6. Seyfried CE and Morris DR, Methods for the study of the physiological effects of inhibitors of polyamine biosynthesis in mitogen-activated lymphocytes. *Methods Enzymol* **94**: 373–389, 1983.
7. Nakaike S, Kashiwagi K, Terao K, Iio K and Igarashi K, Combined use of α-difluoromethylornithine and an inhibitor of *S*-adenosylmethionine decarboxylase in mice bearing P388 leukemia or Lewis lung carcinoma. *Jpn J Cancer Res (Gann)* **79**: 501–508, 1988.
8. Balch WE, Dunphy WG, Braell WA and Rothman JE, Reconstitution of the transport of protein between successive compartments of the Golgi measured by the coupled incorporation of *N*-acetylglucosamine. *Cell* **39**: 405–416, 1984.
9. Bradford MN, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**: 248–254, 1976.
10. Morré DJ, Isolation of Golgi apparatus. *Methods Enzymol* **22**: 130–148, 1971.
11. Igarashi K, Kashiwagi K, Hamasaki H, Miura A, Kakegawa T, Hirose S and Matsuzaki S, Formation of a compensatory polyamine by *Escherichia coli* polyamine-requiring mutants during growth in the absence of polyamines. *J Bacteriol* **166**: 128–134, 1986.
12. Sëppanen P, Alhonen-Hongisto L, Pösö H and Jänne J, Sensitive enzymic determination of methylglyoxal bis(guanylhydrazone) in cultured cells and in animal tissues. *FEBS Lett* **111**: 99–103, 1980.
13. Morrison WR, A fast, simple and reliable method for microdetermination of phosphorus in biological materials. *Anal Biochem* **7**: 218–224, 1964.
14. Jackson RJ and Hunt T, Preparation and use of nuclease-treated rabbit reticulocyte lysates for the translation of eukaryotic messenger RNA. *Methods Enzymol* **96**: 50–74, 1983.
15. Igarashi K, Kojima M, Watanabe Y, Maeda K and Hirose S, Stimulation of polypeptide synthesis by spermidine at the level of initiation in rabbit reticulocyte and wheat germ cell-free systems. *Biochem Biophys Res Commun* **97**: 480–486, 1980.
16. Gregg CT, Preparation and assay of phosphorylating submitochondrial particles: Particles from rat liver prepared by drastic sonication. *Methods Enzymol* **10**: 181–185, 1967.
17. Kakinuma Y, Hoshino K and Igarashi K, Characterization of the inducible polyamine transporter in bovine lymphocytes. *Eur J Biochem* **176**: 409–414, 1988.
18. Kakinuma Y, Sakamaki Y, Ito K, Cragoe Jr EJ and Igarashi K, Relationship among activation of the Na^+/H^+ antiporter, ornithine decarboxylase induction and DNA synthesis. *Arch Biochem Biophys* **259**: 171–178, 1987.
19. Rothman JE, The compartmental organization of the Golgi apparatus. *Sci Am* **253**: 84–89, 1985.
20. Navaratham N, Virk SS, Ward S and Kuhn NJ, Cationic activation of garactosyltransferase from rat mammary Golgi membranes by polyamines and by basic peptides and proteins. *Biochem J* **239**: 423–433, 1986.
21. Ohnishi R, Nagami R, Hirose S and Igarashi K, Methylglyoxal bis(guanylhydrazone) elimination of polyamine effects on protein synthesis. *Arch Biochem Biophys* **242**: 263–268, 1985.
22. Mikles-Robertson F, Feuerstein B, Dave C and Porter CW, The generality of methylglyoxal bis(guanylhydrazone)-induced mitochondrial damage and the dependence of this effect on cell proliferation. *Cancer Res* **39**: 1919–1926, 1979.
23. Oredsson SM, Friend DS and Marton LJ, Changes in mitochondorial structure and function in 9L rat brain tumor cells treated in vitro with 2-difluoromethylornithine, a polyamine biosynthesis inhibitor. *Proc Natl Acad Sci USA* **80**: 780–784, 1983.
24. Kitada M, Igarashi K, Hirose S and Kitagawa H, Inhibition by polyamines of lipid peroxide formation in rat liver microsomes. *Biochem Biophys Res Commun* **87**: 388–394, 1979.
25. Igarashi K, Honma R, Tokuno H, Kitada K, Kitagawa H and Hirose S, Effect of polyamines on prostaglandin synthesis in various cell-free systems. *Biochem Biophys Res Commun* **103**: 659–666, 1981.