Journal of Cellular Biochemistry 66:165–174 (1997)

Polyamine-Dependent Alterations in the Structure of Microfilaments, Golgi Apparatus, Endoplasmic Reticulum, and Proteoglycan Synthesis in BHK Cells

Jyrki J. Parkkinen,[1] Mikko J. Lammi,[2] Ulla Ågren,[2] Markku Tammi,[2] Tuomo A. Keinänen,[3] Tapani Hyvönen,[3]* and Terho O. Eloranta[3]

[1]Department of Pathology, University of Kuopio, Kuopio, Finland
[2]Department of Anatomy, University of Kuopio, Kuopio, Finland
[3]Department of Biochemistry and Biotechnology, University of Kuopio, Kuopio, Finland

Abstract The activity of ornithine decarboxylase, the key enzyme in the synthesis of polyamines, is essential for proliferation and differentiation of all living cells. Two inhibitors of ornithine decarboxylase, α-difluoromethylornithine (DFMO) and 1-aminooxy-3-aminopropane (APA), caused swelling of endoplasmic reticulum (ER) and medial and trans Golgi cisternae, and the disappearance of stress fibers, as visualized by staining with fluorescent concanavalin A (ConA), C6-NBD-ceramide or wheat germ agglutinin (WGA), and phalloidin, respectively. In contrast, the pattern of microtubules, stained with a β-tubulin antibody, was not affected. Rough ER seemed to be especially affected in polyamine deprivation forming whorls and involutions, which were observed by transmission electron microscopy. Since ER and Golgi apparatus are vital parts of the glycosylation and secretory machinery of the cell, we tested the ability of these structurally altered cell organelles to synthesize proteoglycans using [^{3}H]glucosamine and [^{35}S]sulfate as precursors. The total incorporation rate into proteoglycans and hyaluronan was not reduced in polyamine-deprived cells, suggesting that the total glycosylation capacity of cells was not affected. However, the synthesis of a high molecular weight proteoglycan containing chondroitin and keratan sulfate was completely inhibited. The remodeling of cytoskeleton and rough endoplasmic reticulum in polyamine deprivation may perturb the synthesis and secretion of the components of membrane skeleton and of the extracellular matrix, e.g., proteoglycans. Rough ER and cytoskeleton may be the targets where polyamines affect cell proliferation and differentiation. J. Cell Biochem. 66:165–174, 1997.

Key words: cell shape; cytoskeleton; stress fibers; autophagy; vacuolar degradation; hyaluronan; chondroitin sulfate; keratan sulfate

Polyamines, putrescine, spermidine, and spermine are essential to the proliferation and differentiation of all living cells. One of the key enzymes in polyamine biosynthesis is ornithine decarboxylase, the activity of which increases by various growth stimuli [Jänne et al., 1991; Pegg, 1986]. Inhibitors of ornithine decarboxylase, e.g., α-difluoromethylornithine (DFMO) and 1-aminooxy-3-aminopropane (APA), cause the disappearance of cellular putrescine and spermidine, decrease protein and DNA synthesis, and inhibit cell proliferation [McCann and Pegg, 1992].

The cellular cytoskeleton forms a highly organized structure, which regulates many functions of the cell, including proliferation, differentiation, shape, spreading, and movement of anchorage-dependent cells [Bershadsky and Vasiliev, 1988; Luna and Hitt, 1992; Vasiliev, 1985]. Actin microfilaments have interactions with extracellular matrix, plasma membrane, and nucleus. Many extracellular signals, which affect cell growth and proliferation by generating second messengers, require intact actin network [Rao and Cohen, 1991]. Disturbances in the organization of cytoskeleton, notably the microfilament system, may lead to malignant cell transformation [Rao and Cohen, 1991]. There is evidence that polyamine deprivation is

Contract grant sponsors: North Savo Fund of the Finnish Cultural Foundation, the Finnish Foundation for Cancer Research, the Finnish Medical Foundation, and the Kuopio University Foundation.

*Correspondence to: Tapani Hyvönen, Department of Biochemistry and Biotechnology, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland.
E-mail: tapani.hyvonen@uku.fi

Received 15 March 1996; accepted 14 March 1997

accompanied by structural alterations in the cytoskeleton [Balasundaram et al., 1991; Pohjanpelto et al., 1981; Pomidor et al., 1995] and Golgi apparatus [Allen, 1988; Quemener et al., 1993; Sakamaki et al., 1989]. On the other hand, remodeling of the cytoskeleton can affect the activity of ornithine decarboxylase [Pomidor et al., 1995].

These findings prompted us to study the relationship between the structure of cytoskeleton and the function of the secretory cell organelles in polyamine-deprived cells. The ability of the cells to synthesize and secrete proteoglycans, molecules that require extensive posttranslational modifications, was considered a good indicator of possible defects in these processes. Hyaluronan, a high molecular weight glycosaminoglycan synthesized at and secreted through plasma membrane [Prehm, 1984], served as a control for the common endoplasmic reticulum (ER) and Golgi apparatus dependent protein glycosylation and secretory pathway [Calabro and Hascall, 1994]. In this paper, we describe the action of DFMO and APA, two inhibitors of ornithine decarboxylase, on proteoglycan secretion in the context of their influences on the organization of cytoskeleton and on the morphology of cell organelles.

METHODS

Chemicals

α-Difluoromethylornithine (DFMO) was from Orion Oy (Helsinki, Finland) and aminoguanidine from BDH (Poole, UK). 1-Aminooxy-3-aminopropane (APA) was synthesized as described earlier [Khomutov and Khomutov, 1989]. L-[^{14}C(U)]leucine (314.8 mCi/mmol) was from New England Nuclear (Boston, MA), D-[6-^{3}H]glucosamine (28 Ci/mmol) and [^{35}S]sulfate were from Amersham (Arlington Heights, IL). Chondroitinase ABC, heparitinase, and keratanase were purchased from Seikagaku Co (Tokyo, Japan). Tetramethyl rhodamine isothiocyanate-conjugated phalloidin (TRITC-phalloidin) was from Sigma (St. Louis, MO), anti-β-tubulin and fluorescein isothiocyanate-anti mouse IgG (FITC-IgG) from Boehringer (Mannheim, Germany), 3,3′-dihexyloxacarbocyanine iodide and C6-NBD-ceramide from Molecular Probes (Eugene, OR), TRITC-conjugated wheat germ agglutinin (TRITC-WGA) and concanavalin A (TRITC-Con A) from Vector Laboratories Inc. (Burlingame, CA). All other chemicals were of analytical grade.

Cell Culture

BHK21/C13 cells were maintained in Dulbecco's MEM without antibiotics [Hyvönen et al., 1988]. Cell numbers were measured either with haemocytometer or with electronic particle counter (Coulter Electronics Ltd., Dunstable, Bedfordshire, UK). The experiments were started by 8×10^4 cells in culture dishes (diameter 6 cm, Greiner, Frickenhausen, Germany) containing 4 to 5 cover slips (diameter 13 mm). Aminoguanidine (1 mM) was added to cell culture media to inhibit any oxidation of the added putrescine by the serum amine oxidase.

Morphometric Measurements

The cells cultured with or without inhibitors were photographed after 2 and 4 days' treatment using phase-contrast microscopy. The cells were not fixed to avoid shrinkage. The photographs were digitized with a flat-bed scanner and the projected areas of individual cells were measured at 240-fold magnification by the software IMAGE (W. Rasband, NIH, NIMH, Bethesda, MD). At least 70 cells were measured in each group. The statistical significance of the differences was calculated using the Student's two-tailed *t*-test.

Fluorescence Microscopy

The cells were fixed with 3.5% paraformaldehyde and treated with 0.1% Triton X-100 at room temperature before staining the microfilaments with TRITC-phalloidin (130 μg/ml) [Wulf et al., 1979]. The cover slips were mounted on object slides with glycerol in 0.05 M Na-veronal buffer, pH 8.4.

The cells were fixed in methanol at −20°C for microtubules stained with anti-β-tubulin as a primary antibody (1:50) and FITC-labeled anti-mouse IgG as a secondary antibody (1:30).

Golgi apparatus was stained by two different methods using the lectin WGA and C6-NBD-ceramide. For WGA staining, the cells were fixed with 3.5% paraformaldehyde and treated with 0.1% Triton X-100 at room temperature before staining Golgi apparatus with TRITC-WGA (100 μg/ml) [Virtanen et al., 1980]. For C6-NBD-ceramide staining, the cells were fixed with 0.5% glutaraldehyde and stained for 30 min at 2°C with 5 nmol/ml of C6-NBD-Cer/BSA complex. The samples were incubated at room temperature with 3.4 mg/ml defatted BSA to remove the excess C6-NBD-Cer. The samples

were excited at 450–490 nm and the fluorescence was observed at ≥520 nm [Pagano and Martin, 1994]. Hydroxylamine was used to decelerate the fading of the fluorescence during observation under the microscope.

The endoplasmic reticulum was visualized by two different methods. For Con A staining, the cells were fixed with 3.5% paraformaldehyde and treated with 0.1% Triton X-100 at room temperature before staining with with TRITC-ConA (100 µg/ml) [Virtanen et al., 1980]. For staining with 3,3′-dihexyloxacarbocyanine iodide, the cells were fixed gently for 3–5 min in 0.25% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, at room temperature and then stained with 2.5 µg/ml 3,3′-dihexyloxacarbocyanine iodide in cacodylate for 10–20 sec, washed and mounted in the cacodylate buffer [Terasaki et al., 1984].

Transmission Electron Microscopy

Cells on plastic cover slips were fixed in 2% glutaraldehyde in 0.1 M Na-cacodylate (pH 7.4), postfixed in a mixture (1:1) of 2% glutaraldehyde in 0.1 M Na-cacodylate (pH 7.4) and 1% osmium tetroxide in s-collidine buffer (pH 7.3) at 4°C. After staining with 0.5% uranyl acetate, the samples were dehydrated in a graded series of ethanol and embedded in LX-112 (Ladd Research Industries, Burlington, CA). The sections were examined and photographed in a JEOL JEM 1200EX transmission electron microscope (Jeol Ltd., Tokyo, Japan) [Parkkinen et al., 1993].

Metabolic Labeling With [^{14}C(U)Leucine, [^{3}H]Glucosamine, and [^{35}S]Sulfate

Protein synthesis was determined by measuring the incorporation of radiolabeled leucine into acid-insoluble material. Cells were cultured on 24-well plates and labeled for 4 h with 1 µCi/ml of [^{14}C(U)]leucine [Hyvönen et al., 1988]. The labeling with [^{3}H]glucosamine and [^{35}S]sulfate were done on 24-well plates or on 6 cm dishes. Cells were subcultured at a density of 20,000 cells/ml. Synthesis was measured at specified time points by changing the cells into new media with 5 µCi/ml of D-[6-^{3}H]glucosamine or 5 µCi/ml of [^{35}S]sulfate. After incubation for 24 h with the isotopes, the total labeled sulfated glycosaminoglycans and hyaluronan were assayed from the media as described [Ågren et al., 1994]. Cell numbers were measured with electronic particle counter and incorporation of the radioactivity, [^{14}C(U)]leucine, [^{3}H]glucosamine, or [^{35}S]sulfate, was normalized to the cell numbers. For electrophoretic analysis of sulfated macromolecules, the cultures were labeled with 50 µCi/ml of [^{35}S]sulfate.

SDS-Agarose Gel Electrophoresis of [$^{35}SO_4$]Labeled Macromolecules

After labeling, medium was collected and the samples were precipitated overnight at 4°C with 3 volumes of 0.5% (w/v) sodium acetate in 94% (v/v) ethanol and washed three times with 0.5% (w/v) sodium acetate in 70% (v/v) ethanol. Aliquots of the proteoglycans were characterized by overnight digestions with chondroitinase ABC (40 mU/50 µl) and keratanase I (5 mU/50 µl) in 0.1 M Tris-HCl buffer, pH 8.0, at 37°C. Heparitinase digestion (2 mU/50 µl) was performed in 64 mM Tris-HCl, 3.3 mM Ca-acetate, pH 7.0, at 43°C. After heat-inactivation of the enzymes, the samples were freeze-dried and electrophoresed on 0.75% SDS-agarose gel, as described [Säämänen et al., 1990]. Autoradiography was used to localize the [$^{35}SO_4$]labeled molecules (Hyperfilm-βmax, Amersham). Their migration positions were standardized by including HindIII digested λ-DNA stained with ethidium bromide.

RESULTS

Morphometry

BHK cells were treated with the polyamine synthesis inhibitors APA or DFMO for 2 or 4 days. Both inhibitors retarded cell proliferation and also changed cell shape, resulting in slender and elongated cells. To quantify this effect, the cells were photographed in their native, unfixed state and the projected cell areas (µm^2) were measured in each group by image analysis. There were no statistically significant differences between the size of control cells and the cells treated with inhibitors. After culture for 2 days, the areas (mean ± s.d.) of the control cells and those of the cells treated with DFMO and APA were 1,268 ± 528 (n = 117), 1,351 ± 496 (n = 83), and 1,399 ± 474 (n = 87) µm^2, respectively. The area of the cells treated with the drugs did not change by increasing the culture time. After culture for 4 days, the areas of the cells treated with DFMO and APA were 1,264 ± 545 (n = 74) and 1,329 ± 542 (n = 88) µm^2, respectively. Control cells grew at densities too high to allow exact measurements on day 4.

Organization of Microfilaments and Microtubules

The changes in the distribution and organization of stress fibers were visualized with TRITC-phalloidin [Wulf et al., 1979]. In control cells, prominent actin filament stress fibers extended across the spread-out cells but stress fibers disappeared when the cells were treated with APA for 4 days (Fig. 1A,B). Only a thin cortical staining was seen under the plasma membrane. When putrescine was added to the cell cultures with APA there was no change in the organization of microfilament system (Fig. 1D). When cells were grown for 6 days in the presence of APA, but supplemented with putrescine for the last 2 days, stress fibers reappeared and also cell shape returned to normal. The effect of polyamine deprivation on microfilament organization was not yet visible after 2 days. The arrangement of microtubules was not changed in polyamine deficiency.

Endoplasmic Reticulum

Endoplasmic reticulum was localized with the lectin Con A, which specifically binds to the mannose-rich oligosaccharides in glycoproteins before their further processing in Golgi apparatus [Virtanen et al., 1980]. In control cells, the Con A staining was granular (Fig. 2A) but seemed to be more diffuse and shifted towards cell periphery in polyamine-deprived cells, suggesting redistribution of endoplasmic reticulum (Fig. 2B,C). When putrescine was added with APA and DFMO, there was no change in the distribution of ER. This redistribution was confirmed by vital staining of endoplasmic reticulum with 3,3′-dihexyloxacarbocyanine iodide [Terasaki et al., 1984]. Endoplasmic reticulum was expanded in polyamine-deprived cells, almost reaching the plasma membrane whereas there was an endoplasmic reticulum-free space near the plasma membrane in controls (data not shown).

To further clarify the nature and site of the alterations we prepared samples for transmission electron microscopy. The most prominent changes in polyamine-deprived cells were autophagic vesicles and/or lysosomes (Fig. 3A). These vesicles contained cytoplasmic material, very often rough ER. In polyamine-deprived cells, also whorls of the rough ER were seen (Fig. 3B). In control cells, autophagic vesicles were very rare and no whorls were visible.

Golgi Apparatus

In control cells, Golgi apparatus was localized as a bright juxtanuclear staining pattern by C6-NBD-ceramide (Fig. 4A). However, in cells depleted of polyamines for 4 days it was disintegrated and expanded into cell periphery

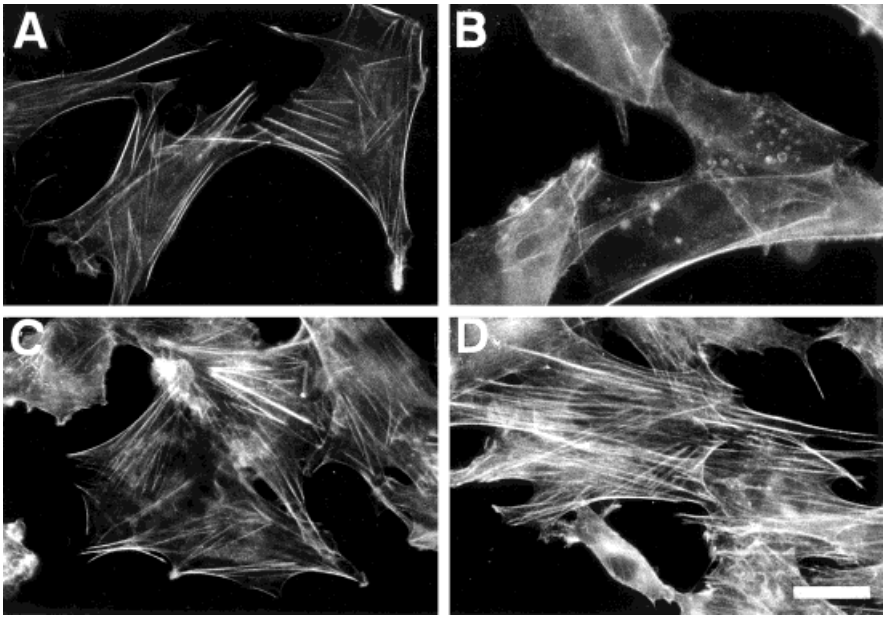

Fig. 1. Microfilament structure after culture for 4 days. Stress fibers were visualized with TRITC-phalloidin. Cells were cultured for 4 days in the following media: (**A**) no additions, (**B**) 1 mM APA, (**C**) 20 μM putrescine, (**D**) 1 mM APA and 20 μM putrescine. Bar = 20 μm.

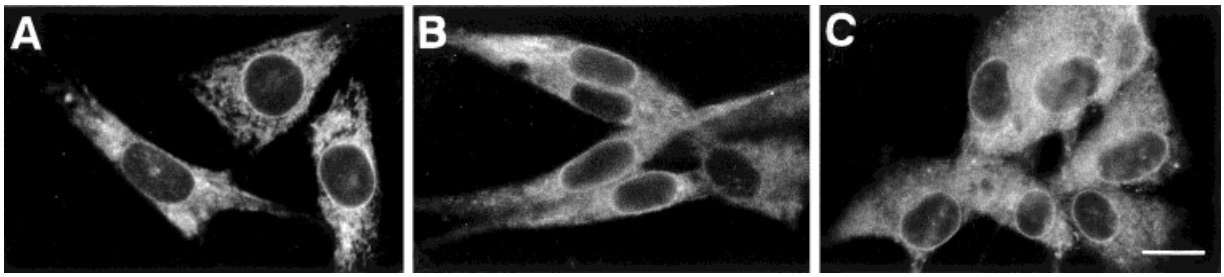

Fig. 2. Endoplasmic reticulum after culture for 4 days. Endoplasmic reticulum was stained with TRITC-Con A. Cells were cultured for 4 days in the following media: (**A**) no additions, (**B**) 5 mM DFMO, (**C**) 1 mM APA. Bar = 40 µm.

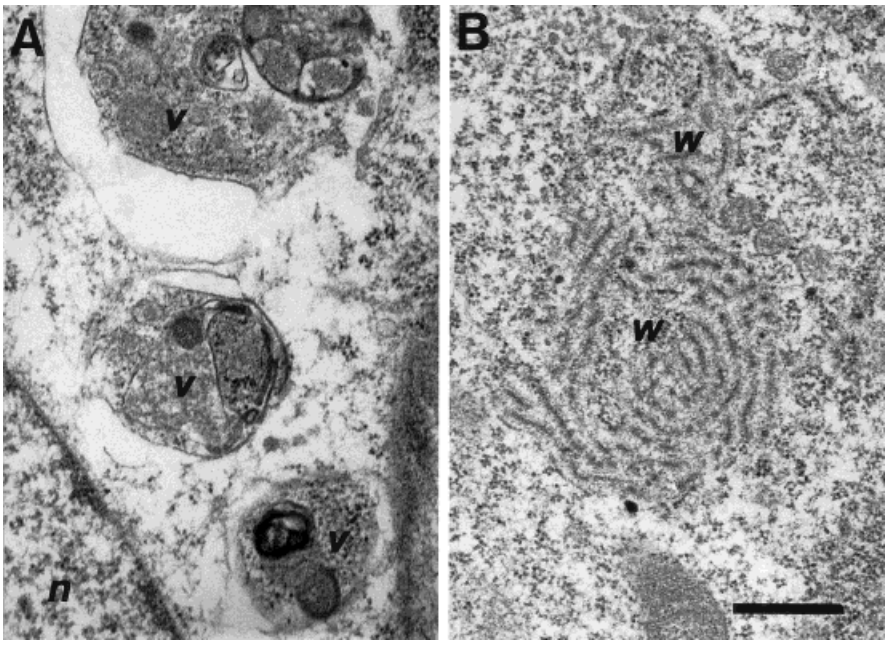

Fig. 3. Alterations in the subcellular structures in APA treated BHK cells visualized by transmission electron microscopy. **A:** Autophagic vesicles. **B:** Whorls of rough endoplasmic reticulum. Magnification, ×15,000. Bar = 0.5 µm. n, nucleus; v, autophagic vesicles; w, whorls.

(Fig. 4B,C). Golgi apparatus was not yet affected by DFMO and APA in 2 days but required 4 days to adopt the expanded structure. Putrescine reversed the effect of DFMO and APA by turning the appearance of Golgi apparatus similar to that in control cells. When cells were grown for 6 days in the presence of DFMO and APA, but supplemented with putrescine for the last 2 days, Golgi apparatus returned to its juxtanuclear position, but was still slightly expanded. Similar alterations in the appearance of Golgi apparatus were found by the lectin WGA.

Protein Synthesis in Polyamine-Deprived Cells

As judged by [^{14}C]leucine incorporation, protein synthesis per cell was decreased by polyamine deprivation. After 2 days' culture with DFMO or APA, protein synthesis was 87 ± 9 and 58 ± 13% of control, respectively. After 4 days' culture with DFMO or APA, it was 33 ± 5 and 48 ± 16% of control cells, respectively. The incorporation of [^{14}C]leucine into extracellular, secreted material was too low to be measured accurately with the method used.

Metabolic Labeling of Proteoglycans and Hyaluronan

We considered it important to know whether the morphological alterations of the ER and Golgi apparatus were associated with a reduced capacity to synthesize and secrete Golgi-dependent macromolecules. For this purpose we chose proteoglycans, highly glycosylated mol-

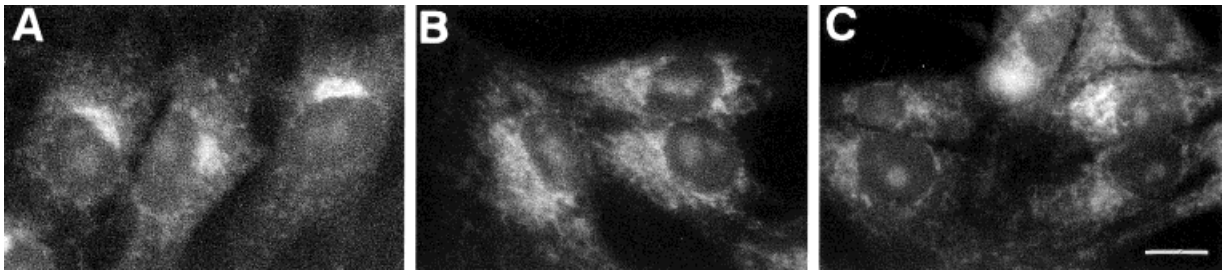

Fig. 4. Visualization of Golgi apparatus with C6-NBD-ceramide after culture for 4 days. Cells were cultured for 4 days in the following media: (**A**) no additions, (**B**) 5 mM DFMO, (**C**) 1 mM APA. Bar = 20 μm.

ecules some of which are secreted and others inserted in the plasma membrane. Hyaluronan, synthesized and secreted directly through plasma membrane [Prehm, 1984], served as a control for a possible block in the protein secretory pathway [Calabro and Hascall, 1994].

The assays of the [^{35}S]sulfate- and D-[6-^{3}H]glucosamine-labeled proteoglycans and hyaluronan in the culture media showed that in polyamine-deprived cells, the synthesis rate per cell is not changed. However, the secretion rates co-varied according to the cell density in control, DFMO- and APA-treated cells (Fig. 5). The synthesis was most active at low cell density, independent of the treatment, and decreased towards confluence. Anyway, as indicated by the level of radiolabeled sulfated glycosaminoglycans in culture medium, the total synthetic capacity was not compromised in polyamine-deprived cells. Thus, the dispersion of the Golgi apparatus towards cell periphery caused no inactivation of total proteoglycan synthesis.

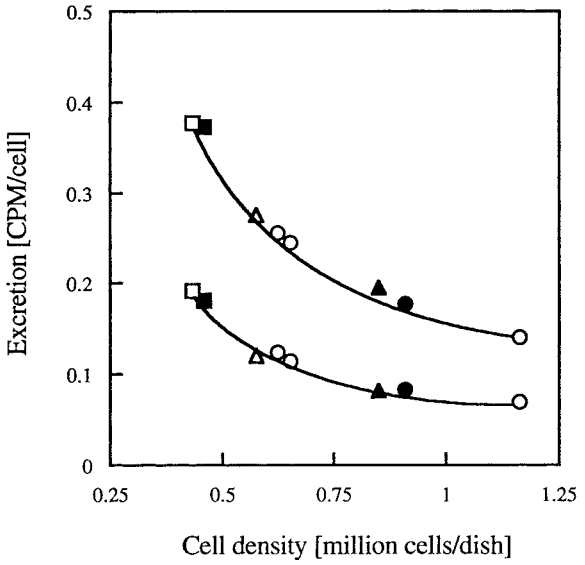

Fig. 5. Secretion of [^{3}H]hyaluronan (upper line) and [^{35}S]glycosaminoglycans (lower line) into growth medium at different cell densities. After 4 days in culture the cells were incubated for 24 h with [^{3}H]glucosamine (upper line) or [^{35}S]sulfate (lower line) and analyzed as described in Methods. Symbols: (○) control, (△) 5 mM DFMO, (□) 1 mM APA. Open symbols are without putrescine, closed symbols with 20 μM putrescine. Each point represents the mean of triplicate cultures.

Proteoglycans and other [^{35}S]sulfate-labeled molecules secreted by the BHK cells were characterized by SDS-agarose electrophoresis. In control cultures, four bands of [^{35}S]sulfate-labeled molecules were detected in the medium samples (Fig. 6, lane 1). Three of the four bands appeared to be proteoglycans since they were susceptible to glycosaminoglycan-degrading enzymes. The largest, slow-mobility band of molecular weight about 1.4×10^6 (DNA ladder as a standard) disappeared by both keratanase and by chondroitinase ABC, while the two faster-migrating bands were partly sensitive to heparitinase and chondroitinase ABC. The fastest migrating band was not digested by any of the enzymes tested and obviously represented sulfated proteins [Miller et al., 1992].

In a sharp contrast to control cultures (Fig. 6, lane 1), the medium from the polyamine-deprived cells showed only 2–3 bands, the one corresponding to the 1.4×10^6 DNA standard being totally absent in the treated cultures (Fig. 6, lanes 2 and 3). Putrescine reversed the effect of DFMO and APA by turning the band pattern similar to control cells (Fig. 6, lanes 5–6). Thus, polyamine deprivation changed the composition of proteoglycans secreted in the growth medium, a high molecular mass chondroitin/keratan sulfate proteoglycan being specifically missing in the treated cultures. The pattern of [^{35}S]sulfate-labeled macromolecules in the medium did not change during the 4 days of growth in control cultures (data not shown), indicating that the alteration in APA and

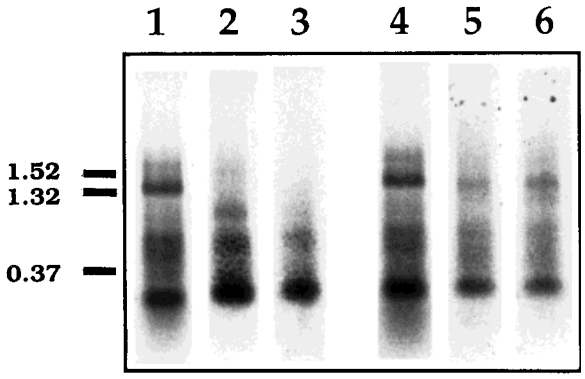

Fig. 6. Effect of APA and DFMO on the electrophoretic distribution of [^{35}S]sulfate labeled macromolecules secreted into culture media after culture for 4 days. Samples of culture medium after 24 h labeling with [^{35}S]sulfate were separated by SDS-agarose electrophoresis and localized by autoradiography. **Lanes:** (**1**) control, (**2**) 5 mM DFMO, (**3**) 1 mM APA, (**4**) 20 μM putrescine, (**5**) 5 mM DFMO and 20 μM putrescine, (**6**) 1 mM APA and 20 μM putrescine. Three bars on the left side indicate DNA standards with the molecular weights 1.52×10^6, 1.32×10^6, and 0.37×10^6 Da.

DFMO-treated cultures was not simply a cell density-dependent modulation of the type of secreted proteoglycans.

DISCUSSION

Organization of Cytoskeleton

Our results confirm that polyamine deprivation causes a disappearance of the stress fibers which traverse through the cytoplasm [McCormack et al., 1994; Pohjanpelto et al., 1981] but the actin cortex (membrane skeleton) just below plasma membrane remains clearly visible [McCormack et al., 1994]. Despite the continued presence of membrane skeleton, its functions such as regulation of cell shape and locomotion or response to growth factors and other external stimuli [Luna and Hitt, 1992] may be deteriorated in polyamine deprivation. Polyamines interact with skeletal membrane proteins, mainly spectrin, in erythrocyte ghosts [Wyse and Butterfield, 1988] and stimulate the polymerization of actin in vitro [Oriol-Audit et al., 1985]. Therefore, a direct effect of polyamines on the actin monomers, filaments, or modifying proteins is possible.

The microtubular system remained intact during polyamine deficiency in our BHK cells like in DFMO treated keratinocytes [Pomidor et al., 1995], but unlike the almost total absence in polyamine-deprived CHO cells [Pohjanpelto et al., 1981]. There are also conflicting reports of whether the synthesis of the precursors of microfilaments and microtubular systems, β-actin and α-tubulin, is affected by polyamines [Kaminska et al., 1992; McCormack et al., 1994]. Because the activity of protein synthesis is slightly decreased in polyamine deficiency, it is difficult to deduce whether impaired protein synthesis results in decreased synthesis of β-actin and α-tubulin or whether the disrupted microfilament system causes diminished protein synthesis. There is strong evidence that at least a part of the polysomes are bound to cytoskeleton during protein synthesis [Bassell, 1993; Hesketh and Pryme, 1991] and disruption of the polyribosome structure has been observed by transmission electron microscopy in lymphocytes treated with DFMO or ethylglyoxal bis(guanylhydrazone) [Sakamaki et al., 1989].

Rough Endoplasmic Reticulum

Redistribution of the rough endoplasmic reticulum was confirmed by two different methods which showed that ER was shifted towards cell periphery in polyamine-deprived cells. However, the most striking feature was the appearance of autophagic vesicles and involutions of the rough ER. Similar vacuoles and whorls have been observed in rat ventral prostate after castration [Helminen and Ericksson, 1971]. Of particular interest was their finding that vacuolar formation is preceded by focal cytoplasmic involutions, such as whorls of rough ER. As polyamine concentration in rat ventral prostate decreases after castration [Pegg et al., 1970], it is likely that the alterations in rat ventral prostate are polyamine-dependent. The yeast polyamine-auxotroph, which has an absolute requirement for spermidine or spermine for growth, also contains vesicle-like bodies as shown by the Nomarski technique [Balasundaram et al., 1991]. Polyamine deprivation is usually cytostatic to cells, not cytotoxic. Therefore, it is very likely that the alteration of the rough ER, which leads to redistribution, whorl formation, and autophagy, does not arise at random but is caused by a specific functional or structural modification of this organelle.

The cell controls the quality of secretory and membrane proteins before they leave the rough ER [Hammond and Helenius, 1995]. Misfolded or incorrectly assembled proteins are degraded in ER [Klausner and Sitia, 1990] or in lysosomes and are not delivered further to the Golgi

apparatus. It has been proposed that protein degradation takes place in a specialized part of ER, "salvage compartment," and that there is a direct pathway from this compartment to lysosomes [Seglen and Bohley, 1992]. The membranes of the smooth and rough ER expand when the synthesis of membrane proteins increases and the amount of membranes correlates also with that of the proteins when the latter are degraded [Lum and Wright, 1995, and references therein]. Hence, the redistribution of rough ER and the appearance of autophagic vesicles in polyamine-deprived cells can reflect the accumulation of proteins that cannot clear out of ER.

Golgi Apparatus

In polyamine-deprived cells, Golgi apparatus was swollen and expanded to the periphery of the cells. However, the microtubular system appeared to be normal, arguing against the possibility that the periferal location results from disrupted microtubules [Rogalski and Singer, 1984]. Swelling of Golgi apparatus was previously observed using transmission electron microscopy in lymphocytes treated with DFMO or ethylglyoxal bis(guanylhydrazone) [Sakamaki et al., 1989] and was accompanied by a reduced activity of galactosyltransferase, a Golgi marker enzyme. By transmission electron microscopy, swelling of endoplasmic reticulum, transport vesicles, Golgi apparatus, or secretory vesicles was also observed in glioblastoma cells treated with putrescine analogues (N,N-tetramethyl-α,ω-diaminoalkanes) [Quemener et al., 1993]. We used a ceramide and a lectin with binding sites on the medial and trans cisternae of the Golgi apparatus and observed that Golgi apparatus was enlarged and partly shifted towards the periphery of the cell. Whether this swelling is a direct effect of polyamine deprivation on Golgi cisternae or reliant on the redistribution of the rough endoplasmic reticulum, cannot be deduced from the present data.

Proteoglycan Synthesis

While the polyamine-deprived cells were able to synthesize [^{35}S]sulfate- and [^{3}H]glucosamine-labeled glycosaminoglycans in total quantities similar to those in the control cells, DFMO- and APA-treatment changed the composition of the proteoglycans. A high molecular weight chondroitin/keratan sulfate proteoglycan, in particular, was missing in the medium of cultures treated with DFMO and APA. Heparan sulfate and chondroitin/dermatan sulfate proteoglycans have been described previously in BHK cells [Bretscher, 1985; Sugahara et al., 1985] but a secreted large chondroitin sulfate proteoglycan also containing keratan sulfate has not been reported earlier. The total disappearance of this band by keratanase and chondroitinase ABC suggests that both keratan and chondroitin sulfate were present at relatively high proportions [Lammi et al., 1994].

Whether the large keratan/chondroitin sulfate proteoglycan secreted by BHK cells is somehow involved in cell migration, cell proliferation, or cell shape, is not known. However, it is interesting that chondroitin sulfate attached on the substratum or added into the growth medium modulates the integrin-mediated adhesion of BHK cells [Sigiura et al., 1993]. Interaction of cell surface components with extracellular matrix controls the organization of actin microfilaments. Especially, formation of stress fibers that traverse through the cell requires proper contact with cell surface components and extracellular matrix [LeBaron et al., 1988]. Stress fibers may not develop at all if transmembrane linking of cytoskeletal components to extracellular matrix does not occur [Izzard et al., 1986; LeBaron et al., 1988]. The actin cortex and anchorage of cells to substrata can be normal in these situations [Izzard et al., 1986].

The modulation of cell structures by DFMO and APA was a slow process, apparent after 4 days. Such a lag is typical for the development of polyamine deficiency. The deprivation of cellular putrescine and spermidine can take as long as 6 days in CHO polyamine auxotroph [Pohjanpelto et al., 1981] or 13 generations in yeast polyamine auxotroph [Balasundaram et al., 1991]. Spermidine forms a hypusine residue in eukaryotic initiation factor 5A (eIF-5A), which has also a long half-life [Park et al., 1993]. Although eIF-5A is indispensable for yeast its role in protein synthesis is still controversial. Whether the structural and functional alterations of ER and Golgi apparatus are related to the shortage of eIF-5A needs further study. Also, whether the altered pattern of proteoglycan secretion is caused by a defect in protein synthesis or in secretion remains to be seen. The role of the yet unidentified, polyamine-dependent extracellular matrix proteoglycan deserves further characterization.

ACKNOWLEDGMENTS

The skillful technical assistance of Eija Antikainen, Eija Korhonen, Eija Rahunen, and Elma Sorsa is gratefully acknowledged. We thank Dr. Alpo Pelttari for helpful discussions and for advice in transmission electron microscopy. The advice of Dr. Jüri Kärner (Tarto University, Estonia) in 3,3′-dihexyloxacarbocyanine iodide staining was valuable.

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