Cytotoxic Effect of Spermine on Retinal Pigment Epithelial Cells

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PURPOSE: A prior study showed inactivation of ornithine-δ-aminotransferase (OAT)-deficient human retinal pigment epithelial (RPE) cells by a specific irreversible inhibitor (5-fluoromethylornithine; 5-FMO) leading to cell death, in an in vitro model of gyrate atrophy (GA) of the choroid and retina. In the present study, the cytotoxicity of metabolites of ornithine, especially spermine, in RPE cells was investigated, to clarify the mechanism of ornithine cytotoxicity in RPE cells.

METHODS. RPE cells were incubated with ornithine or compounds involved in ornithine metabolic pathways. The effects on RPE cell viability and proliferative activity were evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric and [3H]thymidine incorporation assays. Incorporation of spermine into RPE cells was examined using [14C]spermine and dansyl-spermine. To assess spermine-induced RPE cell death, cells were double stained with annexin V and propidium iodide and subjected to flow cytometry.

RESULTS. Ornithine, arginine, glutamate, proline, creatine, glycine, and putrescine exhibited no effects on the viability and proliferative activities of RPE cells, whereas spermidine and spermine (10 mM) inhibited [3H]thymidine incorporation by 13% and 89%, respectively. The inhibition of [3H]thymidine incorporation by spermine was dose dependent and was observed as early as 4 hours after addition. Further, spermine was incorporated and accumulated in the perinuclear region of RPE cells. Apoptotic RPE cell death was induced by spermine in a dose-dependent manner.

CONCLUSIONS. The present results demonstrated that excessive spermine is cytotoxic to RPE cells and suggest that metabolites of ornithine, especially spermine, may be involved in the mechanism of RPE degeneration in GA. (Invest Ophtalmol Vis Sci. 2007;48:455–463) DOI:10.1167/iovs.06-0379

Gyrate atrophy (GA) of the choroid and retina is a rare, inborn defect of ornithine metabolism that is transmitted as an autosomal recessive trait.1 Patients with the disease usually exhibit progressive night blindness and loss of vision leading to blindness, with numerous sharply demarcated circular depigmented areas that gradually spread and become connected, resulting in characteristic findings of GA during an ocular fundus examination. Simell and Takki2 first reported that the biochemical abnormalities of this disorder are hyperornithinemia and overflow ornithinuria. Further, the finding of an association between hyperornithinemia and GA led to the discovery of an enzyme defect, a deficiency of the mitochondrial matrix enzyme ornithine-δ-aminotransferase (OAT).3–5

Genetically engineered mice lacking OAT exhibit symptoms and signs similar to those in patients with GA, such as chronic hyperornithinemia, massive ornithinuria, and progressive retinal degeneration,6 and experiments in such patients have revealed that RPE cells are the initial sites of insult in GA.7 However, the mechanisms by which OAT deficiency and subsequent ornithine accumulation cause retinal pigment epithelial (RPE) degeneration remain unclear.

We have reported that inactivation of OAT in human RPE cell lines by 5-fluoromethylornithine (5-FMO), a specific irreversible inhibitor of OAT, makes them susceptible to ornithine and leads to cell death.8 That system seems to be a useful in vitro model of GA for elucidation of the pathophysiological mechanisms of RPE degeneration.8–10 Because ornithine is one of the components of the urea cycle, it is taken into mitochondria and converted to citrulline by ornithine transcarbamoylase in the liver. However, in the mitochondria of RPE cells, ornithine is mainly converted to glutamate and proline via Δ1-pyrroline-5-carboxylic acid (P5C), which is produced by OAT.11 Under conditions in which OAT is deficient or inactivated, it is probably metabolized to polyamines, such as putrescine, spermidine, and spermine by the ornithine decarboxylase (ODC) present in the cytosol.12

Polyamines are ubiquitous biogenic alkylamines with a normal chain and are highly produced in such tissues as the prostate grand, pancreas, and submandibular gland, as well as in malignant tumors where secretory activity and/or protein and nucleic acid syntheses are active.13,14 Since 1949, when Herbst and Snell15 reported polyamines as regulators of cellular proliferation, several experiments and clinical investigations have been undertaken to elucidate the characteristics of polyamines. These studies demonstrated that polyamines are necessary for cellular proliferation,16–19 including that of RPE cells.20–21 On the contrary, they have been reported to become cytotoxic molecules after oxidation or via the production of acrolein.22–29 Hence, polyamines are thought to serve as both survival and death factors in cells. In the present study, we found that polyamines, especially spermine, had a cytotoxic effect on and induced apoptotic cell death in RPE cells.

MATERIALS AND METHODS

Cell Culture

Bovine eyes were obtained from a local abattoir within 2 hours of death and immediately transported on ice. Each eye was dissected, and the anterior segment, vitreous, and neural retina were removed. The eye cups were washed with phosphate-buffered saline (PBS; pH 7.4)
FIGURE 1. Effects of ornithine and its metabolites on RPE cells. (A) Illustration of ornithine metabolism in mammalian cells. Under healthy conditions, major ornithine metabolic pathways exist in the mitochondria, where ornithine is metabolized to citrulline via ornithine transcarbamoylase (OTC) and to P5C via OAT. Because OTC is lacking in RPE cells, when OAT is deficient or inactivated by 5-FMO, accumulated ornithine can be converted to putrescine, spermidine, and spermine via ODC in RPE cells. AdoMet, S-adenosylmethionine; BH₄, tetrahydrobiopterin; CAP, carbamyl phosphate; NO, nitric oxide. (B) Morphologic changes of bovine RPE cells treated with ornithine and its metabolites. RPE cells were treated with or without 1 and 10 mM of ornithine, arginine, putrescine, spermidine, or spermine for 24 hours. Morphologic changes were assessed using a confocal microscope. Original magnification, ×100.
and incubated with 0.25% porcine trypsin (containing 500 IU BAEE [N-benzyol L-arginine ethyl ester]; Sigma-Aldrich, St. Louis, MO) at 37 °C for 5 minutes. After the incubation, 2 ml Dulbecco’s modified Eagle’s (DMEM; Sigma-Aldrich) was added to the eye cups. RPE cells were removed from the eye cup and collected into a fire-polished Pasteur pipette and transferred to 50-ml centrifuge tubes with single-density gradient (Ficol; Sigma-Aldrich). The tubes were centrifuged at 120g for 5 minutes, after which the cells were resuspended in DMEM containing 4 mM glutamine, 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 µg/mL streptomycin, and 25 µg/mL amphotericin B. The cells were then transferred to a culture dish (Falcon; BD-Biosciences, Franklin Lakes, NJ) and incubated at 37°C in an atmosphere of 5% CO2/95% air. After 5 to 7 days, typical RPE colonies were isolated, and cultured in DMEM with 10% FBS (10% FBS-DMEM) and the same antibiotics. Cells at passages 3 to 7 were used in the experiments.

**Cytotoxic Effect of Ornithine Metabolites**

RPE cells were plated in 12-well culture dishes at 1.2 × 10^3/well in 10% FBS-DMEM. After reaching subconfluence, the cells were treated with 1 or 10 mM ornithine, putrescine, spermidine, spermine, or arginine in 10% FBS-DMEM for 24 hours. The cytotoxicity of ornithine and its metabolites was evaluated morphologically in microphotographs taken with a digital camera (SPOT, Diagnostic Instruments, Sterling Heights, MI) through an inverted confocal microscope (IX70; Olympus, Tokyo, Japan).

**[^3H]Thymidine Incorporation Assay**

To evaluate the effects of ornithine and its metabolites on DNA synthesis, we determined the incorporation of [^3H]thymidine with synchronized RPE cells that had been grown in the G0/G1 phase by 24-hour serum starvation. Briefly, cells were seeded onto 24-well culture dishes at 6 × 10^5/well in 10% FBS-DMEM. After reaching subconfluence, the cells were cultured for 24 hours in DMEM without FBS. The synchronized RPE cells were briefly washed with PBS, and the medium was replaced with DMEM supplemented with 10% horse serum (10% HS-DMEM) containing 0.2 × 10^5/ml [^3H]thymidine (specific activity, 25 Ci/mmol; GE Healthcare, Arlington Heights, IL) through an inverted confocal microscope (IX70; Olympus, Tokyo, Japan).

**[^3H]Thymidine Incorporation in RPE Cells**

<table>
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<tr>
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<th>[^3H]Thymidine Incorporation (% of Control)</th>
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<tr>
<td></td>
<td>1 mM</td>
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<tr>
<td>Vehicle</td>
<td>100.0 ± 10.9</td>
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<tr>
<td>Ornithine</td>
<td>94.1 ± 5.9</td>
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<tr>
<td>Putrescine</td>
<td>91.4 ± 6.2</td>
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<tr>
<td>Spermidine</td>
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<tr>
<td>Spermine</td>
<td>118.2 ± 9.6</td>
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<tr>
<td>Arginine</td>
<td>93.8 ± 5.3</td>
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<tr>
<td>Glutamate</td>
<td>108.9 ± 12.6</td>
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<tr>
<td>Proline</td>
<td>106.1 ± 9.9</td>
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<tr>
<td>Creatine</td>
<td>109.1 ± 20.0</td>
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<tr>
<td>Glycine</td>
<td>108.0 ± 1.3</td>
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Synchronized RPE cells were incubated for 24 hours in DMEM supplemented with 10% HS containing various agents and 0.2 µCi/mL [^3H]thymidine. Data are the mean ± SD of results of three independent experiments.* P < 0.01, as compared with the control.

**Determination of Apoptosis**

RPE cells were plated in 6-cm culture dishes at 6 × 10^5/well in 10% FBS-DMEM. After reaching subconfluence, the cells were treated with 1, 5, or 10 mM spermine in 10% FBS-DMEM and, after 24 hours of incubation, washed with PBS 3 times and resuspended in binding buffer (10 mM Hepes [pH 7.4], 140 mM NaCl, 2.5 mM CaCl2). The cells were then transferred to a culture dish (Falcon; BD-Biosciences) and 0.1 µL aliquots of lysate was lyzed. Further, the radioactivity of [^14]C]spermine incorporated in the RPE cells was measured at 8 hours after the addition of 0.01 to 10 mM of nonlabeled spermine. The incorporation of spermine was determined by the specific activity of [^14]C]spermine.

Because DNA synthesis was inhibited by the cytotoxicity of extra-cellular spermine at 4 and 10 mM after 8 hours, we examined the influence of DNA synthesis on the RPE cells at 1.5, 2.5, 4.5, and 6.5 hours after the addition of 10 mM spermine. In another examination, N1-monodansyl-spermine was synthesized as described previously. Next, RPE cells were plated on glass-bottomed dishes and incubated in 10% HS-DMEM with 1 mM of dansyl-spermine for 30 minutes. After a brief wash with PBS, the medium was substituted with fresh medium. After 2, 8, and 24 hours of incubation, the cells were observed by confocal fluorescence microscope (excitation wavelength, 365 nm).

**Statistics**

Statistical significance was determined by ANOVA with the Bonferroni correction. Values for 50% growth inhibitory concentration (IC50) and 50% LC50 of spermine on cell growth and viability, respectively, were calculated with a computer program in a Probit test. Data are expressed as the mean ± SD of three or more separate experiments. Levels of P < 0.01 were considered significant.
RESULTS

Effects of Ornithine and Its Metabolites on Cell Viability and Proliferation of RPE Cells

It has been reported that ornithine is mainly converted to glutamate and proline in RPE cells; however, we speculated that the synthesis of other metabolites of ornithine would increase under conditions in which OAT was deficient or inactivated (Fig. 1A). To clarify whether newly synthesized metabolites of ornithine have a cytotoxic effect on RPE cells, we first investigated the effects of those compounds on cell viability. Figure 1B shows morphologic changes of RPE cells.

Figure 2. Inhibitory effects of spermine on DNA synthesis in RPE cells. (A) Time course of $[^3]$Hthymidine incorporation into RPE cells. Cells were incubated in 10% HS-DMEM containing 0.2 $\mu$Ci/mL $[^3]$Hthymidine and 0, 4, and 10 mM spermine. The reactions were terminated at the indicated times after a 24-hour incubation in serum-free DMEM. $^P < 0.01$ vs. 0 mM spermine. $^P < 0.01$ vs. 4 mM spermine at each time point. (B) Dose–response effects of spermine (●) and ornithine (○) on $[^3]$Hthymidine incorporation in RPE cells. Cells were incubated in 10% HS-DMEM containing 0.2 $\mu$Ci/mL $[^3]$Hthymidine and various concentrations of spermine or ornithine. The incorporation of $[^3]$Hthymidine into RPE cells after an 8-hour incubation was estimated and compared with that using control medium. The incorporation of $[^3]$Hthymidine was considered to be $100\%$, and the results are expressed as a percentage of the control. $^P < 0.01$ versus 0 mM for the control. Each data point represents the mean ± SD of results of three independent experiments, with similar results obtained in at least two additional experiments.

Figure 3. Effects of spermine on DNA synthesis ($[^3]$Hthymidine incorporation) and cellular viability (MTT colorimetric assay). (A) Time course of the effects of spermine on $[^3]$Hthymidine incorporation and MTT colorimetric assay results. Serum-deprived RPE cells were incubated in 10% HS-DMEM, with or without 10 mM spermine and 0.2 $\mu$Ci/mL $[^3]$Hthymidine (●) for the indicated times. The ratio of $[^3]$Hthymidine incorporated in the presence of 10 mM spermine to that without spermine is expressed as a percentage of the control at each time point. Serum-deprived RPE cells were incubated with 10 mM spermine and 0.5 mg/mL MTT solution (○). (B) Dose–response effects of spermine in an MTT colorimetric assay. $^P < 0.01$ compared with 0 mM. Each data point represents the mean ± SD results of at least three independent experiments.
treated with ornithine and its metabolites. Ornithine, arginine, and putrescine, a metabolite of ornithine via ODC, did not show cytotoxicity on RPE cells. In contrast, RPE cells treated with 10 mM of spermidine showed shrinkage and reduced cell density, whereas those treated with 10 mM spermine became round and detached from the dish. Next, we examined the effects of ornithine and its metabolites on the proliferation of RPE cells for 24 hours, as assessed by [3H]thymidine incorporation. As shown in Table 1, ornithine itself (1 and 10 mM) exhibited no effect on the incorporation of [3H]thymidine into RPE cells compared with the control (94.0% ± 5.9% and 95.4% ± 1.5%, respectively). In addition, other compounds involved in ornithine metabolism, such as arginine, glutamate, proline, creatine, and glycine, as well as putrescine also had no influence on the incorporation of [3H]thymidine. In contrast, spermidine and spermine at 10 mM decreased that incorporation by 13% and 89%, respectively.

To clarify the inhibitory effects of spermine on [3H]thymidine incorporation in RPE cells, we next examined the time course and dose dependency of that inhibition in synchronized cells (Figs. 2A, 2B). Spermine at 4 mM significantly inhibited incorporation at 4 hours after its addition (P < 0.01 vs. 0 mM for the control), and the effect was greater at a dose of 10 mM (P < 0.01 vs. 4 mM spermine). The inhibition of [3H]thymidine incorporation by spermine continued for the entire 24-hour period (Fig. 2A). The dose-dependent effects of ornithine and spermine on [3H]thymidine incorporation in RPE cells are shown in Figure 2B. Whereas 10 mM spermine had a strong cytostatic effect, ornithine scarcely influenced [3H]thymidine incorporation, even at 10 mM. The IC_{50} of spermine for [3H]thymidine incorporation after 8 hours was 6.29 mM.

Effects of Spermine on the Viability of RPE Cells

To clarify the relationship between cell viability and inhibition of DNA synthesis produced by spermine, we examined the effects of spermine on the cellular viability of RPE cells by using an MTT colorimetric assay and compared the results to those obtained in the [3H]thymidine incorporation assay. Whereas the incorporation of [3H]thymidine was significantly inhibited at 4 hours after the addition of 10 mM spermine (Fig.
2A), the MTT colorimetric assay revealed a reduction in RPE cell viability of 50% at 16 hours after addition (Fig. 3A). This result may reflect the inhibition of \(^{3}H\)thymidine incorporation (Fig. 2A). Figure 3B shows the dose dependency of spermine on RPE cell viability. Cell viability decreased in a dose-dependent manner to 20.0% after 24 hours of treatment with 10 mM spermine. The concentration of spermine needed to obtain 50% cellular mortality (LC\(_{50}\)) was calculated to be approximately 6.4 mM.

To determine the time necessary for inhibition of \(^{3}H\)thymidine incorporation by spermine, we pretreated the cells with 10 mM spermine for 0, 1.5, 2.5, 4.5, and 6.5 hours. \(^{3}H\)Thymidine incorporation was inhibited by spermine in a time-dependent manner, with 10 mM spermine causing inhibition by 31.4%, 60.7%, 89.8%, and 94.6% in cells exposed before incorporation for 1.5, 2.5, 4.5, and 6.5 hours, respectively (Fig. 4, open columns). The inhibitory effect of spermine was significantly greater after 0, 1.5, and 2.5 hours in cells that remained exposed to spermine during incorporation (Fig. 4, filled columns).

### Spermine Incorporation by RPE Cells

To elucidate the mechanism by which spermine inhibits \(^{3}H\)thymidine incorporation in RPE cells, we examined whether \(^{14}C\)spermine was incorporated in the RPE cells. As shown in Figure 5A, spermine was linearly incorporated by RPE cells after 24 hours. Further, when nonlabeled spermine (0.01–10 mM) was added to the culture medium in the presence of \(^{14}C\)spermine, the incorporation of \(^{14}C\)spermine into RPE cells increased in a concentration-dependent manner and reached a plateau at 3 mM (Fig. 5B), suggesting that the incorporation of spermine into RPE cells is saturable. Next, spermine accumulation and its distribution in RPE cells were examined using dansyl-spermine. Although fluorescence was nearly absent in the cells before the addition of dansyl-spermine to the culture medium (Fig. 6A), vesicles and fibers in the cytoplasm showed distinct fluorescence at 2 hours after the addition of 1 mM of dansyl-spermine (Fig. 6B). The fluorescence was concentrated in the perinuclear region after 8 hours (Fig. 6C) and accumulated in the perinuclear region after 24 hours (Fig. 6D). Dansyl-spermine inhibited \(^{3}H\)thymidine incorporation with an IC\(_{50}\) that was one order lower than that of spermine, as shown in Figure 7. Thus, dansyl-spermine was rapidly incorporated into the cytoplasm and accumulated around the nuclei in a time-dependent manner. Because \(^{3}H\)thymidine uptake began to be restrained at 4 hours and cell viability was affected after 16 hours, these results suggest that accumulation of intracellular spermine is one of the causes of cytotoxicity.

### Induction of Apoptotic Cell Death in RPE Cells by Spermine

To clarify the mechanism of spermine-induced cell death in RPE cells, we determined both early and late apoptosis using annexin V-FITC and PI labeling of live cells. Annexin V binds to phosphatidylserine exposed on the cell membrane and is one of the earliest indicators of cellular apoptosis. The micrographs in Figure 8 show the morphology of spermine-treated RPE cells. The cells were not affected with 1 mM spermine, whereas they were partially damaged and detached from the dish with 5 mM, and nearly all cells showed extreme damage and were detached with 10 mM. As shown in the plots in Figure 8, flow cytometric analysis revealed that the number of early apoptotic cells, represented by annexin V\(^{-}/\)PI\(^{+}\) (right lower quadrants in the density plot), was increased by 11.2-fold with 5 mM compared with the control cells. Because the number of early apoptotic cells decreased with 10 mM spermine, there was a concomitant increase in late apoptotic cells represented by annexin V\(^{-}/\)PI\(^{-}\) (Fig. 8, right upper quadrants in density plots).

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**Figure 6.** Confocal micrographs of dansyl-spermine–treated bovine RPE cells. Cells were plated on glass-bottomed dishes and incubated for 30 minutes in 10% HS-DMEM containing 1 mM of dansyl-spermine, after which the medium was replaced with fresh medium. (A) Before and after (B) 2, (C) 8, and (D) 24 hours of incubation, the cells were observed with a confocal microscope. Original magnification, ×400.

**Figure 7.** Dose–response effects of spermine and dansyl-spermine on \(^{3}H\)thymidine incorporation. Cells were incubated for 8 hours in 10% HS-DMEM containing 0.2 \(\mu\)Ci/mL \(^{3}H\)thymidine and 0.01 to 10 mM spermine or dansyl-spermine. *\(P < 0.01\) compared with 0 mM (control).
Polyamines have been shown to exert various physiological actions, such as stabilization of nucleic acids,\(^{35,36}\) acceleration of various kinds of nucleic acid synthetic pathways,\(^{37}\) acceleration of protein synthesis,\(^{38}\) acetylation of histone,\(^{39}\) acceleration of phosphorylation of nonhistone chromatin protein,\(^{40}\) and stabilization and enhancement of permeability of the plasma membrane\(^{41}\) and also function as both growth and death factors in cells. The activation of protein synthesis is particularly associated with polyamine-induced cell growth. Yanagihara et al.\(^{22}\) reported that polyamines, especially spermine, compensated for the growth arrest of cultured bovine RPE cells induced by serum deprivation at a concentration of 100 \(\mu\)M and concluded that \(\delta\)-adenosylmethionine decarboxylase activity was more critical than was ODC activity for cell proliferation. However, we speculate that when OAT is inactivated or deficient, the synthesis of polyamines or other metabolites of ornithine that show a cytotoxic effect on RPE cells would be increased, which may be one of the causes of RPE cell death in GA. The present results demonstrated that polyamines, especially spermine, exerted cytotoxicity on cultured bovine RPE cells (Fig. 1). In contrast to the report by Yanagihara et al.,\(^{22}\) the present findings showed that polyamines exert a cytotoxic, rather than proliferative, effect on RPE cells in the millimolar range. The discrepancy between our results and those of the previous report might be explained by the concentrations of polyamines as well as culture conditions used, as serum-added medium was used in our experiments.

Although spermine is known to be an indispensable material for cell growth acceleration,\(^{19-21}\) polyamines become cytotoxic molecules after oxidation by amine oxidase existing in serum.\(^{22-29}\) To investigate the cytostatic effects of spermine, we attempted to determine whether it had an influence on cell growth in a dose- and time-dependent manner. At a concentration of 4 mM, spermine significantly inhibited incorporation of \(^{3}H\)thymidine at 4 hours after addition \((P < 0.01;\) Fig. 2A) and the effect was greater with 10 mM \((P < 0.01\) vs. 4 mM spermine). Spermine also showed a tendency to increase the effect was greater with 10 mM \((P < 0.01\) vs. 4 mM spermine). Spermine also showed a tendency to increase the incorporation of \(^{3}H\)thymidine incorporation at 10 mM and strongly inhibited it at 10 mM (Table 1, Fig. 2B). In contrast, ornithine scarcely influenced thymidine incorporation at a concentration of 10 mM. Thus, in the present study, spermine regulated cell proliferation in both positive and negative directions, as has been shown in several other experimental models.\(^{42}\) Further, DNA synthesis was inhibited by spermine before cell viability decreased (Fig. 3A).

These effects of spermine seemed to occur after it had been incorporated by the RPE cells. However, it is also possible that spermine acts on the cytoplasmic membrane at a high concentration for a long period, subsequently causing inhibition of DNA synthesis. To clarify its mechanisms of action, we also examined the effects of spermine on DNA synthesis in RPE cells temporarily exposed to it before \(^{3}H\)thymidine incorporation, and the results suggested that incorporated spermine may be cytotoxic to RPE cells (Fig. 4). In addition, \(^{14}C\)spermine was linearly incorporated in RPE cells up to 24 hours after its addition and 3 mM of nonlabeled spermine saturated the incorporation of \(^{14}C\)spermine in RPE cells (Fig. 5A, 5B), suggesting the presence of an incorporation mechanism such as a spermine transporter. Although the transport of polyamines has been well characterized in bacteria, genes associated with the transporter have not been identified in mammalian specimens, including RPE cells.\(^{14}\)

To visualize the accumulation and distribution of spermine in RPE cells, we synthesized dansyl-spermine to determine whether intracellular spermine was the main cause of the inhibition of DNA synthesis in RPE cells. Figure 6 shows that dansyl-spermine was incorporated in RPE cells and accumulated in the perinuclear region. Also, DNA synthesis inhibition occurred with dansyl-spermine at one order lower than with spermine (Fig. 7). We considered the reason for this phenomenon to be that the membrane permeability of dansylated spermine is markedly increased by the substitution of an hydrophobic dansyl group for the primary amine group.\(^{32}\) These results support the notion that intracellular accumulation of spermine causes DNA synthesis inhibition, because thymidine incorporation was restrained even after spermine was removed (Fig. 4).

Possible mechanisms of polyamines for apoptosis have been proposed.\(^{42-44}\) Natural polyamines have multiple ways of performing physiological functions, including binding to anionic structures or formation of cytotoxic products from oxidative deamination such as aldehydes and reactive oxygen species. The involvement of polyamines in gene expression and several signaling pathways including apoptotic signaling have also been reported.\(^{45,46}\) In the present study, we found that spermine induced apoptosis in cultured bovine RPE cells.

GA is biochemically characterized by the presence of hyperornithinemia due to a deficiency of OAT. In RPE cells, OAT activity was much higher than that of ODC; therefore, excess ornithine is preferably metabolized into PSC through OAT rather than polyamine synthesis via ODC in normal state RPE.
cells; however, the synthesis of polyamines may be increased in OAT-deficient RPE cells. Plasma ornithine levels in GA patients range from 0.4 to 1.4 mM, ~10 times greater than the normal range, and a chronic reduction of plasma ornithine and an arginine-restricted diet have been shown to slow or stop chorioretinal degeneration.37–39 This correlation between plasma ornithine level and disease progression implies the involvement of an increased production of polyamines by ODC in GA, resulting in chorioretinal degeneration. Although additional experiments are necessary, our results suggest that ornithine metabolites are involved in the mechanism of RPE degeneration in GA, as excess levels of polyamines are cytotoxic to RPE cells.

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References