Low-Affinity Transport of FITC-Albumin in Alveolar Type II Epithelial Cell Line RLE-6TN

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Summary: FITC-albumin uptake by cultured alveolar type II epithelial cells, RLE-6TN, is mediated by high- and low-affinity transport systems. In this study, characteristics of the low-affinity transport system were evaluated. The uptake of FITC-albumin was time and temperature dependent and was inhibited by metabolic inhibitors and bafilomycin A1. Confocal laser scanning microscopic analysis showed punctate localization of the fluorescence in the cells, which was partly localized in lysosomes. FITC-albumin taken up by the cells gradually degraded over time, as shown by fluoroimage analyzer after SDS-PAGE. The uptake of FITC-albumin by RLE-6TN cells was not inhibited by caveolae-mediated endocytosis inhibitors such as nystatin, but was inhibited by clathrin-mediated endocytosis inhibitors such as phenylarsine oxide. The uptake was also inhibited by potassium depletion and hypertonicity, conditions known to inhibit clathrin-mediated endocytosis. In addition, macropinocytosis inhibitors such as 5-(N-ethyl-N-isopropyl) amiloride inhibited the uptake. These results indicate that the low-affinity transport of FITC-albumin in RLE-6TN cells is at least in part mediated by clathrin-mediated endocytosis, but not by caveolae-mediated endocytosis. Possible involvement of macropinocytosis was also suggested.

Keywords: alveolar epithelial cells; RLE-6TN; albumin; clathrin-mediated endocytosis; caveolae-mediated endocytosis; macropinocytosis

Introduction

The alveolar region of the lung is lined with a continuous epithelium comprising two major types of epithelial cells, type I and type II. Type I epithelial cells have a squamous morphology and cover approximately 90–95% of the alveolar surface area. Type II cells are cuboidal epithelial cells and cover about 5–10% of the surface area, though the number of type II cells in alveolar epithelia is similar to or more than that of type I cells. Type II cells also serve as progenitors of type I cells. Alveolar lining fluid contains various physiologically important proteins such as albumin, immunoglobulin G, and transferrin. The concentration of albumin is normally about 5 mg/mL in alveolar fluid and 40 mg/mL in the plasma, while the concentration in alveolar fluid would increase to 40–65% of the plasma level in hydrostatic pulmonary edema and to 75–95% in lung injury pulmonary edema. The clearance of serum proteins from the alveolar space is a critical process in recovery from pulmonary edema as well as in maintaining the normal alveolar milieu. Therefore, understanding the mechanisms of protein transport in alveolar epithelial cells is important for the development of better therapeutic approaches. In addition, such information could provide new insights into the strategies for pulmonary delivery of exogenous protein drugs into the systemic circulation.

Clearance mechanisms of serum proteins from the alveolar space have been studied for years, and among the mechanisms proposed, endocytosis (transcytosis) in alveolar epithelial cells is probably most important. However, detailed mechanisms underlying the protein
transport in alveolar epithelial cells are still unclear. The established cell line is a powerful tool for studying transport events at the cellular level. Driscoll et al.\textsuperscript{3} established an immortalized alveolar epithelial cell line, RLE-6TN, from rats. RLE-6TN cells have several characteristics similar to the lung alveolar type II epithelial cells, such as the expression of cytokeratin 19 and the presence of lipid-containing inclusion bodies in the cells.\textsuperscript{7–9} However, there is little information concerning the transport and/or endocytic functions of RLE-6TN cells. Recently, we examined the usefulness of RLE-6TN cell line as an in-vitro model system of alveolar type II epithelial cells, and reported that FITC-albumin uptake in RLE-6TN cells is mediated by high- and low-affinity transport systems.\textsuperscript{1)} RLE-6TN cells grown on 24-well plates were used. After removal of the culture medium, each well was washed and preincubated with phosphate-buffered saline (137 mM NaCl, 3 mM KCl, 8 mM Na2HPO4, 1.5 mM KH2PO4, 0.1 mM CaCl2, and 0.5 mM MgCl2, pH 7.4) (PBS buffer) supplemented with 5 mM D-glucose (PBS-G buffer) at 37°C for 10 min. Then, PBS-G buffer containing FITC-albumin (4 mg/mL) was added to each well, and the cells were incubated at 37°C or 4°C for a specified period.

For inhibition studies, RLE-6TN cells were preincubated with PBS or PBS-G buffer at 37°C in the absence or presence of the inhibitor as follows: 1 mM 2,4-dinitrophenol for 10 min in PBS buffer, 10 mM sodium azide plus 5 mM 2-deoxy-D-glucose for 10 min in PBS buffer, bafilomycin A1 (25 nM–100 nM) for 30 min in PBS-G buffer containing 0.5% dimethyl sulfoxide (DMSO), nystatin (15–50 μM) for 10 min in PBS-G buffer containing 0.5% DMSO, indomethacin (100–300 μM) for 10 min in PBS-G buffer, phenylarsine oxide (1–5 μM) for 10 min in PBS-G buffer containing 0.5% DMSO, chlorpromazine (30–80 μM) for 10 min in PBS-G buffer, 5-(N-ethyl-N-isopropyl) amiloride (40–160 μM) for 30 min in PBS-G buffer containing 0.5% DMSO, and cytochalasin D (5–20 μM) for 15 min in PBS-G buffer containing 0.5% DMSO. The same vehicles were used for each control experiment. Then, the cells were incubated with 250 μL of the buffer containing FITC-albumin in the absence or presence of the inhibitor at 37°C for 60 min. Bafilomycin A1 and phenylarsine oxide were used only in the preincubation buffer and were not added to the uptake buffer. The effects of potassium depletion and hypertonicity on FITC-albumin uptake in RLE-6TN cells were examined as reported by Hansen et al.\textsuperscript{10} The control buffer used for potassium depletion and hypertonicity experiments consisted of 140 mM NaCl, 10 mM KCl, 20 mM HEPES, 1 mM CaCl2, 1 mM MgCl2, and 1 mg/mL D-glucose (pH 7.4). For the hypertonicity experiment, 450 mM sucrose was added to the control buffer, and for the potassium depletion experiment, KCl was omitted from the control buffer. Uptake of FITC-albumin in these treated or control RLE-6TN cells was examined as de-
At the end of the incubation, the uptake buffer was aspirated and the cells were washed rapidly three times with 1 mL of ice-cold PBS buffer. The cells were scraped with a rubber policeman into 0.75 mL of ice-cold PBS buffer and the wells were rinsed again with 0.75 mL of ice-cold PBS buffer to improve the recovery of the cells. The cells were further washed by centrifugation at 4°C for 3 min at 9,838 g twice. After the supernatant was aspirated, the pellet was solubilized in 1.2 mL of 0.1% Triton X-100 in PBS buffer without CaCl$_2$ and MgCl$_2$ at room temperature for 30 min, and centrifuged for 3 min at 5,600 g. The supernatant was used for fluorescence and protein assays.

Confocal laser scanning microscopy: RLE-6TN cells were grown on 35-mm glass bottom culture dishes for 4 or 5 days. The cells were incubated with FITC-albumin (4 mg/mL) for 60 min at 37°C or at 4°C as described above, and after washing the cells with ice-cold PBS buffer three times for 5 min each, fluorescence in the cells was observed by confocal laser scanning microscopy (LSM5 Pascal, Carl ZEISS, Germany). In some cases, LysoTracker® Red (75 nM), a fluorescent lysosomal marker, and Hoechst 33342 (10 μM), a fluorescent nucleus marker, were added to the uptake medium containing FITC-albumin, and the cells were incubated for 30 min and 60 min, respectively.

Evaluation of intactness of FITC-albumin in RLE-6TN cells: The intactness of FITC-albumin was evaluated in the cells after uptake for 10, 30, or 60 min. In pulse-chase analysis, the cells were incubated with FITC-albumin for 60 min, and after washing, the cells were further incubated with PBS-G buffer without FITC-albumin for 30, 60, 90 or 120 min. These cell samples were solubilized in a loading buffer consisting of 2% SDS, 50 mM Tris-HCl, 10% glycerol, and 6% 2-mercaptoethanol. Then, the sample was subjected to SDS-PAGE with 10% polyacrylamide gel. After SDS-PAGE, the fluorescence intensity of the gel was analyzed by fluoroimage analyzer FLA-2000 (Fuji Photo Film, Tokyo, Japan), and the image was obtained with the aid of a Macintosh personal computer and software provided with the analyzer.

Other analytical methods: The amount of FITC-albumin taken up by RLE-6TN cells was measured using a Hitachi fluorescence spectrophotometer F-3000 (Tokyo, Japan) at an excitation wavelength of 500 nm and an emission wavelength of 520 nm. Protein was determined by the Lowry method with bovine serum albumin served as the standard.

Statistical analysis: Data are expressed as means ± SEM. Statistical analysis was performed by Student’s $t$-test or one-way ANOVA followed by the Tukey’s test for multiple comparisons. The level of significance was set at $^*p<0.05$ or $^{**}p<0.01$.

Results

General characteristics of low-affinity transport of FITC-albumin in RLE-6TN cells: In order to characterize the low-affinity transport system of FITC-albumin in RLE-6TN cells, the concentration of FITC-albumin was set at 4 mg/mL based on our previous report. At this concentration, the contribution of the low-affinity transport system is around 70% of total uptake and contribution dose not increase so much at higher concentrations. Fig. 1 shows the time course of FITC-albumin uptake by RLE-6TN cells. FITC-albumin was taken up by the cells at 37°C, and the uptake increased with time for up to 60 min. In contrast, the uptake was markedly suppressed at 4°C.

The effects of metabolic inhibitors on FITC-albumin uptake by RLE-6TN cells were examined. Pretreatment of the cells with either 2,4-dinitrophenol or sodium azide plus 2-deoxy-D-glucose significantly inhibited FITC-albumin uptake (Fig. 2). The uptake of FITC-albumin was also inhibited by the pretreatment of the cells with bafilomycin A$_1$, an inhibitor of vacuolar H$^+$-ATPase, in a concentration-dependent fashion (Fig. 3). We then measured the uptake of a fluid-phase endocytosis marker, FITC-dextran, and compared with that of FITC-albumin. The uptake of FITC-dextran (4 mg/mL) at 37°C for 60 min was 0.67 ± 0.01 μg/mg protein, which was around 3–4% of that of FITC-albumin. These results indicate that the low-affinity transport of FITC-albumin in RLE-6TN cells is mediated by receptor-mediated endocytosis.

Localization and fate of FITC-albumin taken up by RLE-6TN cells: Intracellular localization of FITC-albumin was examined by confocal laser scanning microscopy. When RLE-6TN cells were incubated with
Fig. 2. Effect of metabolic inhibitors on FITC-albumin uptake by RLE-6TN cells. The cells were treated with 2,4-dinitrophenol (DNP), or sodium azide (NaN3) plus 2-deoxy-D-glucose (2DOG) as described in Materials and methods, and the uptake of FITC-albumin (4 mg/mL) for 60 min was measured at 37°C. Each column represents the mean ± SEM of 3 monolayers. **, P < 0.01; significantly different from the control.

Fig. 3. Effect of a vacuolar H+ -ATPase inhibitor on FITC-albumin uptake by RLE-6TN cells. The cells were pretreated with various concentrations of bafilomycin A1 as described in Materials and methods, and the uptake of FITC-albumin (4 mg/mL) for 60 min was measured at 37°C. Each point represents the mean ± SEM of 3 monolayers. *, P < 0.05, **, P < 0.01; significantly different from each control.

FITC-albumin at 37°C for 60 min, punctate localization of fluorescence was observed in the cells (Fig. 4A). This phenomenon was not observed when incubation was at 4°C (date not shown). Figs. 4B and 4C showed the fluorescence of LysoTracker® Red, a lysosomal marker, and Hoechst 33342, a nucleus marker, simultaneously added to the uptake buffer with FITC-albumin, respectively. As shown in Fig. 4D, colocalization of FITC-albumin and LysoTracker® Red was observed, indicating that the part of FITC-albumin taken up by the cells was targeted to lysosomes.

The intactness of FITC-albumin in the cells was evaluated by fluoroiimage analyzer after SDS-PAGE. As shown in Fig. 5A, the amount of intact FITC-albumin in the cells increased with time during incubation with FITC-albumin. In addition, the increase in the fluorescence was observed at the running top of the gel, which would be free FITC and/or small degradation products. Fig. 5B showed the result of pulse-chase analysis, and FITC-albumin taken up by RLE-6TN cells gradually degraded over time (~ 39% at 2.0 hr). Fig. 5C showed the change in the cellular level of intact FITC-albumin with time during and after incubation with FITC-albumin, which was estimated from the data in Figs. 5A and 5B. Half-life of FITC-albumin in the cells was calculated to be 2.1 hr. These results indicate that FITC-albumin taken up by the cells gradually degraded over time, at least in part, probably in lysosomes.

Role of caveolae- and clathrin-mediated endocytosis and macropinocytosis in low-affinity transport of FITC-albumin in RLE-6TN cells: We then studied the characteristics of endocytic uptake of FITC-albumin by RLE-6TN cells. Pinocytosis can be divided into at least three groups: caveolae-mediated endocytosis, clathrin-mediated endocytosis, and macropinocytosis.11 At first, the role of caveolae-mediated endocytosis, a clathrin-independent process, was examined. For this purpose, nystatin and indomethacin were employed as inhibitors of caveolae-mediated endocytosis. Nystatin at various concentrations did not affect FITC-albumin uptake by RLE-6TN cells (Fig. 6A). In addition, no inhibition was observed by indomethacin (Fig. 6B), although the uptake was slightly stimulated at some concentrations of this compound. Therefore, caveolae-mediated endocytosis would not be involved in FITC-albumin uptake by the low-affinity transport system in RLE-6TN cells.

Next, we examined the role of clathrin-mediated endocytosis by using its inhibitors, phenylarsine oxide and chlorpromazine. The uptake of FITC-albumin was inhibited by the pretreatment of the cells with phenylarsine oxide and chlorpromazine (Fig. 7A). Similarly, FITC-albumin uptake was inhibited by chlorpromazine (Fig. 7B). Furthermore, the effects of potassium depletion and hypertonicity on FITC-albumin uptake, the conditions known to inhibit clathrin-mediated endocytosis,10,12 were examined. As shown in Fig. 7C, potassium depletion and hypertonicity significantly inhibited FITC-albumin uptake by RLE-6TN cells. These results indicate that FITC-albumin uptake by the low-affinity transport system in RLE-6TN cells is partly mediated by clathrin-mediated endocytosis.

However, the inhibitory effects of clathrin-mediated
endocytosis inhibitors described above were somewhat weaker than those by metabolic inhibitors. Therefore, we examined the role of macropinocytosis, a process accompanied by the formation of large, irregular primary endocytic vesicles. As shown in Fig. 8A, 5-(N-ethyl-N-isopropyl) amiloride, a more potent analogue of amiloride which blocks Na⁺/H⁺ exchange and macropinocytosis, inhibited FITC-albumin uptake by RLE-6TN cells in a concentration-dependent fashion. In the presence of cytochalasin D, a specific inhibitor of F-actin elongation involved in macropinocytosis, FITC-albumin uptake was also inhibited (Fig. 8B). These results indicate that macropinocytosis may also be involved in FITC-albumin uptake by the low-affinity transport system in RLE-6TN cells.

**Discussion**

In the present study, the uptake mechanisms of FITC-albumin by the low-affinity transport system were examined in RLE-6TN cells. The uptake of FITC-albumin was almost linear up to 60 min, and showed marked temperature dependence (Fig. 1). The uptake at 37°C was dramatically higher than at 4°C (about 10-fold at 60 min). The apparent uptake of FITC-albumin at 4°C may be the binding to the cell surface, which was about 10% of total uptake at 37°C at 60 min. Therefore, “uptake” values estimated in the present study would partially contain the cell surface binding, in addition to the cellular uptake.

The uptake at 37°C was inhibited by metabolic inhibitors, 2,4-dinitrophenol and sodium azide plus 2-deoxy-D-glucose (Fig. 2). In addition, FITC-albumin uptake was inhibited by bafilomycin A₁ (Fig. 3), which is a specific inhibitor of vacuolar H⁺-ATPase. Vacuolar H⁺-ATPase localized in the endosomal membrane is responsible for lowering pH inside the endosome, which is an essential process for the dissociation of ligands and receptors after receptor-mediated endocytosis. Inhibition of vacuolar H⁺-ATPase results in a decreased activity of the receptor-mediated endocytosis. Thus, these results suggest that the low-affinity transport system of FITC-albumin in RLE-6TN cells is receptor-mediated endocytic process. In addition, when FITC-albumin uptake was compared with
Fig. 5. Estimation of intact FITC-albumin in RLE-6TN cells. The intactness of FITC-albumin was evaluated by fluoroimage analyzer after separation by SDS-PAGE. The data of cell samples after FITC-albumin (4 mg/mL) uptake for 10, 30, or 60 min (A) and after pulse-chase experiment described in Materials and methods (B) were shown, with standards for FITC-albumin and free FITC. The fluorescence intensities of intact FITC-albumin in Figs. A and B were shown in Fig. C. The values at 60 min in Fig. A and at 0 min in Fig. B were set as 100%, respectively.

that of FITC-dextran, a fluid-phase endocytosis marker, it was found that the uptake of FITC-albumin was around 25- to 30-fold more efficient than that of FITC-dextran. This finding would also support our speculation described above.

The intracellular localization of FITC-albumin taken up by RLE-6TN cells was observed by confocal laser scanning microscopy. Using LysoTracker® Red as a lysosomal marker, it was suggested that the part of FITC-albumin was localized in lysosomes (Fig. 4). In addition, a fluoroimage analysis after SDS-PAGE showed that FITC-albumin was taken up by the cells with time, and after cessation of the uptake, it was gradually degraded with the half-life of about 2 hr (Fig. 5). As the localization of FITC-albumin in lysosomes was observed by confocal laser scanning microscopy, it is likely that FITC-albumin was degraded in the lysosomes.
Fig. 7. Effect of inhibitors of clathrin-mediated endocytosis on FITC-albumin uptake by RLE-6TN cells. Effect of phenylarsine oxide (A), chlorpromazine (B), and potassium depletion (K⁺ dep.; C, left) and hypertonicity (Hyper.; C, right) on FITC-albumin (4 mg/mL) uptake for 60 min at 37°C was examined. Experimental conditions to induce potassium depletion and hypertonicity were described in Materials and methods. Each point or column represents the mean ± SEM of 3 monolayers. **, P < 0.01; significantly different from each control.

Fig. 8. Effect of inhibitors of macropinocytosis on FITC-albumin uptake by RLE-6TN cells. Effect of 5-(N-ethyl-N-isopropyl) amiloride (A) and cytochalasin D (B) on FITC-albumin (4 mg/mL) uptake for 60 min at 37°C was examined. Each point represents the mean ± SEM of 3–6 monolayers. **, P < 0.01; significantly different from each control.

We next examined the role of caveolae-mediated endocytosis in FITC-albumin uptake in RLE-6TN cells. The involvement of caveolae-mediated endocytosis has been suggested in albumin uptake in microvessel endothelial cells, and in alveolar type I and type II epithelial cells. John et al. examined albumin transport in cultured alveolar type II cells and in isolated rat lung. They indicated the involvement of gp60, an albumin-binding glycoprotein, in the transport, and showed that filipin, a caveolae disrupting agent, blocked the transport. The important roles of gp60 and caveolae in albumin endocytosis were also reported and are well known in microvessel endothelial cells. Caveolae, which are flask-shaped invaginations of the plasma membrane, are cholesterol- and sphingolipid-rich microdomains. The shape and structure of caveolae are conferred by caveo-
lin, a dimeric protein that binds cholesterol. In this study, we employed nystatin and indomethacin as inhibitors of caveolae-mediated endocytosis. Nystatin inhibits caveolae-mediated endocytosis by interacting with cholesterol in the plasma membrane, and indomethacin inhibits the process by inhibiting the internalization of caveolae and the return of plasmalemmal vesicles to the cell surface. However, FITC-albumin uptake by RLE-6TN cells was not inhibited by these inhibitors (Fig. 6). The presence of caveolae or the expression of caveolin in alveolar type II cells is controversial. Campbell et al. showed that caveolin-1 was not expressed in freshly isolated rat type II cells, but was expressed when type II cells were transdifferentiated to type I-like epithelial cells. On the other hand, John et al. showed the presence of caveolin-1 in cultured alveolar type II cells obtained from rat lung, using anti-caveolin-1 monoclonal antibody. We also examined caveolin-1 mRNA expression in RLE-6TN cells by RT-PCR, and observed its expression (data not shown). Thus, RLE-6TN cells may have a caveolae-mediated endocytic pathway, but the pathway is not involved in FITC-albumin uptake by the low-affinity transport system.

Clathrin-mediated endocytosis is a better-understood endocytic pathway. Clathrin-mediated endocytosis occurs constitutively in all mammalian cells and carries out continuous uptake of nutrients, such as cholesterol-laden low-density lipoprotein and iron-laden transferrin that bind to their own receptors. Clathrin-mediated endocytosis involves the concentration of transmembrane receptors and their bound ligands into coated pits on the plasma membrane. The coated pits are formed by the assembly of cytosolic coat proteins, the main assembly unit being clathrin. In the present study, we examined the effect of clathrin inhibitors, phenylarsine oxide and chlorpromazine, on FITC-albumin uptake in RLE-6TN cells. Phenylarsine oxide inhibits clathrin-mediated endocytosis by reacting with vicinal sulfhydryls to form stable ring structures. Chlorpromazine inhibits the process by inducing the loss of coated pits from the cell surface, probably by interacting with AP-2 binding to membranes. Both compounds inhibited the low-affinity transport of FITC-albumin in RLE-6TN cells in a concentration-dependent manner (Fig. 7A and 7B), indicating the involvement of clathrin-mediated endocytosis. Furthermore, the effects of potassium depletion and hypertonicity on FITC-albumin uptake by RLE-6TN cells were examined. These treatments are known to inhibit clathrin-mediated endocytosis by inducing the disappearance of clathrin-coated pits from the plasma membrane. Both treatments significantly inhibited FITC-albumin uptake by RLE-6TN cells (Fig. 7C). As described already, the contribution of the low-affinity transport system would be around 70% of total uptake under the present condition (4 mg/mL of FITC-albumin), and the rest may represent the high-affinity transport system. On one hand, in our previous study in which 20 μg/mL FITC-albumin was used, the contribution of the high-affinity transport system would be around 90% of total uptake. Based on the inhibitory potencies of various endocytosis inhibitors under these two experimental conditions, the sensitivity of the low- and high-affinity transport systems to each inhibitor can be roughly estimated. Such estimation also supported our speculation that endocytosis inhibitors employed in the present study would inhibit not only the high-affinity transport system but also the low-affinity transport system of FITC-albumin in RLE-6TN cells. Taken together, these results strongly suggest that the low-affinity transport system of FITC-albumin in RLE-6TN cells is, at least in part, mediated by clathrin-mediated endocytosis.

Hackstein et al. reported that the immunosuppressive macrolide rapamycin impaired macropinocytosis of FITC-albumin in murine bone marrow-derived dendritic cells. In addition, it was shown that amiloride inhibited FITC-albumin uptake via macropinocytosis stimulated by IL-10 in human dendritic cells. Therefore, macropinocytosis may also be involved in FITC-albumin uptake by RLE-6TN cells. Macropinocytosis is one of endocytic processes that is accompanied by cell surface ruffling, and is characteristically different from other endocytic processes including clathrin-mediated endocytosis. Because macropinosomes are relatively large in size, they provide an efficient route for non-selective endocytosis of solute macromolecules. A ruffle is formed by a linear band of outward-directed actin polymerization near the plasma membrane, which lengthens into a roughly planar extension of the cell surface. Thus, macropinocytosis can be inhibited by cytochalasin D which inhibits F-actin polymerization. In the present study, we observed that cytochalasin D inhibited FITC-albumin uptake by RLE-6TN cells (Fig. 8B). Fretz et al. reported that Na+/H+ exchanger in the plasma membrane is blocked by amiloride and its analogues such as 5-(N-ethyl-N-isopropyl) amiloride. These agents would lower cytoplasmic pH and inhibit macropinocytosis. In this study, 5-(N-ethyl-N-isopropyl) amiloride was shown to inhibit FITC-albumin uptake by RLE-6TN cells (Fig. 8A). These results suggest that the low-affinity transport system of FITC-albumin in RLE-6TN cells is mediated by macropinocytosis, in addition to clathrin-mediated endocytosis. We also attempted to examine the effect of chlorpromazine and 5-(N-ethyl-N-isopropyl) amiloride added together in the incubation buffer on FITC-albumin uptake by RLE-6TN cells, in order to clarify the relative contribution of these two transport systems. However, because of the cytotoxicity, the combined effect of these inhibitors could not be estimated. On one hand, both amiloride and 5-(N-ethyl-N-isopropyl) amiloride are reported to inhibit clathrin-mediated uptake of albumin...
in the kidney cells.\(^3\)\(^4\) Also, F-actin may play an important role in clathrin-mediated endocytosis in mammalian cells.\(^3\)\(^5\)\(^6\) Therefore, possible interaction of 5-(N-ethyl-N-isopropyl) amiloride and cytochalasin D with clathrin-mediated endocytosis cannot be ruled out. Further studies are needed to clarify the involvement of macropinocytosis and its contribution to FITC-albumin uptake by RLE-6TN cells.

Characteristics of the low-affinity transport system of FITC-albumin uptake in RLE-6TN cells described above were quite similar with those observed in the high-affinity transport system.\(^1\) Both transport systems were mediated by clathrin-mediated endocytosis, but not by caveolae-mediated endocytosis. In addition, 5-(N-ethyl-N-isopropyl) amiloride also inhibited the high-affinity transport system of FITC-albumin (data not shown). Thus, qualitative characteristics of both transport systems are very close. Recently, we examined the activity and qualitative characteristics of both transport systems are very close. Recently, we examined the activity and qualitative characteristics of both transport systems are very close. Recently, we examined the activity and qualitative characteristics of both transport systems are very close.

In conclusion, uptake mechanisms of FITC-albumin via low-affinity transport system were examined using the cultured alveolar type II epithelial cell line, RLE-6TN. The low-affinity transport system of FITC-albumin in RLE-6TN cells was suggested to be mediated at least in part by clathrin-mediated endocytosis, but not by caveolae-mediated endocytosis. Possible involvement of macropinocytosis was also suggested. After taken up by the cells, part of FITC-albumin was gradually degraded in lysosomes.

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References


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