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2-Phenyl-4-quinolone prevents serotonin-induced increases in endothelial permeability to albumin

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Abstract

To investigate the role of 2-phenyl-4-quinolone in enhancing endothelial monolayer paracellular barrier function and preventing the disturbance of paracellular barrier function by vasoactive agents, the study examined the effect of 2-phenyl-4-quinolone on serotonin-mediated macromolecule transfer and microfilament changes in cultured rat heart endothelial cells. Serotonin-treated endothelial cells induced concentration-dependent increases in the passage of Evans blue dye-bound bovine serum albumin. Incubation of the endothelial monolayers with 2-phenyl-4-quinolone antagonized serotonin- and cytochalasin B-induced macromolecular permeability. 2-Phenyl-4-quinolone also opposed the effect of serotonin or cytochalasin B on the distribution and quantity of actin filaments in the endothelial cytoskeleton. Furthermore, 2-phenyl-4-quinolone alone led to an apparent quantitative increase in F actin fluorescence in endothelial cells. The addition of 10^{-7} M 2-phenyl-4-quinolone had an effect on serotonin-induced changes in the myosin and distribution of myosin were comparable to that on serotonin monolayers. In conclusion, 2-phenyl-4-quinolone attenuated the serotonin-induced permeability of rat heart endothelial cells and this was associated with stabilization of F actin microfilaments and changes in the myosin organization. This result suggests that influences on cytoskeletal assembly may be involved in this process.

Keywords: 2-Phenyl-4-quinolone; 5-HT (5-hydroxytryptamine, serotonin); Endothelial cell, rat; Permeability; Cytochalasin B; Actin microfilament; Myosin

1. Introduction

The integrity of the endothelial cell monolayers, a critical requirement for barrier maintenance, is needed for the prevention of edema formation. A breach in the integrity of the endothelial cell monolayer results in interstitial edema and can be induced by bioactive agents (Majno and Palade, 1961; Majno et al., 1969; Sharma and Cervos Navarro, 1990). Vasoactive agents have been reported to increase endothelial permeability in association with a change in cell shape and the development of intercellular gaps (Rotrosen and Gallin, 1986; Phillips et al., 1989; Patterson et al., 1994; Goeckeler and Wysolmeraki, 1995). These changes in cell shape may reflect changes in actin, the predominant cytoplasmic microfilament in non-muscle cells, whose biophysical properties may contribute to the maintenance of structural integrity. Therefore, a role of the cytoskeleton in determining endothelial cell permeability is well documented. Many investigators have indicated that actin and myosin in endothelial cells play a central role in regulating the width of the intercellular clefts, thereby controlling the paracellular pathway of vascular permeability (Shasby et al., 1982; Wong and Gottlieb, 1986, 1990; Schnittler et al., 1990; Gottlieb et al., 1991; Patterson et al., 1994). It also has been reported that the drugs, ethchlorvynol and cytochalasin D, disrupt the cytoskeleton, resulting in increased transendothelial permeability (Shasby et al., 1982; Wysolmerski et al., 1984) and the agents, phallacidin and phalloidin, stabilize the cytoskeleton, preventing agonist-mediated increases in transendothelial permeability (Alexander et al., 1988; Phillips et al., 1989; Langeler and Van Hinsbergh, 1991; Alexander et al., 1993; Ma and Pedram, 1996). In other studies, 3′,5′-cyclic adenosine monophosphate (cAMP) activation was found to be an important determinant of endothelial macromolecular barrier function in vitro. The cAMP-induced effects were

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associated with increases in the polymerized actin pool in endothelial cells (Bensch et al., 1983; Stelzner et al., 1989).

2-Phenyl-4-quinolone is a synthetic compound (Kuo et al., 1993). Wang et al. (1994) have reported that 2-phenyl-4-quinolone plays a role in inhibiting local edema formation and in protection of the vasculature against serotonin-induced plasma extravasation in vivo. Our previous results suggested that 2-phenyl-4-quinolone attenuates the serotonin-induced permeability of endothelial cells to macromolecules in association with elevated cAMP levels (Lee, 1997). Therefore, we now determined whether such effects of 2-phenyl-4-quinolone are associated with cytoskeletal changes. The results showed that 2-phenyl-4-quinolone, probably like other cAMP enhancing agents, modulates the mediators of acute inflammation such as serotonin which induces permeability of endothelial cells by increasing the polymerized actin pool and inhibiting disruption or disassembly of actin microfilaments.

2. Materials and methods

2.1. Materials

All culture media and fetal bovine serum were obtained from Gibco BRL (Grand Island, NY, USA). Endothelial cell growth supplement, bovine serum albumin, cytochalasin B, Evans blue dye, fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin G (IgG), monoclonal anti-myosin immunoglobulin and serotonin were purchased from Sigma (St. Louis, MO, USA); Transwells (diameter: 0.65 cm; pore size: 3 μm) were from Corning Costar (Cambrige, MA, USA); anti-Von Willebrand factor, human fibronectin, and lactate dehydrogenase (LDH) Detection Kit were from Boehringer Mannheim (Germany); 7-nitrobenz-2-oxa-1,3-diazole (NBD)-conjugated phallacidin and rhodamine-conjugated phalloidin were purchased from Molecular Probes (Eugene, OR, USA). Serotonin was dissolved in Hank's Balanced Salt Solution (HBSS) and others substances were dissolved in less than 0.5% dimethylsulfoxide (DMSO).

2.2. Rat heart endothelial cells culture preparation

Rat heart endothelial cells were isolated as described previously (Lee, 1997). Briefly, ventricles of three hearts were removed from 4-day-old donor rats. This tissue was washed four times with HBSS, and finely minced with dissecting scissors. The tissue was then subjected to four successive trypsinizations (10 ml 0.125% trypsin in calcium (Ca^{2+}) and magnesium (Mg^{2+})-free HBSS) with stirring, using a 50-ml trypsinization flask. After each trypsinization, free cells (myocardial and endothelial) were removed and supplemented with 2 ml of endothelial cell culture medium consisting of Dulbecco's modified essential medium (DMEM) and 20% fetal bovine serum. The cells were recovered by centrifugation at $1000 \times g$, resuspended in endothelial cell culture medium, and allowed to adhere to the bottom of fibronectin-coated wells in a 24-well tissue culture cluster plate (Falcon) for 90 min. The culture medium was then removed and the culture wells were washed twice with HBSS to remove non-adherent (mostly myocardial) cells. Thereafter, culture medium supplemented with 150 μg of endothelial cell growth supplement and 1000 U penicillin–1000 μg streptomycin/ml as well as 20% fetal bovine serum was added to the remaining adherent cells. At 90 min, the adherent cells are primarily endothelial cells, since the myocardial cells require more time (approximately 24 h) to adhere to a culture surface. All cultures demonstrated typical contact-inhibited cobblestone appearance. Factor VIII-related antigen was confirmed by indirect immunofluorescence using human factor VIII antiserum and FITC-conjugated goat anti-mouse IgG (Vehar and Davie, 1977).

2.3. Measurement of rat heart endothelial cell monolayers barrier function

Rat heart endothelial cells cultured on filters were used 3 days after seeding (1×10^5 cells/well). Exchange of marcromolecules through the endothelial cell monolayers was investigated by assaying the transfer of Evans blue dye-bound bovine serum albumin (4% final concentration). Passage of macromolecules through endothelial cell monolayers was performed as described previously (Lee, 1997) with some modifications. Briefly, endothelial cell monolayers were cultured onto 48 Transwell polycarbonate membrane assemblies. The membrane assemblies were then placed in 0.6 ml of DMEM containing 20% fetal bovine serum in 24-well plates and the cells were allowed to grow a tight monolayer. For experimentation, membrane assemblies with cells attached were washed twice by immersion in HBSS, and transferred to 24-well plates. Thereafter, 600 μl of the HBSS was placed in each of the wells which formed the lower chamber and 100 μl of HBSS was placed above the endothelial monolayers. These volumes were chosen so as to avoid creating a hydrostatic gradient across the monolayers. Drugs were then added to the top and bottom chambers, and the lower chamber was stirred for rapid mixing. The entire system was kept at 37°C by a thermostatically regulated water bath. At the end of the experiment, a 200-μl aliquot was removed from each of the lower chambers and transfer of bovine serum albumin across the monolayers was quantified by measuring optical density at 600 nm.

2.4. F actin measurement

Endothelial cells were plated at 2×10^5 cells/well in 24-well plates and grown to confluence in DMEM supplemented with 20% fetal bovine serum, over 3 days. Old

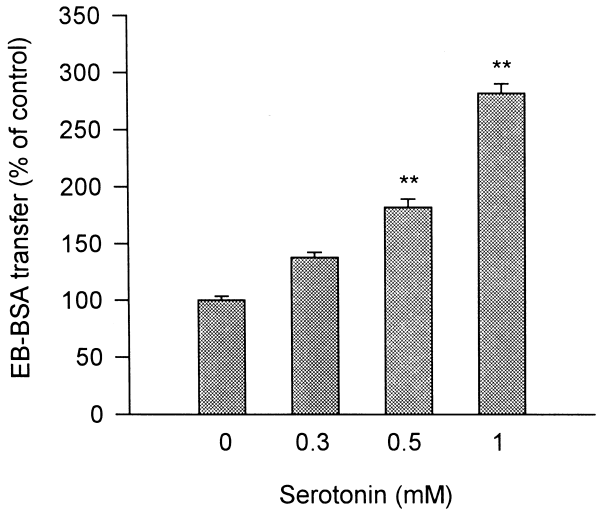

Fig. 1. Effects of increasing concentrations of serotonin (0.3, 0.5, 1 mM) on the transfer of macromolecules across the filter-grown rat heart endothelial cell monolayers ($n = 23$ to 27). Albumin transfer was measured at 30 min. The results are expressed as the mean percent control ± S.E.M. ** $P < 0.01$ compared to the control values.

medium was removed and replaced with either fresh HBSS alone or HBSS containing serotonin (1 mM), 2-phenyl-4-quinolone (10^{-7} M), or cytochalasin B (10^{-6} M) for the time intervals indicated. HBSS was aspirated and the cells were directly fixed in 3.7% formaldehyde in HBSS for 15 min at room temperature. The formaldehyde was then removed and cells were incubated in 300 μl HBSS containing lyso-phosphatidylcholine (100 μg/ml) and the F actin fluorescent stain, NBD–phallicidin (1.65×10^{-7} M), for 40 min at 25°C in the dark. The NBD–phallicidin was then extracted with 500 μl methanol in the dark for 90 min. The supernatant was aspirated off and centrifuged to remove any cell debris. Fluorescence of 300 μl of the supernatant was measured with a fluorescence spectrophotometer set to excitation 465 nm, emission 525 nm. Cells stimulated with the various reagents but not incubated with NBD–phallicidin were handled similarly to determine background autofluorescence, which was subtracted from the appropriate samples. The results were then expressed as relative F actin content (Stelzner et al., 1989; Goeckeler and Wysolmeraki, 1995).

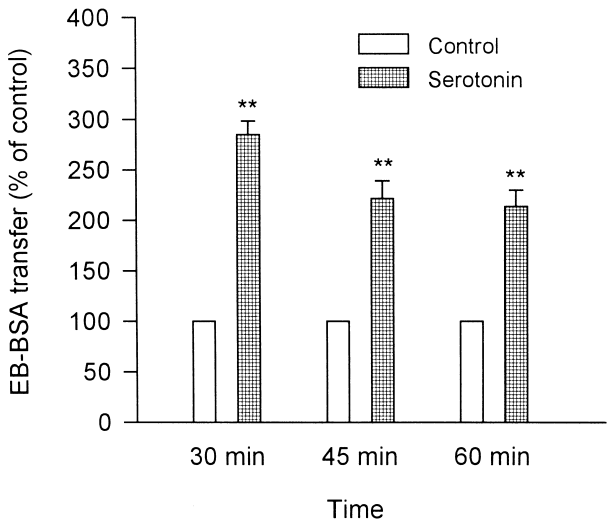

Fig. 2. The time course of serotonin (1 mM) effect on endothelial cell monolayer permeability. Permeability increase induced by serotonin at various incubation times. Endothelial cell monolayers were incubated with serotonin for 30, 45 and 60 min. Monolayers incubated with HBSS alone served as control. Evans blue-bovine serum albumin transfer was measured at 30, 45 and 60 min. Graph depicting the relative amount of individual control. Data are expressed as the mean percent control ± S.E.M. of 12 independent experiments. ** $P < 0.01$ compared to the corresponding control values.

2.5. Immunolocalization of F actin and myosin

Qualitative F actin and myosin staining were obtained by plating endothelial cells at 1×10^5 cells/slip. Since an obvious view of the morphological difference induced by serotonin and 2-phenyl-4-quinolone would be obtained, subconfluent endothelial cells were used. Endothelial cell cultured on coverslips were incubated with vehicle, serotonin (1 mM), 2-phenyl-4-quinolone (10^{-7} M), or cytochalasin B (10^{-6} M) in HBSS for the time intervals indicated. HBSS was aspirated off and cells were directly fixed in 3.7% formaldehyde in HBSS for 15 min, followed by 3–5 min in acetone at −20°C. The cells were washed gently with HBSS. To visualize F actin, the cells were incubated with 1.65×10^{-7} M rhodamine–phalloidin in HBSS for 30 min. To visualize myosin, the cells were incubated with mouse anti-myosin (non-muscle) immunoglobulin (37°C, 45 min), washed, and subsequently stained

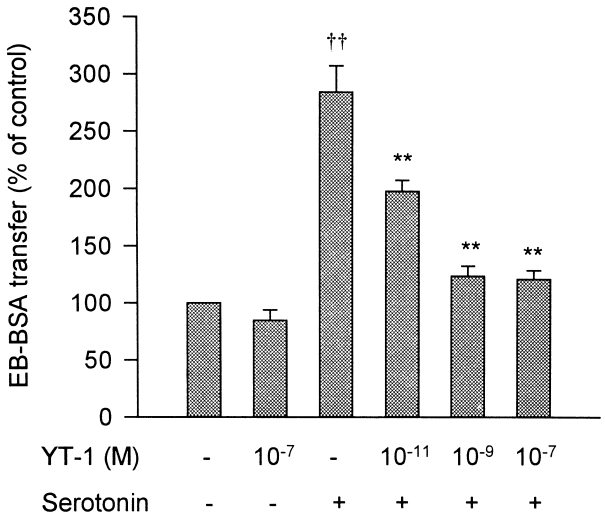

Fig. 3. The effects of 10^{-11}, 10^{-9}, 10^{-7} M 2-phenyl-4-quinolone (YT-1) on serotonin-induced increases in the permeability of endothelial cell monolayers to Evans blue-bovine serum albumin. Each concentration of 2-phenyl-4-quinolone was added for 10 min before incubation with serotonin (1 mM) for 30 min. Results are the means percent control ± S.E.M. for 25 separate experiments. †† $P < 0.01$ compared to the control values. ** $P < 0.01$ compared to the serotonin-treated monolayers.

with FITC-conjugated goat anti-mouse IgG. After the cells were stained, they were washed gently, glycerin was added to each coverslip and the cells were viewed with a fluorescence microscope (Zeiss Axioskop).

2.6. Cytotoxicity assay

Lactate dehydrogenase (LDH) release from rat heart endothelial cell monolayers was determined. The endothelial cells grown on the plastic tissue culture plates were exposed to various concentrations of reagent in DMSO in HBSS with 0.1% bovine serum albumin for 30 min. The supernatant was removed and centrifuged at $1000 \times g$ for 10 min. LDH activity in the supernatant was determined with the commercial LDH Detection Kit.

2.7. Data analysis

The results were analyzed for statistical significance by analysis of variance with repeated measures and a New-

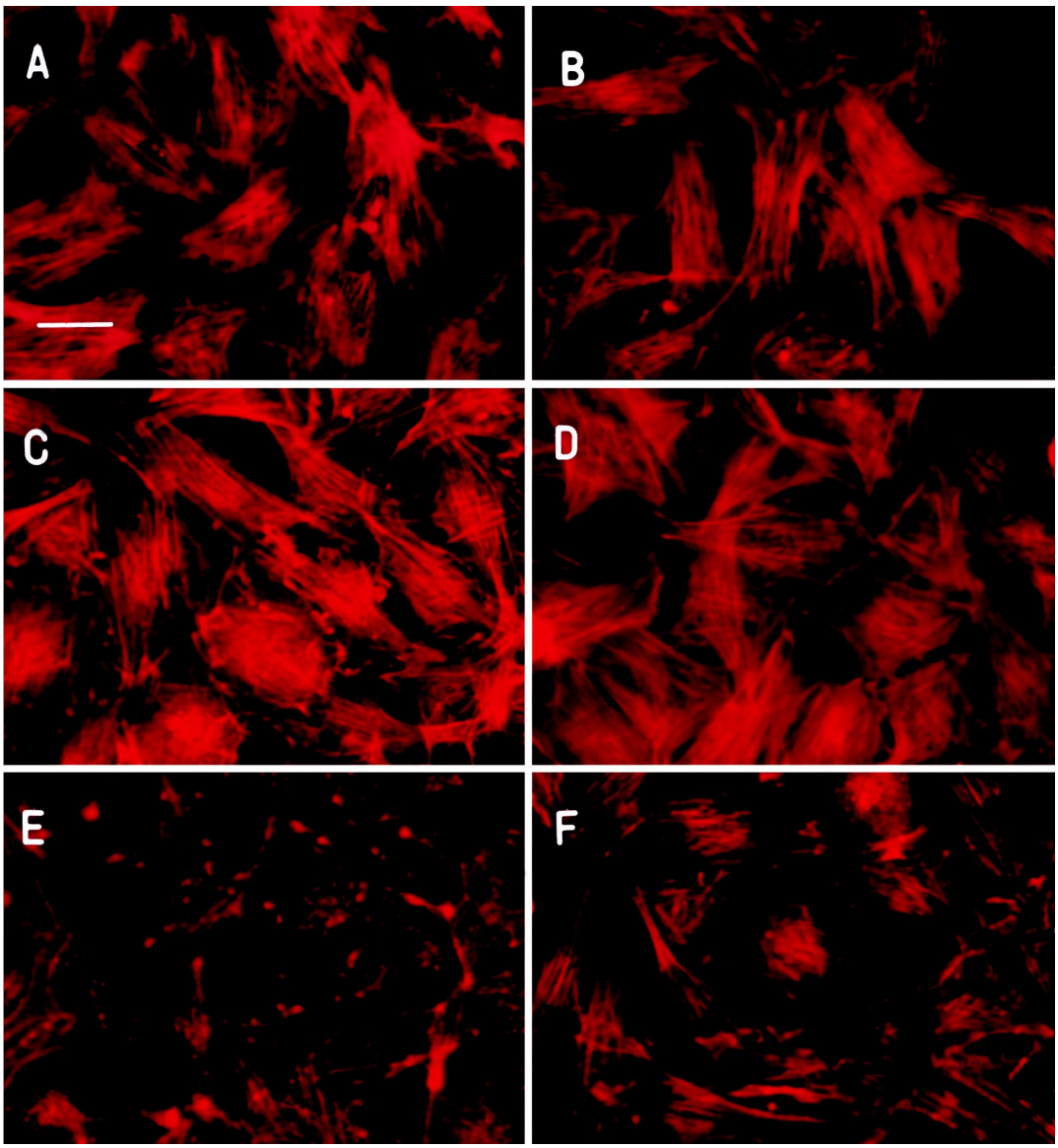

Fig. 4. Fluorescence photomicrographs of the rat heart endothelial cells. F actin microfilaments in endothelial cell monolayers grown on glass coverslips visualized by rhodamine-labeled phalloidin binding. (A) Control monolayers. Actin micro-filaments were present diffusely as short microfilaments throughout the endothelial cells. (B) Thirty minutes following challenge with serotonin (1 mM), cells were spindle-shaped and there was redistribution of actin. Fibers were denser and often aligned parallel to the long axis of the cell. Large clefts are evident between the cells. (C) Monolayers were incubated with 10^{-7} M 2-phenyl-4-quinolone for 10 min. Cells had a similar appearance to those in the control. (D) Monolayer treated with 2-phenyl-4-quinolone (10^{-7} M) 10 min before 30-min incubation with serotonin (1 mM). The number of stress fibers and intensity of fluorescence were comparable to those in serotonin-treated cells. (E) After incubation with cytochalasin B (10^{-6} M) for 10 min, stress fibers were disrupted in many cells. (F) Monolayers were treated with 10^{-7} M 2-phenyl-4-quinolone for 10 min and then challenged for an additional 10 min with 10^{-6} M cytochalasin B. All photographic exposures were for 30 s (Bar = 25 μm).

man–Keuls test. A P value less than 0.05 was considered significant for all tests.

3. Results

3.1. Effect of 2-phenyl-4-quinolone on the serotonin-induced macromolecular permeability across the rat heart endothelial cell monolayers

To investigate the mechanisms of 2-phenyl-4-quinolone protection in serotonin-induced exudation, this study used filter-grown rat heart endothelial cells. The filter-grown endothelial cell monolayers were established as a useful in vitro model system for studying endothelial permeability. Incubation of endothelial cell monolayers with serotonin induced concentration-dependent increases in the passage of bovine serum albumin (Fig. 1). Serotonin 1 mM at times 30, 45, and 60 min significantly increased albumin transfer rates to $285 \pm 13\%$ (the albumin transfer of control: 1.465 ± 0.131 μg Evans blue/ml HBSS), $221 \pm 18\%$ (the albumin transfer of control: 2.369 ± 0.179 μg Evans blue/ml HBSS), and $214 \pm 16\%$ (the albumin transfer of control: 3.432 ± 0.303 μg Evans blue/ml HBSS) of the relative control, respectively (Fig. 2). The serotonin-induced increase in the albumin that passed across the monolayers was significant with 1 mM serotonin and a 30-min incubation period. Therefore, 1 mM serotonin and a 30-min incubation period were chosen for further experiments. The serotonin (1 mM)-caused, about three-fold, increase in the passage of albumin in 30 min was adequately blocked by 10-min pretreatment of the monolayer with 2-phenyl-4-quinolone (10^{-11}, 10^{-9}, 10^{-7} M) (Fig. 3).

3.2. Effect of 2-phenyl-4-quinolone stimulation on endothelial cells F actin

In the experiments following, the effect of 2-phenyl-4-quinolone on endothelial actin microfilaments was examined. Subconfluent endothelial cells were incubated in either media alone, 2-phenyl-4-quinolone (10^{-7} M), or 1 mM serotonin, and F actin was identified with rhodamine–phalloidin, a specific F actin fluorescent probe. In the control cells (Fig. 4A), actin microfilaments were present diffusely as short microfilaments throughout the endothelial cells. In response to serotonin, the actin reorganized into prominent stress fibers aligned parallel to each other and to the long axis of the cell. Cells retracted from one another, exhibiting large clefts between adjacent cells while retaining their polygonal morphology (Fig. 4B). Cells exposed to 2-phenyl-4-quinolone showed clear structural changes, including greater diffusion of actin microfilaments and closer cell-to-cell apposition, as suggested in Fig. 4C. The change in distribution of rhodamine–phalloidin-labeled actin from a diffuse pattern to numerous parallel stress fibers commonly seen with serotonin activation was largely prevented by 2-phenyl-4-quinolone pretreatment (Fig. 4D). In the next experiment, 2-phenyl-4-

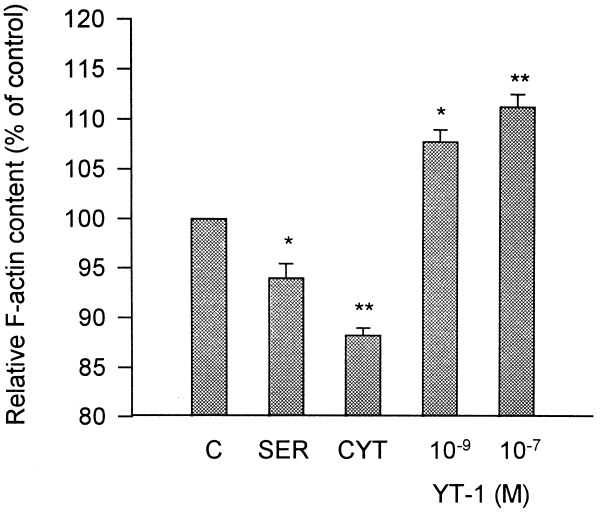

Fig. 5. The effects of a 15-min incubation with serotonin (SER: 1 mM), cytochalasin B (CYT: 10^{-6} M), or 2-phenyl-4-quinolone (YT-1: 10^{-9}, 10^{-7} M) on endothelial cell F actin ($n = 5–7$) are shown. Following incubation with these agents, endothelial cells were stained with the fluorescent F actin probe, NBD–phallicidin. The F actin was then methanol-extracted over 60 min, and the extracted fluorescence was measured in a fluorescence spectrophotometer. Results are expressed as relative F actin content (RFC): the ratio of fluorescence intensity (FI) of stimulated cultures to fluorescence intensity of control cultures ($RFC = FI_{stimulated}/FI_{control} \times 100\%$). Results are expressed as the mean ± S.E.M. of six separate experiments. Asterisks indicate values significantly different from control values ($*$ $P < 0.05$, $**$ $P < 0.01$).

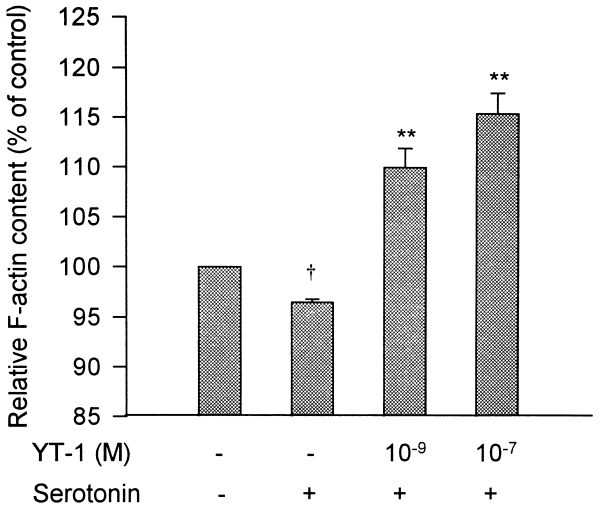

Fig. 6. The effects of 2-phenyl-4-quinolone (YT-1: 10^{-9}, 10^{-7} M) on serotonin-induced decreases in the F actin content of endothelial cell monolayers. Monolayers treated with 2-phenyl-4-quinolone (YT-1) 10 min before 30-min incubation with serotonin (1 mM), fixed, permeabilized, and stained with NBD–phallicidin. Results are expressed as relative F actin content. Each value is the mean percent control ± S.E.M. of six separate experiments. † $P < 0.05$ compared to the control values. $**$ $P < 0.01$ compared to the serotonin-treated monolayers.

quinolone alone also caused significant increases in quantitative endothelial cell F actin fluorescence compared to that in control cells, indicating a larger polymerized actin pool (Fig. 5). On the other hand, the serotonin-mediated decrease in F actin quantity was prevented by pretreatment with 10^{-9}, 10^{-7} M 2-phenyl-4-quinolone (Fig. 6).

3.3. Effect of 2-phenyl-4-quinolone on the cytochalasin B-induced endothelial cell monolayer paracellular permeability

Based on the above results, 2-phenyl-4-quinolone modulation of endothelial paracellular permeability appeared to be mediated by actin microfilaments. To further evaluate the role of actin microfilaments in 2-phenyl-4-quinolone modulation of paracellular barrier function, the effect of 2-phenyl-4-quinolone on cytochalasin B (an actin depolymerizing agent)-mediated macromolecular permeability in endothelial cell was examined. Cytochalasin B (10^{-6} M) induced a marked decrease in quantitative endothelial cell F actin fluorescence compared to that in control cells (Fig. 5). Furthermore, cytochalasin B (10^{-6} M) also caused severe disruption and breakage of endothelial actin microfilaments (Fig. 4E). This indicates that the peripheral actin microfilaments are disrupted and gaps form between cells and permeability of the endothelial cell increases. Pretreatment of endothelial cell monolayers with 2-phenyl-4-quinolone prevented the cytochalasin B-induced disruption of actin microfilaments (Fig. 4F) and increase in macromolecular permeability (Fig. 7), providing further support for the role of actin microfilaments as a mediating factor of 2-phenyl-4-quinolone modulation of paracellular barrier function.

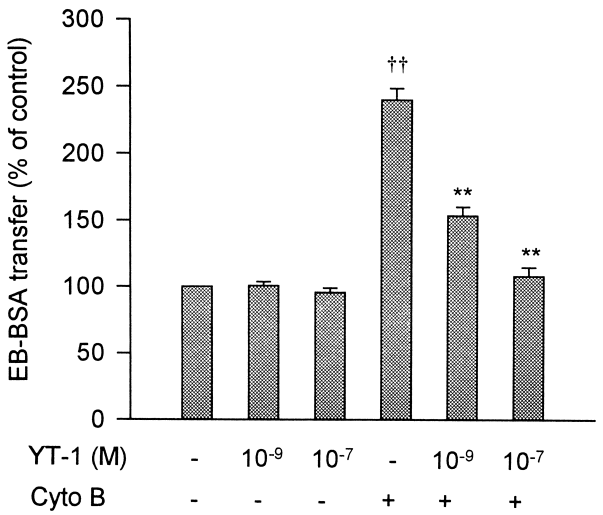

Fig. 7. The effects of 10^{-9}, 10^{-7} M 2-phenyl-4-quinolone (YT-1) on resting transfer of macromolecular and 2-phenyl-4-quinolone on cytochalasin B-induced increase in the permeability of endothelial cell monolayers to macromolecules. Each concentration of 2-phenyl-4-quinolone was added 10 min before 10-min incubation with cytochalasin B (10^{-6} M). Results are the mean percent control ± S.E.M. for 10 separate experiments. †† $P < 0.01$ compared to the control values. ** $P < 0.01$ compared to the cytochalasin B-treated monolayers.

3.4. Effect of 2-phenyl-4-quinolone on the serotonin-induced changes in myosin in endothelial cell monolayers

Fig. 8A illustrates the myosin distribution in endothelial cell monolayers while, in the control, myosin is localized diffusely throughout the cytoplasm with no obvious organization. In endothelial cell monolayers treated with 1 mM

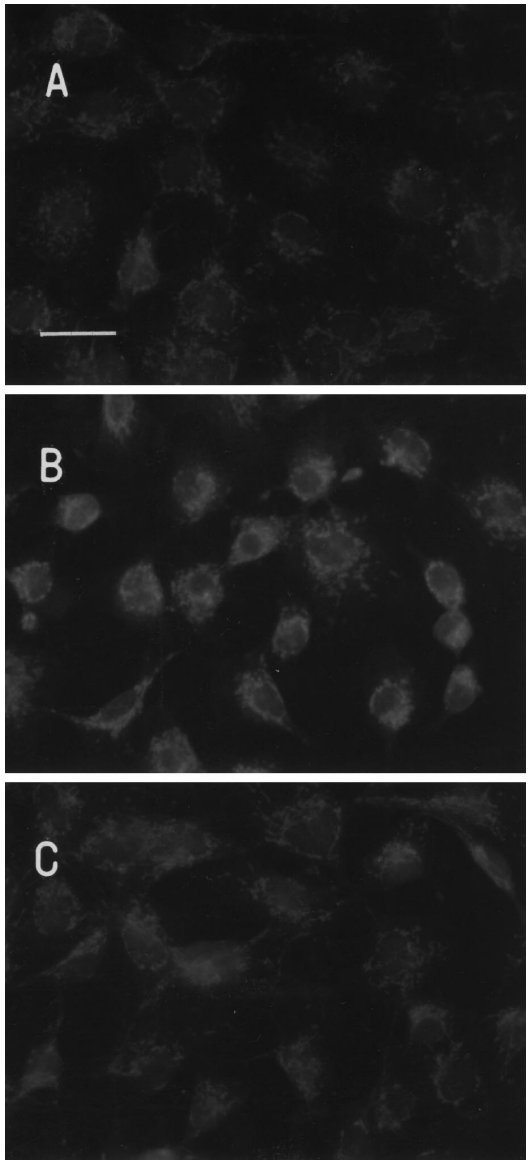

Fig. 8. Immunofluorescent localization of myosin in rat heart endothelial cells. Cells were labeled for myosin with a specific antibody. (A) Control monolayer. Myosin was diffusely localized throughout the cytoplasm. (B) Endothelial cell monolayers treated with 1 mM serotonin, myosin concentrated prominently on the nuclear margins. (C) Monolayers treated with 2-phenyl-4-quinolone (10^{-7} M) 10 min before 30-min incubation with serotonin (1 mM). The distribution of myosin was comparable to that in serotonin monolayers. All photographic exposures were for 30 s (Bar = 25 μm).

serotonin, myosin organized into discrete aggregates dispersed randomly throughout the cytoplasm and concentrated prominently on the nuclear margins (Fig. 8B). The effect of addition of 10^{-7} M 2-phenyl-4-quinolone on serotonin-induced changes in the myosin organization and distribution of myosin was comparable to the effect in serotonin monolayers (Fig. 8C).

3.5. Cytotoxicity assay

Treatment of rat heart endothelial cells with various reagents for 30 min did not increase LDH release (data not shown). This finding indicated that the increase in endothelial permeability was not due to a cytolytic effect of these interventions.

4. Discussion

Vascular endothelial cells separate the circulating blood from the surrounding vessel wall and tissues. The endothelium functions as a critical and selective barrier to macromolecular permeability, protecting the underlying tissues from edema and preserving organ function. Endothelial cell contraction plays a pivotal role in the increased extravasation of fluid and macromolecules in vascular leakage. Various vasoactive substances, such as thrombin and histamine, have been demonstrated to induce an increase of endothelial permeability in vivo (Majno et al., 1967) and in vitro (Killackey et al., 1986; Langeler et al., 1989). In the present study, serotonin induced dose-dependent increases in the passage of macromolecules in rat heart endothelial cells as well as of other vasoactive agents in various cell cultures. In vivo, 2-phenyl-4-quinolone plays a role in inhibiting local edema formation and in the protection of the vasculature against the serotonin-induced plasma extravasation (Wang et al., 1994); the mechanisms of this protective effect are, however, not clear. To investigate the mechanisms of 2-phenyl-4-quinolone prevention of edema formation, this study used filter-grown rat heart endothelial cells to measure the effect of 2-phenyl-4-quinolone on the serotonin-mediated passage of albumin across endothelial monolayers. The result indicates that 2-phenyl-4-quinolone reverses serotonin-mediated increases in endothelial permeability to macromolecules; the ability of 2-phenyl-4-quinolone to produce this effect in endothelial cells with low basal permeability was confirmed. However, the mechanisms of 2-phenyl-4-quinolone enhancement of endothelial paracellular barrier function still needs to be investigated. Results of our previous study had suggested that 2-phenyl-4-quinolone attenuates the serotonin-induced permeability of rat heart endothelial cells to macromolecules in association with elevated cAMP levels (Lee, 1997). Recently, Garcia and Schaphorst (1995) reported that agonist-induced endothelial cell contraction is attenuated by increases in cAMP, at least in part, via myosin light chain kinase inhibition. Furthermore, other investigators demonstrated that the cAMP-induced effects were associated with increases in the polymerized actin pool in endothelial cells (Bensch et al., 1983; Stelzner et al., 1989). It also has been reported that endothelial barrier function is regulated by F actin-dependent changes in cell shape, coincident with the appearance of intercellular gaps (Phillips et al., 1989; Langeler and Van Hinsbergh, 1991; Goldblum et al., 1994). In addition, other studies also found that the assembly and disassembly of actin could be important determinants of endothelial barrier properties (Shasby et al., 1982; Stelzner et al., 1989). Therefore, enhancement of the barrier is related to the endothelial cytoskeletal changes and the apposition or quantitative increases in polymerized actin, which contrasts with the increased permeability associated with disruption or disassembly of actin microfilaments. Since endothelial actin polymerization is an important factor of endothelial barrier function, the present study served to determine whether the 2-phenyl-4-quinolone-induced effects were associated with increases in the F actin filaments.

In these experiments, the fact that serotonin increased the assembly and length of stress fibers in endothelial cells indicates that the serotonin-induced increase in endothelial paracellular permeability is mediated in part through its effect on actin microfilaments. These results are consistent with those of previous investigators that have shown an association between histamine- and thrombin-induced increases in permeability and changes in microfilaments (Garcia et al., 1986; Rotrosen and Gallin, 1986). The present results provide direct evidence that 2-phenyl-4-quinolone acts on endothelial monolayers to prevent serotonin-mediated increases in macromolecular permeability and changes in actin distribution. In addition, 2-phenyl-4-quinolone alone caused significant increases in quantitative endothelial cell F actin fluorescence compared to that in control cells. Our study also demonstrated that treatment of the actin microfilaments with a depolymerizing agent, cytochalasin B, disrupted the endothelial microfilaments, decreased F actin content, and increased the permeability in endothelial cell monolayers. Consistent with such results, other studies have suggested that disruption of endothelial cell actin microfilaments with cytochalasins increased endothelial permeability (Rotrosen and Gallin, 1986; Stasek and Garcia, 1992; Stasek et al., 1992; Garcia and Schaphorst, 1995). In our study, 2-phenyl-4-quinolone prevented the cytochalasin B-mediated disruption of F actin microfilaments and increases in paracellular permeability. These data suggest that pretreatment with 2-phenyl-4-quinolone prevents the serotonin- and cytochalasin B-mediated rearrangements of the actin-containing stress fibers and support the possibility that 2-phenyl-4-quinolone, directly or indirectly, stabilizes the endothelial cell cytoskeleton and intercellular junction. These results are consistent with previous observations that actin stabilizers enhance the endothelial barrier (Alexander et

al., 1988), and suggest that enhancement of the barrier may be related to endothelial cytoskeletal changes.

Schnittler et al. (1989, 1990) have suggested that endothelial gap formation depends on the availability of free myosin binding sites on the endothelial actin filaments. Garcia and Schaphorst (1995) also indicated that the actin–myosin cytoskeleton plays a critical role in maintenance of the structural and mechanical integrity of the endothelial cell monolayer and that activation of the contractile apparatus is a key event in agonist-induced endothelial cell barrier dysfunction. These results indicated that gap formation was caused by the myosin filaments reorganization. Therefore, the mechanism of 2-phenyl-4-quinolone modulation of serotonin-induced endothelial permeability is also dependent on the distribution of myosin. 2-Phenyl-4-quinolone obviously changed serotonin-induced endothelial cells myosin organization. The results of the present experiments demonstrated that one mechanism by which 2-phenyl-4-quinolone accomplishes these protective effects on endothelial cell permeability may be partial attenuation of serotonin-induced myosin filament reorganization.

In conclusion, the study showed that serotonin caused an increase in rat heart endothelial cell monolayer paracellular permeability; the serotonin-induced increase in endothelial permeability was prevented by 2-phenyl-4-quinolone. The results indicated that endothelial monolayer paracellular permeability modulation by 2-phenyl-4-quinolone was regulated in part by actin and myosin microfilaments. The results support the hypothesis that 2-phenyl-4-quinolone has an enhancing effect on endothelial barrier function and prevents the disruption of endothelial barrier function by vasoactive agents such as serotonin. These findings suggest a possible mechanism by which 2-phenyl-4-quinolone protects endothelial function and preserves the microvasculature from pharmacologic injury by injurious agents.

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