Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily and bind to specific DNA sequences to induce transcriptional activation of specific genes (6). In three subtypes of PPARs (PPARα, -β, and -γ), PPARγ is found in adipose tissues, skeletal muscle, liver, pancreas, macrophages, T cells, and vascular cells (7–10). PPARγ stimulation by thiazolidinediones, such as pioglitazone and rosiglitazone, resulted in improved insulin sensitivity; thus, the thiazolidinediones are widely used as antidiabetic agents (11).

Recent investigations suggested that PPARγ ligands have inhibitory effects on tumor cell lines, but the effects appear not to be entirely elicited by the direct action on tumor cells. Similarly, Panigrahy et al. (12) reported that PPARγ is expressed in tumor endothelium and in cultured endothelial cells and that rosiglitazone inhibits angiogenesis in vitro and in vivo via its effects on endothelium. In addition, 15-deoxy-

Δ-12,14-prostaglandin J2, an endogenous PPARγ ligand, inhibited VEGF-induced angiogenesis in the rat cornea (13) and induced caspase-mediated endothelial cell apoptosis via a PPAR-dependent pathway (9). Possible mechanisms of antiangiogenic actions by PPARγ activation include the inhibition of mitogen-activated protein kinase-dependent activation (14); up-regulation of angiogenesis inhibitor maspin and CD36 (15), the receptor for antiangiogenic thrombospondin; inhibition of matrix metalloproteinase, VEGF, and VEGF receptor expression; and increase of plasminogen activator inhibitor and matrix metalloproteinase inhibitor expression (12, 13, 16). However, the direct mechanism for the antiangiogenic effects of PPARγ activation in the endothelial cells remains currently unclear.

The endothelium plays a pivotal role in a variety of vascular functions such as blood pressure control, vascular remodeling, and angiogenesis, where ion channels, particularly large conductance Ca2+-activated K+ channels (also known as the BK channel or maxi-K channel), are key mediators (17). Maxi-K channels are distributed in various tissues such as human brain tumors, vascular smooth muscle cells, and endothelial cells, and their activations were shown to inhibit migration of human glioma cells (18). The maxi-K channels are synergistically regulated by various intracellular second messengers, such as cytoplasmic Ca2+ concentration, cGMP, hydrogen peroxide, and nitric oxide (NO) (19–21). NO is produced by a group of enzymes called nitric-oxide synthases (NOS), and endothelial NOS (eNOS or NOS3) is responsible for the production of NO in vascular endothelial cells. Although the expression and activity of eNOS seems to be constitutive, the activity was shown to be regulated by serine phosphorylation (22).

In this study, we investigated the possible involvement of maxi-K channels in the antiangiogenic effects of rosiglitazone in human umbilical vein endothelial cells (HUVECs). The inhibitory action of rosiglitazone on VEGF-stimulated angiogenesis was blocked either by iberio-
Maxi-K Channel Opening by Rosiglitazone for Antiangiogenesis

toxin or by knockdown of maxi-K channel expression, and the effect was associated with apoptotic action as shown by DNA fragmentation and Bcl-2 and Bax regulation. The mediator involved in the maxi-K channel opening by rosiglitazone appears to be NO, produced by PPARγ-activated eNOS. These findings provide important understanding that maxi-K channel plays a critical role in the rosiglitazone-induced antiangiogenesis in HUVECs.

EXPERIMENTAL PROCEDURES

Cell Cultures—HUVEC (ATCC CRL-1730; endothelial cell line derived from vein of human umbilical cord) were cultured in Kaighn’s F12K medium supplemented with 10% heat-inactivated fetal bovine serum, 0.1 mg/ml heparin sodium, 0.03–0.05 mg/ml endothelial cell growth supplement, and 1% antibiotics (100 units/ml penicillin and 100 µg/ml streptomycin). Cells were grown to confluence at 37 °C in 5% CO2 on 0.1% gelatin-coated culture dishes and used for experiments at no greater than passage 8.

Angiogenesis Assays—The tube formation assay was performed on 24-well plates coated with 250 µl of Matrigel Basement Membrane Matrix (10 mg/ml)/well and polymerized for 30 min at 37 °C. Cells treated with either bisphenol A diglycidyl ether (BADGE) or iberiotoxin for 30 min at 37 °C were plated onto a layer of Matrigel at a density of 1 × 10⁴ cells/well and followed by the addition of either rosiglitazone or NS-1619. Matrigel cultures were incubated at 37 °C for 18 h and photographed (∗100).

An in vivo Matrigel plug assay was carried out by injecting 0.5 ml of Matrigel containing VEGF₁₆₅ (5 ng/ml) and heparin (40 units/ml) into C57BL/6 mice subcutaneously. After 5 days, mice were sacrificed, and Matrigel plug was recovered, fixed with 10% formaldehyde/phosphate-buffered saline (pH 7.4) and examined with hematoxylin/eosin stain after paraffin embedment. To quantify the formation of new blood vessels, the amount of hemoglobin was measured using the total hemoglobin kit. The concentration of hemoglobin was calculated from a known amount of hemoglobin assayed in parallel.

Cell Proliferation Assay—HUVECs seeded at a density of 1 × 10⁴ cells/well in 96-well plates were incubated for 3 h in Kaighn’s F12K medium containing 1% fetal bovine serum and exposed to rosiglitazone or NS-1619 (0.1–10 µM) for 3 h, followed by VEGF₁₆₅ (10 ng/ml) stimulation for 4 days. After incubation, an MTT assay was carried out (23), and the optical density was measured at 570–630 nm using a microplate spectrophotometer (Bio-Rad).

Apoptosis Assays—Cells were treated with either rosiglitazone (10 µM) or NS-1619 (10 µM) for 3 h in the presence or absence of either BADGE (20 µM), iberiotoxin (0.3 µM), clotrimazole (10 µM), or glibenclamide (10 µM). Then cells were stimulated with 10 ng/ml VEGF₁₆₅ for 2 days. DNA fragmentation was detected by electrophoresis of DNA (15–20 µg/well) on 1% agarose gel, followed by visualization using ethidium bromide staining, and gel pictures were taken by UV transillumination.

Apoptotic index was further quantified by acridine orange/ethidium bromide uptake, as previously reported (24). Cells treated as described above were stained by using acridine orange (final 25 nM) and ethidium bromide (final 25 µg/ml) mixture for 10 min, and then cells were photographed (∗100).

Western Blotting Assay—Cells treated as described above were stimulated with 10 ng/ml VEGF₁₆₅ for 1 h (eNOS) or 24 h (Bcl-2 and Bax) or 48 h (PPARγ). Following centrifugation of cell lysates at 12,000 rpm, 10 µg of total protein was loaded into an 8% SDS-polyacrylamide gel and transferred to nitrocellulose membrane (Amersham Biosciences). Protein bands were visualized using chemiluminescence (Pierce) and quantified with UN-SCAN-IT gel 5.1 software (Silk Scientific, Inc., Orem, UT). Polyclonal antibodies against Bax, PPARγ, phospho-eNOS-Ser¹¹⁷⁹, phospho-eNOS-Thr⁴⁹⁷, glyceraldehyde-3-phosphate dehydrogenase, actin, and monoclonal antibody against Bcl-2 were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

Membrane Potential Measurement Using Voltage-sensitive Fluorescent Dye—Cells (1.5 × 10⁴ cells/well) were incubated with 200 nM DiBAC in Dulbecco’s phosphate-buffered saline for 30 min at 37 °C and exposed to BADGE (20 µM), iberiotoxin (0.3 µM), or N⁵,¹⁰-monomethyl-l-arginine acetate (L-NMMA, 10 µM) for 1 h and then stimulated by rosiglitazone or NS-1619 (10 µM) for 3 h, followed by VEGF₁₆₅ (10 ng/ml) for 1 h. The fluorescence intensities of DiBAC molecules were recorded at 545 nm using a fluorescence plate reader (Bio-Tek Instruments, Inc. Winooski, VT). Hyperpolarization resulted in the extrusion of the dye from cells, causing a subsequent decrease in fluorescence intensity.

Maxi-K Channel Knockdown Using Stealth™ RNAi Oligonucleotide—The Stealth™ RNAi oligonucleotide was synthesized by Invitrogen with the following sequence complementary to human maxi-K mRNA (GenBank™ accession number nm 002547, beginning at the position 1726 target sequence site): sense, 5’-CCG AAG AUA AGA AUC AUC ACUCAA A; antisense, 5’-UUU GAG UGA UGA UUC UUA UCU UCG G. The Stealth™ RNAi negative control Duplex (Invitrogen) was used as a control oligonucleotide. Transfection efficiency was monitored using a fluorescent oligonucleotide (BLOCK-iT; Invitrogen) and estimated to be 80–90%.

The Stealth™ RNAi molecules were transfected into HUVECs using Lipofectamine and Plus reagent following Invitrogen’s protocols. The final concentration of 100 nM Stealth™ RNAi oligonucleotide was
Maxi-K Channel Opening by Rosiglitazone for Antiangiogenesis

Empirically determined to maximally suppress target RNA expression, and the Stealth™ RNAi oligonucleotide was transfected to the medium 48 h prior to the treatment of rosiglitazone. The ability of the Stealth™ RNAi oligonucleotide to knock down maxi-K channel expression was analyzed by Western blot and real time PCR on whole cell extracts.

Measurement of Cytosolic Ca²⁺ and NO Concentrations—Cells transfected with either control RNAi or Maxi-K channel Stealth™ RNAi were seeded at 5 × 10⁴ cells/well in 12-well tissue culture plates and incubated with Kaighn’s F12K medium containing 1% fetal bovine serum plus BADGE or iberiotoxin for 1 h and then exposed to rosiglitazone or NS-1619 for 3 h. Thereafter, VEGF165 was added and incubated for 3 h. Cytosolic Ca²⁺ concentration was determined by measuring the fluorescence of fluo-3 AM (Molecular Probes, Inc., Eugene, OR) at 545 nm using a fluorescence plate reader (Bio-Tek Instruments).

Statistical Analysis—Recombinant human VEGF165 was purchased from R&D Systems. Rosiglitazone was synthesized in the Korea Research Institute of Chemical Technology (Daejeon, Korea). NS-1619, iberiotoxin, BADGE, clomizamole, endothelial cell growth supplement, heparin, acridine orange, ethidium bromide, and MITT were from Sigma. The Hemo-s reagent kit was from YD Diagnostics (Seoul, Korea). 1-NMMA and glibenclamide were from Tocris. Rosiglitazone, NS-1619, BADGE, iberiotoxin, clomizamole, glibenclamide, and 1-NMMA were dissolved in dimethyl sulfoxide as a 20 mM stock solution and then diluted with phosphate-buffered saline.

Statistics—The results are expressed as means ± S.E. Student’s t test was used for analyzing values between vehicle groups and compound-treated groups. p < 0.05 was considered to be statistically significant.

RESULTS

Effects of Maxi-K Channel Modulators and Rosiglitazone on VEGF-induced Angiogenesis—Matrigel-plated HUVECs elongated and migrated in the presence of VEGF, forming a tubular network, as evidenced by morphological changes. Both rosiglitazone and NS-1619, a maxi-K channel opener, markedly suppressed the formation of tube-like structures at 10 μM (Fig. 1). The suppression of tube formation by rosiglitazone (10 μM) was reversed by either BADGE (20 μM), a PPARγ antagonist, or iberiotoxin (0.3 μM), a maxi-K channel blocker. The concentrations of BADGE and iberiotoxin were selected as 20 and 0.3 μM, respectively, which showed maximum effect without cytotoxicity.

Effects of Maxi-K Channel Modulators and Rosiglitazone on the VEGF-induced Cell Proliferation—When HUVECs were incubated in the medium containing 10 ng/ml VEGF165 for 4 days, cell proliferation was increased to about 2-fold. Rosiglitazone (0.1–10 μM) suppressed the cell proliferation in a concentration-dependent manner with about 70% inhibition at 10 μM (Fig. 3). The effect of rosiglitazone was partially reversed by BADGE (20 μM) and iberiotoxin (0.3 μM), suggesting that...
Maxi-K Channel Opening by Rosiglitazone for Antiangiogenesis

The DNA fragmentation by rosiglitazone was accompanied by suppression of Bcl-2 (antiapoptotic protein) expression and increased Bax (proapoptotic protein) expression (Fig. 5), further suggesting that the suppression of endothelial cell viability is associated with apoptotic effects. These effects were also reversed by BADGE (20 μM) and iberiotoxin (0.3 μM).

Effects of Knockdown of Maxi-K Channel Expression on the Rosiglitazone-induced Antiangiogenic and Antiproliferative Actions—The transfection of Stealth™ RNAi oligonucleotide in HUVECs resulted in the reduction of maxi-K channel expression to 10% of control (Fig. 6A). In contrast to the antiangiogenic and antiproliferative effects of rosiglitazone in negative control Stealth™ RNAi-transfected HUVECs, rosiglitazone (10 μM) was ineffective on VEGF165-induced tube formation and cell proliferation in maxi-K channel-knocked down cells (Fig. 6B and C). These results provide conclusive evidence that maxi-K channel activation is required for the antiangiogenic and antiproliferative effects of rosiglitazone on endothelial cells.

Rosi

The antiproliferative effects of rosiglitazone may be, at least in part, mediated by PPARγ activation and by maxi-K channel opening (Fig. 3). The extent of the observed inhibitory effects of rosiglitazone was similar to that described in previous reports (13, 25). In parallel, NS-1619 (0.1–10 μM) significantly suppressed the cell proliferation induced by VEGF165 (10 ng/ml) in a concentration-dependent manner, which was reversed by iberiotoxin but was not reversed by BADGE.

A. Apoptotic actions of rosiglitazone (Rosi) in HUVECs. A and B, DNA fragmentation assay. BADGE (20 μM), IBTX (0.3 μM), clotrimazole (10 μM), or glibenclamide (10 μM) was added to HUVECs for 1 h, and then rosiglitazone or NS-1619 (10 μM) was incubated for 3 h. After incubation, cells were stimulated with 10 ng/ml VEGF165 for 1 day (C) or 2 days (A and B). Experiments were carried out three times. C, acridine orange/ethidium bromide staining. Cells treated as described above were stained by using acridine orange (final 25 μg/ml) and ethidium bromide (final 25 μg/ml) mixture for 10 min and photographed (×100). a, control; b, 10 ng/ml VEGF165; c, 10 ng/ml VEGF165 + 20 μM BADGE; d, 10 ng/ml VEGF165 + 0.3 μM IBTX; e, 10 ng/ml VEGF165 + 1 μM rosiglitazone; f, 10 ng/ml VEGF165 + 10 μM rosiglitazone; g, 10 ng/ml VEGF165 + 10 μM rosiglitazone + 20 μM BADGE; h, 10 ng/ml VEGF165 + 10 μM rosiglitazone + 0.3 μM IBTX; i, 10 ng/ml VEGF165 + 1 μM NS-1619; j, 10 ng/ml VEGF165 + 10 μM NS-1619; k, 10 ng/ml VEGF165 + 10 μM NS-1619 + 20 μM BADGE; l, 10 ng/ml VEGF165 + 10 μM NS-1619 + 0.3 μM IBTX.
expected, but this effect was not affected by iberiotoxin (Fig. 7B), indicating that activation of maxi-K channel is indeed the consequence of PPARγ activation by rosiglitazone. In addition, iberiotoxin had no effect on the endogenous PPARγ transcriptional activity stimulated by rosiglitazone when determined with a transcription reporter assay using PPAR-responsive element reporter genes (data not shown).

Effect of Rosiglitazone on eNOS Phosphorylation and NO Release—To determine whether rosiglitazone opened the maxi-K channel via
NO production, we examined the eNOS phosphorylation by rosiglitazone. The eNOS-Ser\textsuperscript{1179} phosphorylation was significantly and concentration dependently elevated by rosiglitazone (1 and 10 \textmu M). Increased eNOS-Ser\textsuperscript{1179} expression was antagonized by 20 \textmu M BADGE but not antagonized by 0.3 \textmu M iberiotoxin (Fig. 8A). On the other hand, eNOS-Thr\textsuperscript{497} phosphorylation was little affected by rosiglitazone treatment.

Concurrent with increased eNOS-Ser\textsuperscript{1179} phosphorylation, rosiglitazone increased NO production, as shown in Fig. 8B. Rosiglitazone (10 \textmu M) increased NO production to about 900 nM, which approximates the required concentration to activate maxi-K channels (19). Increased NO production by rosiglitazone was antagonized by either 20 \textmu M BADGE or 10 \textmu M L-NMMA (data not shown) but not antagonized by 0.3 \textmu M iberiotoxin or by knockdown of maxi-K channel expression (Fig. 8B). In addition, L-NMMA pretreatment prevented rosiglitazone-induced hyperpolarization (Fig. 7A), suggesting that NO is an important player for PPAR\textgamma-mediated maxi-K channel activation. The DNA fragmentation and suppression of in vitro tube formation (Fig. 8, C and D) by rosiglitazone were antagonized by 10 \textmu M L-NMMA, further indicating that NO is involved as a key mediator of rosiglitazone-induced apoptotic and antiangiogenic effects. L-NMMA itself had no effect on VEGF\textsubscript{165}-induced in vitro tube formation and DNA fragmentation. The concentration of 10 \textmu M of L-NMMA was chosen, because 10 \textmu M L-NMMA exhibited maximum effect without cytotoxicity.

**Effect of Rosiglitazone on Cytosolic Ca\textsuperscript{2+} Concentrations**—We studied the effects of rosiglitazone and NS-1619 on the cytosolic Ca\textsuperscript{2+} concentration based on the previous observation that maxi-K channel activation resulted in the increase of intracellular Ca\textsuperscript{2+} concentration (26). Rosiglitazone elevated cytosolic Ca\textsuperscript{2+} concentration (45 \pm 3% increase),...
which was antagonized either by 0.3 μM iberiotoxin or by 20 μM BADGE (Fig. 9). In parallel, NS-1619 treatment also increased intracellular Ca²⁺ concentration (results not shown).

To determine whether increased Ca²⁺ is the upstream or downstream effect of maxi-K channel activation by rosiglitazone, we checked the effects of rosiglitazone on the cytosolic Ca²⁺ in the maxi-K channel RNAi-transfected cells. The increased Ca²⁺ by rosiglitazone was abolished in maxi-K channel RNAi-transfected cells, suggesting that increased Ca²⁺ may be the consequence of the maxi-K channel activation by rosiglitazone.

DISCUSSION

PPARγ has been recognized as a potential therapeutic target for the treatment of pathologic neovascularization (13, 25), since various PPARγ ligands inhibited growth and/or migration of vascular endothelial cells, smooth muscle cells, monocytes, and certain tumor cells (27, 28). The present study demonstrates the novel finding that the antiangiogenic effect of rosiglitazone is mediated by PPARγ-induced maxi-K channel opening and subsequent apoptosis in HUVECs.

Among many cell types involved in the PPARγ-mediated antiangiogenic action, recent investigations supported the importance of endothelial cells in the inhibitory activity of PPARγ ligands with the identification of functionally active PPARγ expression in the tumor endothelium and in the immortalized endothelial cells (9, 12, 13). An endogenous ligand, 15-deoxy-Delta-12,14-prostaglandin J-2, and thiazolidinediones inhibited endothelial differentiation into tube-like structures and proliferation, and suppressed VEGF-induced angiogenesis (13, 29). Similarly, troglitazone and rosiglitazone inhibited VEGF-induced proliferation, migration, and tube formation of bovine choroidal endothelial cells (13), and rosiglitazone directly suppressed growth of a variety of primary tumors and metastatic invasion by antiangiogenic effect (12). Our results confirmed the previous reports (13) that rosiglitazone, a PPARγ agonist, induced a strong concentration-dependent inhibition of neovascularization in response to VEGF165 in vitro and in vivo.

Consistent with the reports showing PPARγ-mediated apoptosis in endothelial cells (9), we also observed that the antiproliferative effects of rosiglitazone were accompanied by DNA fragmentation with decreased Bcl-2 and increased Bax expression. In addition, the apoptotic cell death by rosiglitazone was further confirmed by acridine orange and ethidium bromide staining. Control and VEGF165-treated cells had a uniform
green color in nuclei, but rosiglitazone-treated cells had bright orange areas of condensed chromatin in the nucleus. The effects of rosiglitazone were abolished by pretreatment with BADGE, a PPAR\textsubscript{γ} antagonist, suggesting that rosiglitazone-induced anti-angiogenesis via apoptosis is predominantly mediated through PPAR\textsubscript{γ} activation in HUVECs.

Maxi-K channels, widely expressed channels in various cell types, regulate membrane action potential, neurotransmitter release, and cell death in neurons (30, 31), and may induce cell shrinkage and apoptosis due to increased loss of cytosolic K\textsuperscript{+} in unexcitable endothelial cells. Thus, we postulated that maxi-K channels might be involved in the inhibitory action of rosiglitazone in VEGF\textsubscript{165}-induced angiogenesis of HUVECs. As confirmed from previous reports (12, 32), maxi-K channels as well as PPAR\textsubscript{γ} were expressed in HUVECs by Western blot in our experiments (data not shown).

Several lines of our experiments supported the involvement of maxi-K channels in rosiglitazone-induced anti-angiogenesis in endothelial cells in a PPAR\textsubscript{γ}-dependent manner. First, the antiangiogenic effect by rosiglitazone was reversed by either iberiotoxin, a maxi-K channel blocker, or BADGE, a PPAR\textsubscript{γ} antagonist. Second, NS-1619, a specific maxi-K channel opener exhibited similar antiangiogenic effects, which was reversed by iberiotoxin but not by BADGE. Third and most importantly, knockdown of maxi-K channel expression by RNAi abolished the antiangiogenic effects of rosiglitazone. On the other hand, either clotrimazole, an intermediate conductor of Ca\textsuperscript{2+}-activated K\textsuperscript{+} channel blocker, or glibenclamide, an ATP-sensitive K\textsuperscript{+} channel blocker, was ineffective on the action of rosiglitazone, strongly indicating that PPAR\textsubscript{γ} activation induced specifically the maxi-K channel opening, at least in part, resulting in the proapoptotic and antiangiogenic effects in endothelial cells.
The reversal of rosiglitazone action by maxi-K channel inhibition (i.e. iberiotoxin and RNAi) could be due to either the suppression of the rosiglitazone-induced activation of PPARγ or the downstream effects of PPARγ activation by rosiglitazone. In the present study, we observed that the effects of rosiglitazone on PPARγ activation were not interfered with by iberiotoxin. Furthermore, we demonstrated that rosiglitazone opened maxi-K channel, as determined by membrane hyperpolarization being reversed in the presence of either iberiotoxin or BADGE. All of these results suggest that rosiglitazone induced maxi-K channel opening via PPARγ activation, subsequently exhibiting antiangiogenic effects in endothelial cells.

Maxi-K channels are regulated by various intracellular second messengers, including NO (19–21). For example, NO appears to stimulate maxi-K channel activity either via indirect cGMP generation or via direct fashion (19, 20), although the regulatory role of NO in the endothelial maxi-K channel is still controversial (21, 33). Recently, several studies reported that PPARγ activation increased endothelial NO production (22, 34), possibly via regulation of eNOS phosphorylation at serine 1179 and at threonine 497. In comparison, other studies suggested that eNOS phosphorylation is not involved in the maxi-K channel opening. We believe that these data represent the first evidence of maxi-K channel activity in the rosiglitazone-induced anti-angiogenesis in endothelial cells. Although in vivo antiangiogenic activity of rosiglitazone seems to be a coordinated phenomenon from the effects on many different cell types, the results of the present study would provide an important insight on the antiangiogenic mechanism of rosiglitazone in the endothelial cells. Furthermore, the novel compounds acting as maxi-K channel openers in endothelial cells would potentially provide a useful approach for antiangiogenic therapy.

**REFERENCES**

1. Cao, Y., Ji, R. W., Davidson, D., Schaller, J., Marti, D., Sohnidel, S., McCance, S. G., O-Reilly, M. S., Llinas, M., and Folkman, J. (1996) *J. Biol. Chem.* 271, 29461–29467
2. Folkman, J. (1995) *Nat. Med.* 1, 27–31
3. Margeli, A., Kouraklis, G., and Theocharis, S. (2003) *Angiogenesis* 6, 165–169
4. Risau, W. (1997) *Nature* 386, 671–674
5. Neufeld, G., Cohen, T., Gengrinovitch, S., and Politzer, Z. (1999) *FASEB J.* 13, 9–22
6. Schoonjans, K., Martin, G., Staels, B., and Auwerx, J. (1997) *Curr. Opin. Lipidol.* 8, 159–166
7. Vidal-Puig, A., Jimenez-Linan, M., Lowell, B. B., Hamann, A., Hu, E., Speigelman, B., Flier, J. S., and Moller, D. E. (1996) *J. Clin. Invest.* 97, 2553–2561
8. Tontonoz, P., Nagy, L., Alvarez, J. G., Thomazy, V. A., and Evans, R. M. (1998) Cell 93, 241–252
9. Bishop-Bailey, D., and Hla, T. (1999) *J. Biol. Chem.* 274, 17042–17048
10. Loviscach, M., Rehman, N., Carter, L., Mudahlar, S., Mohadeen, P., Ciaraldi, T. P., Peerlamp, J. H., and Henry, R. R. (2000) *Diabetologia* 43, 304–311
11. Wilton, T. M., Cobb, J. E., Cowan, D. J., Wiethe, R. W., Correa, I. D., Prakash, S. R., Beck, K. D., Moore, L. B., Kliever, S. A., and Lehmann, J. M. (1996) *J. Med. Chem.* 39, 665–668
12. Panigrahy, D., Singer, S., Shen, L. Q., Butterfield, C. E., Freedman, D. A., Chen, E. J., Moses, M. A., Kilroy, S., Duensing, S., Fletcher, C., Fletcher, J. A., Hatly, L., Hahnfeld, P., Folkman, J., and Kaipainen, A. (2002) *J. Clin. Invest.* 110, 923–932
13. Xin, X., Yang, S., Kowalski, J., and Gervitsen, M. E. (1999) *J. Biol. Chem.* 13, 9116–9119
14. Goetz, S., Bungenstock, A., Czapilla, D., Eilers, F., Stawowy, P., Kintscher, U., Spencer-Hansch, C., Graf, K., Nurnberg, B., Law, R. E., Fleck, E., and Grafe, M. (2002) *Hypertension* 40, 748–754
15. Huang, H., Campbell, S. C., Bedford, D. F., Nelin, T., Veliceasa, D., Shroff, E. H., Henkin, J., Schneider, A., Bouck, N., and Volpert, O. V. (2004) *Mol. Cancer Res.* 2, 541–550
16. Sierra-Honigmann, M. R., Nath, A. K., Murakami, C., Garcia-Cardenas, G., Papapetropoulos, A., Sessa, W. C., Madge, L. A., Schechner, J. S., Schwabb, M. B., Polverini, P. J., and Flores-Riveros, J. R. (1998) *Science* 281, 1683–1686
17. Ling, S., Woromuk, G., Sy, L., Lev, S., and Braun, A. P. (2000) *J. Biol. Chem.* 275, 30683–30689
18. Krafl, K., Krause, P., Jung, S., Basrai, D., Liebmann, L., Bolz, J., and Pant, S. (2003) *Pflugers Arch.* 446, 248–255
19. Bolotina, V. M., Najibi, S., Palacin, J. I., Pagano, P. J., and Cohen, R. A. (1994) *Nature* 368, 850–853
20. Fukao, M., Mason, H. S., Britton, F. C., Kenyon, J. L., Horowitz, B., and Keef, K. D. (1999) *J. Biol. Chem.* 274, 10927–10935
Maxi-K Channel Opening by Rosiglitazone for Antiangiogenesis

21. Brakemeier, S., Eichler, I., Knorr, A., Fassheber, T., Kohler, R., and Hoyer, J. (2003) *Kidney Int.* **64**, 199–207
22. Cho, D. H., Choi, Y. J., Jo, S. A., and Jo, I. (2004) *J. Biol. Chem.* **279**, 2499–2506
23. Mosmann, T. (1983) *J. Immunol. Methods* **65**, 55–63
24. Kalvelyte, A., Imbrasaitė, A., Bukauskiene, A., Verselis, V. K., and Bukauskas, F. F. (2003) *Biochem. Pharmacol.* **66**, 1661–1672
25. Murata, T., Hata, Y., Ishibashi, T., Kim, S., Hsueh, W. A., Law, R. E., and Hinton, D. R. (2003) *Arch. Opthal. Mol.* **119**, 709–717
26. Kuhlmann, C. R., Trumper, J. R., Abdallah, Y., Wiebke Ludders, D., Schaefer, C. A., Most, A. K., Backenkohler, U., Neumann, T., Walther, S., Piper, H. M., Tillmanns, H., and Erdogan, A. (2004) *Thromb. Haemost.* **92**, 1099–1107
27. Hsueh, W. A., and Law, R. E. (2001) *Arterioscler. Thromb. Vasc. Biol.* **21**, 1891–1895
28. Willon, T. M., Lambert, M. H., and Kliewer, S. A. (2001) *Annu. Rev. Biochem.* **70**, 341–367
29. Bourcier, M. N., Sukhova, G. K., Libby, P., and Plutzky, J. (1999) *Arterioscler. Thromb. Vasc. Biol.* **19**, 546–551
30. Poolos, N. P., and Johnston, D. (1999) *J. Neurosci.* **19**, 5205–5212
31. Kim, K. Y., Lee, J. H., Park, J. H., Yoo, M. A., Kwak, Y. G., Kim, S. O., Yoo, S. E., and Hong, K. W. (2004) *Eur. J. Pharmacol.* **497**, 267–277
32. Frieden, M., Malli, R., Samardzija, M., Demaures, N., and Graier, W. F. (2002) *J. Physiol.* **540**, 73–84
33. Haburcak, M., Wei, L., Viana, F., Prenen, J., Droogmans, G., and Nilius, B. (1997) *Cell Calcium* **21**, 291–300
34. Calnek, D. S., Mazzella, L., Roser, S., Roman, J., and Hart, C. M. (2003) *Arterioscler. Thromb. Vasc. Biol.* **23**, 52–57
35. Krick, S., Platoshyn, O., Sweeney, M., Kim, H., and Yuan, J.X. (2001) *Am. J. Physiol.* **280**, C970–C979
36. Zhang, X. F., Gopalakrishnan, M., and Shieh, C. C. (2003) *Neuroscience* **122**, 1003–1011