Inhibition of Protein Kinase C Is Not Sufficient to Prevent or Reverse Effects of VEGF<sub>165</sub> on Claudin-1 and Permeability in Microvascular Retinal Endothelial Cells

Heidrun L. Deissler, Helmut Deissler, and Gabriele E. Lang

Purpose: Pathogenesis of diabetic macular edema is driven by deregulated expression of VEGF. A study of long-term exposure of immortalized bovine retinal endothelial cells (iBRECs) to VEGF<sub>165</sub> clearly confirmed the role of the tight junction protein claudin-1, which almost completely disappeared within 24 hours, an effect that was completely reversed by addition of the VEGF-binding Fab fragment ranibizumab. This study was conducted to investigate whether the VEGF<sub>165</sub>-induced loss of claudin-1 is regulated by protein kinase C (PKC) and indeed affects the barrier function of iBRECs.

Methods: The effects of various PKC inhibitors on claudin-1 expression and cellular localization in iBRECs treated with VEGF<sub>165</sub> for up to 2 days were studied by Western blot analyses and immunofluorescence microscopy. The permeability of the cell layers was determined by transepithelial electrical resistance measurements.

Results: Activation of PKC led to decreased expression of claudin-1, which was blocked by inhibitors of PKC. However, none of the PKC inhibitors significantly affected VEGF<sub>165</sub>-induced effects on cellular localization or expression of claudin-1. Also VEGF<sub>165</sub>-induced higher permeability of iBREC layers could be reversed or prevented by ranibizumab but not by PKC inhibitors. In addition, low claudin-1 expression and its delocalization from the plasma membrane were significantly associated with elevated permeability.

Conclusions: In iBRECs, PKC isoforms are not crucially involved in the VEGF<sub>165</sub>-initiated signal transduction that affects permeability and expression of claudin-1. This finding is in contrast to published results concerning only short-term effects of VEGF<sub>165</sub>. The results also confirmed that claudin-1 is a highly relevant component of functional tight junctions in retinal endothelial cells. (Invest Ophthalmol Vis Sci. 2010;51:535–542) DOI:10.1167/iovs.09-3917

Diabetic retinopathy (DR) is one of the major complications of diabetes mellitus and the main cause of blindness in industrialized countries. Events in the genesis and early development of DR include the loss of retinal pericytes, thickening of the capillary basement membrane, and increased leukocyte adhesion to retinal endothelial cells (RECs). It is now a generally accepted concept that the pathogenesis of DR is driven by deregulated expression of growth factors. Of these, vascular endothelial growth factor (VEGF) is considered most important because its levels are substantially elevated in the vitreous fluid and in the retinal vasculature of patients with DR.1–3 Although different VEGF-binding receptors have been identified, its effects on cellular proliferation, migration, and permeability are mainly mediated through VEGF receptor 2.4,5 VEGF is also an activator of several isoforms of serine/threonine-specific protein kinase C (PKC) in endothelial cells (ECs), and it seems possible that pathogenic processes depend on this pathway.6–9 The PKC family includes several members that differ in their structure, required cofactors, and substrate specificity. They can be divided into three subgroups: conventional (α, βI, II, and γ), novel (δ, ε, and θ), and atypical (ζ and η/θ) PKC.

Activation of PKC by hyperglycemia, especially of isoform β, has been implicated in the processes deregulated in DR.6,8,10 Hyperglycemia also results in upregulated expression of VEGF and components of the extracellular matrix that contribute to elevated permeability of RECs, probably leading to macular edema in DR.11,12–15 Several in vitro studies showed that altered permeability of ECs induced by VEGF<sub>165</sub> was associated with changed cellular localization, expression, and/or modification of tight junction (TJ) proteins.11–17 These complexes of membrane proteins like claudins or occludin and membrane-associated proteins (e.g., ZO-1) are crucial determinants of paracellular signaling of ECs.18,19 Site-specific phosphorylation of occludin induced by short-term treatment with VEGF<sub>165</sub> of bovine retinal endothelial cells (BRECs) may interfere with TJ assembly.14,16,20 However, in these previous investigations, the long-term effects of VEGF that should be more relevant in the pathologic situation of vivo were neglected. We showed that long-term treatment of immortalized (i)BRECs with VEGF<sub>165</sub> indeed resulted in delocalization of the TJ proteins occludin and claudin-1 from the plasma membrane. This result was not associated with a change in expression or a modification of occludin, but expression of claudin-1 was markedly reduced. Claudin-1 is expressed by microvascular endothelial cells of the brain and retina, although at a lower level than other claudins.17,21,22 It is an essential part of TJ in the mammalian epidermal barrier and claudin-1-deficient mice die soon after birth.23 In human Caco-2 cells, claudin-1 is phosphorylated at serine/threonine residues, and PKC/β is involved in the regulation of its expression and cellular localization.24 Based on these previous observations, we studied whether PKC is involved in long-term effects of VEGF<sub>165</sub> on claudin-1 and permeability of iBRECs, and whether the presence of claudin-1 is important in proper barrier function in these cells.
MATERIALS AND METHODS
Reagents, Antibodies, and Media
Recombinant human growth factor rhVEGF165 (SF21-expressed) was from R&D Systems (Wiesbaden, Germany). The EDTA-free protease inhibitor cocktail (Complete) was purchased from Roche Applied Science (Mannheim, Germany). Brij 97, sodium deoxycholate and the phosphatase inhibitor cocktail 2 were from Sigma-Aldrich (Deisenhofen, Germany). The PKC inhibitors Go6976, Go6983, GF109203X (also known as BIM 1 or G66850), rottlerin (mallotoxin), and Ro 31-8245 (negative control) were obtained from Merck-Kalbiochem (Darmstadt, Germany), as were the inhibitor of PKCB (IAPD; 3-(1-(3-imidazol-1-ylpropyl)-1H-indol-3-yl)-4-anilino-1H-pyrrole-2,5-dione), the PKC activator phorbol-12-myristate-13-acetate (PMA), and the corresponding negative control substance 4α-phorbol-12,13-didecanoate (4αPDD). Ranibizumab (Lucentis; Genentech, South San Francisco, CA), the Fab fragment of a humanized VEGF-binding antibody, was purchased from Novartis Pharma GmbH (Nuremberg, Germany).25 Rabbit polyclonal antibodies binding to human claudin-1 (JAY.8), claudin-5 (Z23.JM), claudin-5 (Z43.JK), occludin (Z-T22), and ZO-1 (Z.R1) were from Invitrogen (Karlsruhe, Germany). Horseradish peroxidase-conjugated anti-rabbit secondary antibodies were included in a detection kit (Opti4CN) from Bio-Rad (Munich, Germany). Horseradish peroxidase–conjugated anti-rabbit secondary antibodies were included in a detection kit (Opti4CN) from Bio-Rad (Munich, Germany). Mg2+/-Ca2+-free PBS (PBlsd) and PBS were obtained from Invitrogen. Complete microvascular endothelial cell growth medium (ECGM; Promocell, Heidelberg, Germany) was supplemented with co-delivered, premixed additives, resulting in final concentrations of 0.4% ECGS/H, 5% fetal calf serum, 10 ng/mL epidermal growth factor, and 103 nM hydrocortisone. Serum-reduced basal medium (EBGM; Promocell) was supplemented with 0.4% ECGS/H, 0.25% fetal calf serum, and 103 nM hydrocortisone.

Cell Cultivation
Telomerase-immortalized microvascular endothelial cells from bovine retina (iBRECs) were cultivated on fibronectin-coated surfaces in cell culture flasks (BD Biosciences, Heidelberg, Germany) in ECGM with 2.8 μM hydrocortisone, as previously described.26 Cultures were washed with PBlsd to support enzymatic cell detachment for subcultivation. In all experiments, the concentration of hydrocortisone in the medium was 103 nM.

Treatment of iBRECs with VEGF165 or PMA and Inhibitors of PKC
Before the experiment with confluent iBRECs, ECGM was replaced by EBGM for 24 hours. The cells were then either pretreated with inhibitors of PKC in various concentrations or 100 μg/mL ranibizumab in EBGM—supplemented with 1 μg/mL fibronectin to improve adhesion of cells, especially to glass surfaces—for 2 hours before 100 ng/mL VEGF165 was added for an additional 20 to 48 hours. Alternatively, the cells were incubated with 100 ng/mL VEGF165 for 2 days and PKC inhibitors or 100 μg/mL ranibizumab was then added to the medium (still containing VEGF165) for an additional 24 hours.

The confluent iBRECs were pretreated with inhibitors of PKC or with ranibizumab for 2 hours, as described earlier, before 100 nM PMA (or 100 nM 4αPDD in control experiments) was added for an additional 20 hours.

Immunofluorescence Staining
iBRECs at a density of 2 × 105 cells/cm² were allowed to adhere to two-chamber slides (BD Biosciences or Nunc, Wiesbaden, Germany) coated with fibronectin in ECGM. After having formed a confluent monolayer, the cells were treated with the effectors as just described. The cells were fixed in methanol/acetic acid at −20°C, and the antigens were detected by immunofluorescence staining.27 Primary antibod-
were used at the lowest still-sufficient concentrations. For the anti-claudin-1 antibody used, we confirmed that it was not cross-reactive with other bovine TJ proteins (data not shown).

**Effect of PKC Inhibitors on Loss of Claudin-1 Induced by Treatment with the PKC Activator PMA**

To narrow down the PKC isoforms that are involved in the regulation of claudin-1 expression or modification, we studied the effects of PKC inhibitors of various specificities (Table 1) in iBRECs. The cells were incubated with PKC inhibitors or the VEGF inhibitor ranibizumab for 24 hours before Western blot analyses of the claudins were performed. Whereas most PKC inhibitors did not significantly influence the expression or cellular localization of claudin-1, rottlerin (inhibitor of PKCδ and -θ at 1–5 μM) abolished claudin-1 expression in iBRECs (Table 1; Fig. 1A). This effect is most likely not due to inhibition of PKCδ, because it was not observed in experiments with other inhibitors of PKCδ (GF109203X and G66983). Treatment with rottlerin also led to changes in iBREC morphology (i.e., development of spikelike extensions). In contrast to untreated iBRECs, expression of TJ proteins in the plasma membrane was seen only at cell–cell contacts. Expression of claudin-5 was not significantly affected by the PKC inhibitors tested (Fig. 1A).

Activation of PKC was achieved by treatment of iBRECs with 100 nM PMA for up to 24 hours. After 6 hours, expression of claudin-1 analyzed by Western blot had declined and was undetectable after 24 hours (Fig. 1B). Pretreatment of iBRECs for 2 hours with PKC inhibitors GF109203X (specific for the α, β, γ, δ and ε isoforms), G66983 (specific for the α, β, γ, δ and ε isoforms) or rottlerin prevented loss of claudin-1 induced by PMA in a dose-dependent manner. This result was not observed after similar treatment with the PKC inhibitors IAPD (inhibits mainly β) and G66976 (specific for α and β), or with the control compound Ro 31-6045 (Table 1, Fig. 2). The VEGF-binding Fab fragment ranibizumab also failed to inhibit the PMA-induced loss of claudin-1. In contrast to claudin-1, expression of claudin-5 was not affected by PMA (Fig. 2). From these results and specificities of the inhibitors used, it can be concluded that PKCδ may play a role in regulation of claudin-1 but not PKCα and -β.

**Effect of PKC Inhibitors on Loss of Claudin-1 Induced by Long-Term Treatment with VEGF165**

Based on our observation that long-term treatment of iBRECs with VEGF165 resulted in downregulation of claudin-1 (which completely disappeared from the plasma membrane, see Fig. 5) and reports on VEGF-induced activation of PKC isoforms in endothelial cells, we investigated whether long-term effects of VEGF165 on claudin-1 are mediated through PKC isoforms.7,9,17 Loss of claudin-1 was induced by treatment of iBRECs with

**Table 1. Inhibition of PMA-Induced Lower Expression of Claudin-1 by G6983, GF109203X, and Rottlerin**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Affected PKC Isoforms at Concentration Used</th>
<th>Effect on Basal Expression of Claudin-1?</th>
<th>Prevention of PMA-Induced Lower Expression of Claudin-1?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α</td>
<td>β</td>
<td>βI</td>
</tr>
<tr>
<td>GF109203X</td>
<td>X</td>
<td>X</td>
<td>–</td>
</tr>
<tr>
<td>G66983</td>
<td>X</td>
<td>X</td>
<td>–</td>
</tr>
<tr>
<td>G66976</td>
<td>X</td>
<td>X</td>
<td>–</td>
</tr>
<tr>
<td>IAPD</td>
<td>–</td>
<td>–</td>
<td>X</td>
</tr>
<tr>
<td>Rottlerin</td>
<td>–</td>
<td>–</td>
<td>X</td>
</tr>
<tr>
<td>Ro 31-6045</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ranibizumab†</td>
<td>–</td>
<td>–</td>
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</tr>
</tbody>
</table>

↓↓↓↓, strongly reduced expression of claudin-1.
* Inhibition (X) or no inhibition (−) of the indicated PKC isoform at the concentration used, according to information provided by the supplier.
† Ranibizumab binds to VEGF and prevents its interaction with VEGF receptors.
TABLE 2. Influence of PKC Inhibition on VEGF-Induced Effects

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Affected PKC Isoforms at Concentration Used*</th>
<th>Prevention/Reversal of VEGF-Induced Loss of Claudin-1?</th>
<th>Prevention/Reversal of VEGF-Induced Delocalization of Claudin-1?</th>
<th>Prevention/Reversal of VEGF-Induced Elevation of Permeability?</th>
</tr>
</thead>
<tbody>
<tr>
<td>GF109203X</td>
<td>X</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Gö6983</td>
<td>X</td>
<td>No</td>
<td>Partly</td>
<td>No</td>
</tr>
<tr>
<td>Gö6976</td>
<td>X</td>
<td>No</td>
<td>No</td>
<td>ND</td>
</tr>
<tr>
<td>IAPD</td>
<td>X</td>
<td>No</td>
<td>No</td>
<td>ND</td>
</tr>
<tr>
<td>Rottlerin</td>
<td>X</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
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<td>Ro31–6045</td>
<td>X</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Ranibizumab†</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

ND, not determined.
* Inhibition (X) or no inhibition (−) of indicated PKC isoform at concentration used according to information provided by the supplier.
† Ranibizumab binds to VEGF and prevents its interaction with VEGF receptors.

VEGF<sub>165</sub> for 2 days before PKC inhibitors (or ranibizumab) were added for an additional 20 hours (Table 2). In accordance with our previous investigations, reduced expression of claudin-1, analyzed by Western blot analysis in cell extracts, was completely reversed after the addition of ranibizumab. Of all tested PKC inhibitors, only Gö6983 slightly increased claudin-1 expression (Fig. 3A; Table 2)—that is, in these experiments inhibition of PKC was not sufficient to reverse the VEGF<sub>165</sub>-induced loss of claudin-1. Similar results were obtained in a series of experiments in which iBRECs were pre-treated with the different PKC inhibitors for 2 hours before they were incubated with VEGF<sub>165</sub> for 1 to 2 days. Only ranibizumab prevented complete loss of claudin-1, and of the inhibitors only Gö6983 showed a very weak effect (Fig. 3B). These results confirmed that PKC isoforms are not crucially involved.

Effect of PKC Inhibitors GF109203X and Gö6983 on Increased Permeability of iBRECs Induced by Long-Term Treatment with VEGF<sub>165</sub>

To study the long-term effects of VEGF<sub>165</sub> and PKC inhibitors on cell permeability, we performed TER measurements of iBREC layers. The inhibitors GF109203X and Gö6983 were chosen because their overlapping spectra of specificity covered most PKC isoforms. Confluent monolayers of iBRECs seeded on porous membrane filters were pretreated for 2 hours with GF109203X, Gö6983, or ranibizumab. VEGF<sub>165</sub> was then added for 2 days, and changes in the permeability of the cell layers were measured (Fig. 4; Table 2 for summary). Inhibitors alone did not significantly influence the permeability of iBRECs (Fig. 4, t = 2 hours). Within 20 hours after addition of VEGF<sub>165</sub>, permeability of iBRECs was significantly elevated (Fig. 4, t = 22 hours). Preincubation with ranibizumab completely prevented this VEGF<sub>165</sub>-induced effect, whereas permeability was not affected by GF109203X or Gö6983 (Fig. 4, t = 22 hours and t = 46 hours). To detect potential changes in the cellular localization of TJ proteins, these were visualized after TER measurements by immunofluorescence staining (Fig. 5).

Long-term treatment of iBRECs with VEGF<sub>165</sub> led to the delocalization of claudin-1 and occludin from the plasma membrane, whereas other TJ proteins were not affected (Fig. 5, second column from the left). This effect on claudin-1 and occludin was completely reversed or prevented by addition of ranibizumab (Fig. 5, right column). Of interest, pretreatment of iBRECs with GF109203X or Gö6983 did not prevent VEGF-induced delocalization of claudin-1, but did prevent that of occludin (Fig. 5). Cellular localization of the TJ proteins claudin-3 and ZO-1 was not significantly altered (data not shown).

Similar experiments in which iBRECs were treated with VEGF<sub>165</sub> for 2 days before ranibizumab, GF109203X, or Gö6983 was added for another 24 hours confirmed that VEGF<sub>165</sub>-induced changes in permeability could be prevented by treatment with ranibizumab but not with any of the PKC inhibitors used.
Diabetic retinopathy is a major microvascular complication of diabetes. Activation of PKC, especially its \( \beta \)-isotype, by hyperglycemia is considered to be critically involved in some processes deregulated in this disease.\(^6\,\!^8\,\!^{10}\) But despite promising results in in vitro studies and animal models, inhibitors affecting PKC\( \beta \) were not particularly successful in the treatment of diabetic macular edema, perhaps because of the limited relevance of the mostly used rodent models of diabetes in which nonconserved putative phosphorylation sites of claudins could have played a role.\(^{30}\,\!^{31}\) In addition, highly relevant long-term effects of involved growth factors like VEGF were neglected in most of the in vitro studies. It is revealing that a significant change in protein expression or modification of occludin was not found after long-term exposure of BRECs to VEGF\(_{165}\), although short-term exposure led to subtly changed phosphorylation at Ser\(^{490}\).\(^{16}\,\!^{17}\,\!^{20}\) In contrast, studying long-term exposure to VEGF\(_{165}\) clearly confirmed the role of claudin-1, which almost completely disappeared within 24 hours, an effect that could be completely reversed by addition of the VEGF-binding Fab fragment ranibizumab.\(^{17}\) In this study, we investigated whether this VEGF\(_{165}\)-induced loss of claudin-1 is regulated by PKC and indeed affects the barrier function of iBRECs.

Although the iBRECs used in this study were of bovine origin, these cells were shown to behave like human retinal ECs and therefore represent a relevant model system. Compared with primary cells, it is a distinct advantage of iBRECs that these are free of contaminating cells of other types, allowing more accurate and detailed studies.\(^{17}\,\!^{26}\,\!^{27}\) Immortalizing primary cells by ectopic expression of human telomerase reverse transcriptase (TRT) is usually not associated with significant changes in important cellular processes, and the amount of human TRT in iBRECs is similar to that of the bovine homologue expressed in primary BRECs. iBRECs express the TJ proteins claudin-1, claudin-3, claudin-5, ZO-1, and occludin, as well as VEGF receptor 2 and PKC\( \beta I\), PKC\( \beta II\), and PKC\( \theta\), as shown by immunohistochemical staining of the bovine retina.\(^{32}\) Furthermore, of the most relevant TJ protein claudin-1, most putative phosphorylation sites are conserved between the human and bovine homologues.
It is interesting that both inhibition and activation of PKC led to lower expression of claudin-1 and its delocalization from the iBREC plasma membrane. This finding again shows that substantial additional efforts are needed to replace the current simplistic view by a more detailed knowledge of the interactions of numerous signaling molecules, including PKC isoforms, in the complex and intertwined pathways involved in the regulation of cellular functions. Effects of PKC inhibitors suggest that PKC\(\beta\) and/or \(\delta\) is the PKC isoform involved in this modulation of claudin-1. This conclusion is in accordance with similar regulation of claudin-1 by PKC\(\beta\) in human Caco-2 cells in which direct phosphorylation of claudin-1 was observed.\(^{24}\) The effects of PKC activation on claudin-1, however, seem to depend on the type of cells analyzed. In human melanoma cells without any functional TJs, the low basal amount of claudin-1 located in the cytoplasm was increased after addition of PMA which correlated with an enhanced motility of the cells.\(^{25}\) Stimulation of PKC with PMA in macrovascular ECs led to synthesis of more VEGF mRNA.\(^{34}\) Whether VEGF expression can be directly induced or enhanced by activated PKC in retinal microvascular ECs, as in retinal pericytes, remains to be shown\(^{10,55}\); but the observation that PMA-induced effects on claudin-1 expression in iBRECs were not prevented by the VEGF-inhibitor ranibizumab supported the assumption that in these cells, production or secretion of significant amounts of VEGF were not induced. Indeed, VEGF could not be detected in cell lysates or supernatants of PMA-stimulated iBRECs (data not shown).

To evaluate how permeability of iBRECs was affected by VEGF\(_{165}\), TER measurements of a confluent monolayer were performed. Although confluent cultured ECs are extremely flattened compared with their cuboidal form in vivo, this assay is considered an accurate model for assessing chronic vascular hyperpermeability associated with pathologic neoangiogenesis in malignant diseases and DR.\(^{56}\) In iBRECs, the presence of plasma membrane-localized claudin-1 strongly correlated with barrier function (Figs. 4, 5). This finding is in accordance with the results obtained with canine epithelial MDKC and human CaCo-2 cells.\(^{24,55-57}\) Remarkably, in contrast to claudin-1, the presence of the TJ protein occludin seemed not to be necessary for proper barrier function in iBRECs. In addition, localization of other TJ proteins claudin-3, claudin-5, or ZO-1 in the plasma membrane of iBRECs could also not compensate for the loss of claudin-1. In view of the hypothesis that PKC is crucially involved in the regulation of VEGF\(_{165}\)-induced changes of permeability in RECs, this study now surprisingly showed that PKC inhibitors covering all relevant isoforms were not sufficient to prevent or reverse this effect in long-term experiments. In previous studies, the slightly elevated permeability of BRECs induced by treatment with VEGF\(_{165}\) for 1 hour could be prevented by treatment with the PKC inhibitor GF109203X.\(^{16}\) In iBRECs permeability was slightly but not significantly elevated after such short treatment with VEGF\(_{165}\), and a significantly higher permeability was observed 20 hours after addition of VEGF\(_{165}\). However, this increase could not be affected with PKC inhibitors. In addition, none of the PKC inhibitors

![Figure 5](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933452/ on 04/18/2017)
tested completely reversed or prevented the effect of VEGF165 on claudin-1 expression or localization under these conditions. The results of our investigation therefore strongly suggest that in retinal endothelial cells, PKC is most likely not involved in VEGF165-induced signaling leading to modulation of claudin-1 expression and increased permeability, at least during prolonged exposure to VEGF165, which resembles the in vivo situation in patients with DR. This result is also in accordance with established mechanisms of tumor angiogenesis in which activation of PKC by VEGF165 is assumed not to result in changed permeability of new or preexisting vasculature. However, it seems possible that reported short-term effects of VEGF165 on permeability or TJ were transmitted through PKC, whereas cellular responses to prolonged treatment with VEGF165 were triggered by other signaling pathways. Cellular localization of PKCa in macrovascular ECs was also found to depend on the duration of VEGF treatment: After 3 minutes, it was localized at the plasma membrane but after 30 minutes, again in the cytoplasm. Accordingly, our preliminary results of immunofluorescence staining of iBREC treated with VEGF165 for 2 days indicated that PKCa and - were indeed localized exclusively in the cytoplasm, where a direct interaction with TJ proteins was not possible.

In summary, our in vitro model of immobilized retinal endothelial cells provided evidence for the assumption that PKC is most likely not involved in VEGF165-induced signaling leading to increased cellular permeability and expression of the TJ protein claudin-1. This result is in contrast to published results concerning only short-term effects of VEGF165. However, the prolonged treatment with VEGF165 in our experiments might reflect the pathologic situation in DR more closely. Our results also help to understand why the extensively studied PKCβ inhibitors were not beneficial to patients with diabetic macular edema.

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References


