Endothelin-Induced Changes in the Physiology of Retinal Pericytes

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PURPOSE. Pericytes are positioned on the abluminal wall of capillaries and are thought to play a role in regulating retinal blood flow. Although endothelin (ET)-1 is a putative endothelium–pericyte signal, the mechanisms by which this molecule regulates pericyte function remain unclear. Because ion channels play a vital role in the response of pericytes to extracellular signals, this study was undertaken to assess the effects of ET-1 on ionic currents.

METHODS. The perforated-patch configuration of the patch-clamp technique was used to monitor whole-cell currents of pericytes located on microvessels freshly isolated from the rat retina. To assay cell-to-cell coupling within retinal microvessels, a gap junction–permeant tracer was loaded through patch pipettes into pericytes and the spreading of the tracer detected by immunohistochemistry.

RESULTS. ET-1 acting through ET1 receptors altered pericyte currents and caused depolarization of the membrane potential. The effects on pericyte currents were dynamic over time. Initially, the nonspecific cation (NSC) and calcium-activated chloride (ClCa) currents were activated and the adenosine triphosphate (ATP)-sensitive potassium (KATP) current inhibited. Subsequently, by a mechanism sensitive to a protein kinase C (PKC) inhibitor, the NSC, ClCa, and voltage-dependent potassium currents diminished as gap junction pathways closed within the microvessels.

CONCLUSIONS. ET-1 regulates pericyte conductances by multiple mechanisms. One process involves a PKC-dependent closure of gap junction pathways resulting in loss of electrotonic input from neighboring cells. Thus, ET-1 not only affects individual microvascular cells, but also regulates the effective size of the multicellular functional units that may serve to control capillary blood flow. This regulation of intercellular communication within pericyte-containing microvessels may be an important, previously unrecognized, action of ET-1. (Invest Ophthalmol Vis Sci. 2002;43:882–888)

Having a low density of retinal capillaries1 is a successful adaptation that lessens interference by blood vessels with light passing through the retina. However, because this vascular organization provides little functional reserve, the regulation of retinal blood flow must be highly responsive to local metabolic demands. This task is facilitated by the independence of the retinal circulation from systemic influences. For example, the presence of a tight blood–retinal barrier and the absence of autonomic innervation1–3 prevent circulating vasoactive molecules and extrinsic neural input from directly influencing the contractile cells that regulate blood flow by adjusting lumen diameter. As a result, the retinal microcirculation is almost exclusively under the local control of molecules that are released by the vascular endothelium, neurons, and glia.

A specialized feature of the retinal microvasculature is an absence of the smooth muscle sphincters4 that control capillary perfusion in other tissues. The responsiveness of retinal capillaries to vasoactive signals4,5 despite the absence of precapillary myocytes, suggests that the control of retinal blood flow occurs, in part, at the capillary level. Regulation of microperfusion at distal locations in the vasculature may be an effective mechanism to efficiently distribute oxygen and nutrients to metabolically active neurons within the inner retina.

Candidates for playing a role in adjusting capillary blood flow are the pericytes, which envelope small blood vessels and change their contractile tone, at least in culture, during exposure to vasoactive molecules.6–10 Contraction and relaxation of these cells may adjust lumen size.1,4,9 Although pericytes are present in almost all vascular beds, the ratio of pericytes to endothelial cells is greatest in the retina,10 suggesting the particular importance of these cells in the retinal microcirculation. However, the mechanisms by which vasoactive molecules influence the function of these cells remain uncertain.

In this study, we focused on the effects of endothelin (ET)-1, which is a putative endothelial cell-to-pericyte signal. This molecule is expressed by retinal vascular endothelial cells11, and pericytes have ET receptors.11,12 Consistent with its role as a vasoconstrictor, ET-1 increases calcium levels in pericytes.13–15 Causes these cells to contract,1,3,14 and evokes a constriction of pericyte-containing microvessels of the retina.3

Because ion channels are likely to be important in mediating the functional responses of pericytes to vasoactive signals, we examined the effects of ET-1 on the ionic currents of these cells. Using the perforated-patch technique, we monitored currents in pericytes located on microvessels freshly isolated from the adult rat retina. We now report that exposure of retinal microvessels to ET-1 alters four types of ionic currents in the pericytes. Multiple mechanisms appeared to mediate the ET-induced changes in pericyte physiology. One process involved the regulation of electronic input from neighboring cells through gap junction pathways within the retinal microvasculature.

METHODS

Microvessel Isolation

Microvessels from 6- to 8-week-old rat retinas were freshly isolated using a tissue-print method.5,16 Animal use conformed to the guidelines of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and of the University of Michigan Committee on the Use and Care of Animals. For each experiment, a rat (Harlan Sprague-Dawley, Inc., Indianapolis, IN) was killed with carbon dioxide, and the...
retinas were rapidly removed and incubated in 2.5 mL Earle's balanced salt solution (catalog no. 24010; Life Technologies, Grand Island, NY), supplemented with (in millimolar) 0.5 EDTA, 20 glucose, 15 U papain (Worthington Biochemicals, Freehold, NJ), 0.04% DNase, and 2 mM cysteine, for 30 minutes at 30°C, while 95% oxygen-5% CO2 was bubbled through to maintain pH and oxygenation. Subsequently, each retina was carefully transferred through a large-bore, fire-polished Pasteur pipette (3-mm inner diameter) to a 60-mm Petri dish containing approximately 3 mL of solution A, containing (in millimolar) 140 NaCl, 3 KCl, 1.8 CaCl2, 0.8 MgCl2, 10 Na-HEPES, 15 mannitol, and 5 glucose at pH 7.4, with osmolarity adjusted to 310 mOsm. Without much delay, each retina was again gently drawn up into the large-bore pipette and, with minimal trauma, was expelled along with approximately 0.2 mL of solution A onto a clean glass coverslip (diameter, 15 mm; Warner Instrument Corp., Hamden, CT), which was positioned in a specially built chamber. While viewing the specimen at a magnification of ×6.4 with a dissection microscope, we carefully unfolded the retina so that its vitreal surface faced upward. A second coverslip was then placed over the vitreal surface of the retina. Forceps touching the upper coverslip provided gentle downward pressure that sandwiched the retina between the two coverslips. During this tissue-print step, vessels adhered to the upper coverslip. After a few seconds, the upper coverslip was carefully removed and placed, with its vessel-containing surface up, in a 60-mm Petri dish partially filled with solution A. This tissue-print step was repeated, so that several coverslips containing microvessels could be obtained from each retina. The coverslips were viewed at ×100 magnification with an inverted microscope equipped with phase-contrast optics. Pericytes were identified by their characteristic location on the abluminal wall of microvessels that had outer diameters of less than 7 μm.16,17 Coverslips with a plentiful number of pericyte-containing microvessels were selected for our experiments.

Electrophysiology

A coverslip containing microvessels was recorded in a recording chamber that was perfused at 2 mL/min with solution A plus additives, as noted. During the recording session, vessels were examined at ×1000 magnification with an inverted microscope equipped with phase-contrast optics. As detailed previously,5,16,18 the perforated-patch technique was used to monitor the ionic currents of pericytes located on microvessels that had been isolated from a retina within 2 hours. The pipette solution for these experiments contained 50 mM KCl, 65 mM K2SO4, 6 mM MgCl2, 10 mM HEPES, 240 μg/mL amphotericin B, and 240 μg/mL nystatin at pH 7.4, with the osmolarity adjusted to 280 mOsm. Pipettes, which had resistances of approximately 5 MΩ, were mounted in the holder of a patch-clamp amplifier (Dagan Corp., Minneapolis, MN). After the tip of a pipette was positioned with the aid of a piezoelectric micromanipulator (Burleigh, Victor, NY) onto the soma of a pericyte, application of gentle suction to the back end of the pipette created a more than 10-GΩ seal. As the amphotericin-nystatin perforated the patch, the access resistance to the pericytes studied decreased to less than 20 MΩ. For the generation of current–voltage (I–V) plots, currents were evoked by voltage step protocols controlled by computer (pClamp 8 software; Axon Instruments, Inc., Foster, CA) and were filtered at 1 kHz with a four-pole Bessel filter and digitally sampled at 400-μs intervals, using a data acquisition system (Digidata 1200B; Axon Instruments). Digitized data were stored in a computer for data analysis and graphics display (pClamp 8; Axon, and Origin 6.1; OriginLab). For the generation of I–V plots, currents were sampled at 50–ms intervals; curve-fitting software (Origin 6.1; OriginLab) calculated exponential functions that described the decay of the membrane capacitive currents.

Tracer Labeling

As detailed previously,20 a patch pipette containing 0.5% of the gap junction-permeant tracer N-(2-aminoethyl) biotinamide chloride (Neurobiotin: Vector Laboratories, Burlingame, CA) plus (in millimolar) 25 KCl, 105 potassium aspartate, 1.5 CaCl2, 2 MgCl2, 3 K-EGTA, and 10 K-HEPES at pH 7.4 and osmolarity of approximately 280 mOsm was mounted in a holder. The tip was positioned onto the soma of a retinal pericyte located on a freshly isolated retinal microvessel, and an approximately 10-GΩ seal was created. After the patch of cell membrane at the tip of the pipette was broken by applying progressively increasing amounts of suction through a pneumatic transducer (Bio-Tek, Winooski, VT), a voltage of +50 mV was applied to the pipette for 5 minutes to enhance movement of the tracer from the pipette into the sampled pericyte. The tracer-containing pipette was then removed, and the microvessel was left undisturbed for 40 minutes. The location of the sampled pericyte was documented in a sketch. After approximately 18 hours of fixation at 4°C in phosphate-buffered saline (PBS) containing 4% paraformaldehyde, the microvessel-containing coverslip was washed in PBS, and the endogenous peroxidase activity of the vessels was blocked by exposure to 0.3% hydrogen peroxide in PBS for 30 minutes. After a 60-minute incubation in 0.5% Triton X-100, the coverslip was exposed for 40 to 65 hours at 4°C to 3,3′-diaminobenzidine plus nickel (DAB kit; Vector Laboratories). After the vessels were counterstained with methyl green, they were viewed at ×100 magnification with a microscope equipped with bright-field optics, and the extent of staining by the tracer was measured.

Chemicals

Chemicals were from Sigma/RBI (St. Louis, MO) unless noted otherwise.

Statistics

Data are expressed as the mean ± SEM. Probability was evaluated by Student’s t-test.

Results

ET-Modulated Currents

To help elucidate the mechanisms by which ET-1 regulates the function of the retinal microvasculature, we used the perforated-patch technique to monitor the ionic currents of pericytes located on microvessels freshly isolated from the rat retina.
From our earlier studies of retinal microvessels, we reported that both sustained and transient conductances are detected in fresh pericytes. The sustained conductances recorded under control conditions are due chiefly to the activity of NSC and voltage-dependent potassium ( Kv ) channels. The transient depolarizing currents detected in pericytes are generated by Cl Ca channels.

In the present study, we found that exposure of isolated microvessels to ET-1 altered both the sustained and transient currents of retinal pericytes. This is illustrated in Figure 1 and quantified in Figure 2. Initially, ET-1 induced an increase in the amplitudes of the NSC and Cl Ca currents, but did not significantly ( P = 0.4 ) affect the Kv conductance. Associated with the early changes in the ionic conductances, the membrane potential of the pericytes decreased from -46 ± 1 to -31 ± 2 mV ( n = 13 ). After approximately 5 minutes of exposure to ET-1, the amplitudes of the NSC, Kv , and Cl Ca currents began to decrease. New steady state levels were reached within approximately the next 10 minutes (Figs. 1, 2). Despite these conductance changes, the mean membrane potential of the sampled pericytes remained at -31 ± 1 mV. The ET-induced changes in the NSC, Kv , and Cl Ca currents were similar ( P > 0.3 ) at ET-1 concentrations of 0.25 ( n = 8 ), 1 ( n = 13 ), and 100 nM ( n = 8 ). The effects of ET-1 on the pericyte currents and membrane potential were reversible (Fig. 1), although up to 1 hour or more was required to return to control values.

The effect of ET-1 was not limited to changes in the NSC, Kv , and Cl Ca currents. In addition to these basal currents, a hyperpolarizing ATP-sensitive potassium (K ATP ) current can be activated in pericytes. As illustrated and quantified in Figure 3, we found that ET-1 rapidly and profoundly inhibited the K ATP current induced by pinacidil, which is a K ATP channel opener. The inhibitory effect of ET-1 on the pericyte K ATP current persisted throughout the period of ET exposure (Fig. 3C). Similar to the effects of ET-1 on the basal currents of pericytes, the inhibition of the induced K ATP conductance reversed slowly.

Role of ET A Receptors

We found that BQ123, which is a specific antagonist of ET A receptors, blocked the effects of ET-1 on the ionic currents of fresh pericytes. Specifically, in the presence of BQ123 (500 nM), ET-1 (1 nM) did not significantly ( P = 0.25, n = 4 ) alter the NSC, Kv , Cl Ca , or K ATP currents in pericytes. Further support for an ET A rather than an ET B mechanism, was our observation that IRL-1620 (100 nM), which is a specific ET B receptor agonist, had no significant ( P > 0.4 , n = 3 ) effect on the pericyte currents. Taken together, these findings indicate that ET-1 acts through ET A receptors to regulate the conductances of retinal pericytes.

Effect of a PKC Inhibitor

In a number of cell types, ET-1 activates protein kinase C (PKC). To assess whether this enzyme is involved in mediating the effects of ET-1 on the ionic conductances of pericytes, we tested the effect of chelerythrine, which is a PKC inhibitor. In a series of experiments (Fig. 4), the early effects of ET-1 on pericyte conductances were not significantly ( P > 0.3 , n = 5 ) altered when isolated microvessels were exposed to 1 μM chelerythrine for 45 to 90 minutes before supplementing the bathing solution with 1 nM ET-1. This finding suggests that during ET exposure, PKC does not have a critical role in either the initial activation of the NSC and Cl Ca channels or the early inhibition of K ATP channels.

However, although the PKC inhibitor did not alter the initial effects of ET-1, chelerythrine significantly blocked the subsequent decreases in the NSC, Kv , and Cl Ca currents during
prolonged exposure to ET-1 (Fig. 4). These observations are consistent with the hypothesis that activation of PKC plays a role in mediating the late inhibitory effects of ET-1 on the NSC, KV, and ClCa conductances recorded in the pericytes of retinal microvessels.

ET-Induced Inhibition of Cell Coupling

The question arose as to the mechanism by which PKC mediates the late ET-induced decrease in NSC, KV, and ClCa conductances. We considered the hypothesis that ET-1 reduces electrotonic coupling between pericytes and neighboring microvascular cells. This seemed to be a reasonable possibility, because we have demonstrated that there is an extensive network of cell-to-cell coupling within pericyte-containing microvessels of the retina and that this intercellular communication is markedly reduced in the presence of an activator PKC.20

The uncoupling of the pericyte-containing microvasculature may involve a phosphorylation of connexin 43, which is expressed by retinal pericytes26 and is regulated by PKC.27 Closure of gap junction pathways would prevent the electrotonic spread to sampled pericytes of ionic currents that are generated in neighboring cells.

To help assess the possibility that ET-1 reduces cell coupling, we analyzed the rate of decay of the transient membrane capacitive current evoked by a voltage step (Fig. 5). A complex rate of decay is predicted for cells that are interconnected in series.28–30 In contrast, the decay of this capacitive current should be fit by a single exponential function for a single cell.31 Figure 5A shows an example of a recording under control conditions in which the decay of the capacitive current (solid line) in a pericyte was poorly fit by a single exponential function (dotted line). However, after 16 minutes of exposure to ET-1, a single exponential function (dotted line) described the pericyte’s capacitive current (Fig. 5B). Similar results were observed in five other recordings from pericytes located on isolated microvessels. In this series of recordings, the decay of the capacitive current was fit by a single exponential function at 13 ± 3 minutes after the onset of ET exposure. However, when the perfusate also contained 500 nM BQ123, the ET A receptor antagonist, we did not detect a change in the capacitive current during an exposure of 12 minutes or more (n = 5) to ET-1 (1 nM). Consistent with a role for PKC in closing gap junction pathways during exposure to 1 nM ET-1, cheleryth-

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**Figure 2.** Effect of ET-1 on the basal ionic conductances of pericytes located on isolated microvessels. Columns show chord conductances before ET exposure (Control), 3 to 5 minutes after the onset of exposure to 1 nM ET-1 (ET [early]), and 12 to 15 minutes after the onset of ET exposure (ET [late]). n = 13. Compared with the column to the left: *P ≤ 0.04, **P ≤ 0.001; ns, not significant (P = 0.4). During ET exposure, the amplitudes of the NSC and ClCa currents initially increased. Later, the NSC, KV, and ClCa currents of the sampled pericytes decreased.

**Figure 3.** Effect of ET-1 on the ionic currents of a retinal pericyte exposed to a KATP channel opener, pinacidil. (A) Current traces obtained under control conditions, during exposure to 5 μM pinacidil, and during exposure to pinacidil and 1 nM ET-1. The clamp protocol is shown below. B For the same pericyte as in (A), IV relations in the presence of pinacidil (A) and 6 minutes after the onset of exposure to 1 nM ET-1 (C). (B, inset) IV plots from this cell under control conditions (B) and 5 minutes after the addition of 5 μM pinacidil to the perfusate (A). (C) Mean chord conductances of the KATP current before (Control) and during exposure to 1 nM ET-1 in a series of eight sampled pericytes. ET(early), KATP conductance measured 2 to 5 minutes after the onset of ET exposure; ET(late), KATP conductance 12 to 15 minutes after onset of ET exposure. ET(early) and ET(late) conductances were not significantly different (P = 0.9). **P ≤ 0.001 when compared with the control value. ET-1 inhibited the pinacidil-induced KATP currents in retinal pericytes.
rine (1 μM) prevented the ET-induced transition to an exponentially decaying membrane capacitive current in each of five sampled pericytes. Thus, it appears likely that the ET-induced uncoupling of pericytes from neighboring vascular cells is mediated by a mechanism involving ETA receptors and PKC activation.

Use of a gap junction–permeant tracer (Neurobiotin; Vector Laboratories) provided further evidence that ET-1 reduces cell-to-cell communication within pericyte-containing microvessels (Fig. 6). In our previous studies in which this tracer was loaded into pericytes through a patch pipette, we demonstrated that the tracer spreads hundreds of micrometers from a sampled pericyte.20 In our present study, we found that exposure of microvessels to ET-1 (0.5 nM) reduced the spread of the tracer by 72% (P < 0.001, n = 9), from 457 ± 29 to 127 ± 50 μm.

Quantification of the incidence of cases in which the tracer appeared to remain exclusively within the tracer-loaded pericyte (e.g., Figure 6B) provided further evidence that ET-1 reduces intercellular communication. In a control group, no evidence for cell-to-cell coupling was observed in 2.5% (1/31) of the microvessels studied. In contrast, we did not detect diffusion of tracer from 33% (3/9) of the sampled pericytes located on microvessels exposed to ET-1 (a significant difference at P = 0.05, Fisher exact test). We conclude from our tracer studies and electrophysiological analyses that one mechanism by which ET-1 may reduce pericyte currents is to diminish cell-to-cell coupling with neighboring microvascular cells.

DISCUSSION

The results show that ET-1 acting through ET_A receptors significantly alters the physiology of retinal pericytes. Initially, ET-1 inhibited the K_ATP current and activated the NSC and Cl_Ca currents of the sampled pericytes. Subsequently, during sustained ET exposure, the NSC, Cl_Ca, and K_V currents of the sampled pericytes diminished as ET-1 induced closure of the gap junction pathways that couple cells within the retinal microvasculature.20 Throughout the period of ET exposure, the pericyte membrane potential was approximately −30 mV, which is approximately 15 mV depolarized from the resting level.
The initial inhibition of KATP currents and activation of NSC and ClCa currents in retinal pericytes is similar to the effects of ET-1 on these types of currents in vascular smooth muscle cells. For example, ET-1 inhibits KATP channels in cerebral pial arteries, as well as in other vascular myocytes. This vasoactive molecule is also known to activate NSC channels of cerebral arterioles and of other larger vessels. In addition, ET-1 increases ClCa currents in smooth muscle cells, including those of the choroidal vasculature in the eye. Thus, it appears that the initial response of retinal pericytes to this vasoconstrictor is similar to that of other contractile cells within the vascular system.

However, despite the initial similarities of pericyte and vascular smooth muscle responses, there appeared to be differences during prolonged exposure to ET-1. Specifically, our electrophysiological and tracer assays of intercellular communication revealed that sustained exposure to ET-1 was associated with a loss of cell-to-cell coupling within the pericyte-containing microvasculature. With the uncoupling of pericytes from neighboring cells, the currents recorded from the sampled pericytes decreased. This is the first report of an ET-mediated regulation of gap junction pathways in the vascular system.

Control of gap junction pathways within the retinal microvasculature may be an important mechanism by which ET-1 influences pericycle physiology. There is likely to be a causal link between the ET-induced loss of cell coupling and the decrease in the NSC, ClCa, and KV currents recorded in pericytes during exposure to this vasoconstrictor. Consistent with this possibility, we found that pericyte uncoupling, which occurred between approximately 5 and 15 minutes after the onset of exposure to ET-1, was temporally associated with a reduction in the pericyte conductances. Also supporting the hypothesis that closure of gap junction pathways causes a reduction in pericyte currents, exposure to the PKC inhibitor chelerythrine prevented both the uncoupling of pericytes and the decrease in the NSC, ClCa, and KV currents. A parsimonious explanation is that a PKC-dependent closure of gap junction pathways prevents currents generated in neighboring vascular cells from spreading electrotonically to the sampled pericytes.

Although it appears that control of cell-to-cell coupling plays an important role in the late decrease in pericyte NSC, ClCa, and KV currents, the rapid and sustained inhibition by ET-1 of the KATP current is not regulated by this mechanism. In agreement with this, the decrease in the KATP current was maximal before the loss of cell-to-cell coupling. Also, PKC activation appeared not to be essential for the ET-induced inhibition of KATP channels, because chelerythrine did not prevent the ET-induced decrease in KATP current. Thus, it seems clear that ET-1 regulates pericyte physiology by multiple mechanisms.

The significance of the ET-induced decrease in cell-to-cell coupling within the pericyte-containing microvasculature is uncertain. One likely consequence is to limit the number of vascular cells that are depolarized when ET-1 is released from a localized area of the vascular endothelium. Because of the extensive gap junction pathways interconnecting cells of the retinal capillaries, the ET-induced depolarization of a pericyte could initially spread electrotonically through widespread portions of the microvascular tree. This may cause a rather generalized vasoconstriction, which, we predict, would become significantly more restricted as gap junction pathways are closed during a sustained exposure to ET-1. Thus, ET-1 not only affects individual cells of the retinal microvasculature, but also regulates the effective size of the multicellular functional units that may serve to control capillary blood flow. However, more studies are needed to establish that the regulation of intercellular communication is an important mechanism for the autoregulation of retinal blood flow.

The ET-induced decrease in the membrane potential of pericytes is likely to have functional consequences. With depolarization, the membrane potential enters the “window of current” for the voltage-gated calcium channels (VGCCs) that are expressed by these cells. At voltages within this window (i.e., approximately −35 ± 15 mV) there is sufficient depolarization to activate these calcium channels, but not enough to cause their complete inactivation. Consistent with ET-1 activating VGCCs, a substantial portion of the ET-induced increase in intracellular calcium levels in cultured retinal pericytes is blocked by an inhibitor of these ion channels.

A likely response of pericytes to an ET-activated calcium influx is cellular contraction. The expression of calcium-sensitive contractile protein by pericytes and the demonstration of pericyte contraction, at least in culture, supports the idea that intracellular calcium levels regulate the contractile tone of these cells. A contraction of pericytes may constrict the microvascular lumen and thereby reduce capillary blood flow.

In addition to regulating pericyte function under physiological conditions, ET-1 may have pathophysiological effects. For example, elevated levels of ET-1 are detected in the retinas of diabetic animals. An excess of this vasoactive molecule may contribute to the closure of gap junctions and to the disruption of blood flow regulation observed early in the course of diabetes. Also, the ability of microvessels in the diabetic retina to respond to metabolic compromise may be limited if an overexpression of ET-1 prevents the activation of KATP channels by vasodilators, such as adenosine.

Our use of isolated microvessels permitted electrophysiological studies of fresh, rather than cultured, pericytes. This may be particularly important in elucidating the mechanisms by which ET-1 regulates the function of the retinal microvasculature. For example, although this vasoactive molecule is reported to have no major effects on the membrane potential of cultured pericytes, we found that ET-1 reversibly induced a significant depolarization of these cells when they were located on freshly isolated microvessels. Of course, because an in vivo application of the electrophysiological techniques used in this study seems impractical at present, it remains to be demonstrated that the effects of ET-1 observed in isolated microvessels also occur in the retina in vivo. However, despite some limitations, experiments using freshly isolated retinal microvessels should help to clarify how vasoactive molecules regulate the function of the pericyte-containing microvasculature under physiological and pathophysiological conditions.

Acknowledgment

The authors thank David Wu and Tatsuo Kodama for helpful discussions.

References


