PDGF-Induced Coupling of Function with Metabolism in Microvascular Pericytes of the Retina

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Purpose. The aim of this study was to test the hypothesis that platelet-derived growth factor (PDGF)-BB regulates the physiology of retinal pericytes, which are contractile cells located on the ablumenal surface of capillaries. The expression of PDGF-BB and its cognate receptor in retinal vessels suggests a vasoactive function. However, although endothelium-derived PDGF-BB appears vital for the development of pericyte-containing microvessels, its role in the mature vasculature remains uncertain.

Methods. Based on the premise that ion channels mediate the responses of pericytes to vasoactive signals, the perforated-patch configuration of the patch-clamp technique was used to determine the effect of PDGF-BB on the ionic currents and membrane potential of pericytes located on microvessels freshly isolated from the adult rat retina. Changes in pericyte calcium levels were monitored with the calcium indicator fluo-4. Differential interference contrast optics and image analysis software aided in assessing the effects of PDGF-BB on the lumens of isolated pericyte-containing microvessels. In some experiments, blockers of adenosine triphosphate (ATP) synthesis created chemical ischemia.

Results. Electrophysiological recordings from pericytes showed that PDGF-BB can activate nonspecific cation channels, chloride channels, and ATP-sensitive potassium channels. The metabolic status of an isolated capillary determined which of these ion channels were activated by PDGF-BB and thereby whether the membrane potential decreased or increased, the cell calcium rose or fell, and the vessel lumen constricted or dilated.

Conclusions. The ability of PDGF-BB to be a vasoconstrictor when energy supplies are ample and to be a vasodilator under ischemic conditions may provide an efficient mechanism to link capillary function to local metabolic needs. (Invest Ophthalmol Vis Sci. 2001;42:1939–1944)

An essential task of the circulatory system is to efficiently distribute oxygen, glucose, and other nutrients to regions of need. In the central nervous system, local metabolic demand plays a major role in regulating blood flow. This process of metabolic autoregulation is particularly important in the retina, because its vasculature has no extrinsic autonomic innervation, and, thus, local conditions exclusively control its microcirculation. Whereas capillary perfusion in many tissues is regulated by precapillary sphincters, the absence of these muscles in the retinal vasculature suggests that autoregulation in the retina occurs, at least in part, at the capillary level.

Prime candidates for regulating capillary blood flow in the retina are the contractile pericytes, which are positioned on the ablumenal surface of the endothelium-lined lumen. By contracting and relaxing, pericytes may regulate lumen size and thereby control capillary perfusion. Reflecting the likely importance of pericytes in regulating retinal blood flow, the ratio of pericytes to endothelial cells is greater in the retina than in any other vascular bed.

Although pericytes appear to play a role in controlling the retinal microcirculation, knowledge of the mechanisms by which vasoactive molecules influence the function of these cells is limited. Current models of blood flow regulation in pericyte-containing microvessels focus on molecules that are either vasodilators (e.g., adenosine) or vasoconstrictors (e.g., endothelin-1). Herein, we present evidence suggesting an additional mechanism—namely, that certain molecules can serve as both a dilator and a constrictor, depending on the local metabolic conditions.

In this study, we focused on the effects of PDGF-BB, because the expression of this molecule by vascular endothelial cells and the presence of PDGF-β receptors on pericytes suggest a paracrine role in the vasculature. Consistent with this possibility, developmental studies indicate that PDGF-BB released by newly formed vascular endothelial tubes promotes the directed migration onto immature capillaries of pericyte progenitor cells. In mice without PDGF-BB or its cognate receptor, microvessels have few pericytes and rupture in utero, causing fatal hemorrhages. Despite the importance of PDGF-BB during angiogenesis, the function of this molecule in the mature vascular system is less certain. However, the expression of PDGF-BB and its receptor in adult retinal vessels points to a continuing functional role. Furthermore, the increased expression of PDGF-BB by hypoxic endothelial cells supports the possibility that this molecule serves to link capillary function with metabolism.

Because ion channels are likely to be important in mediating the responses of pericytes to vasoactive signals, we examined the effect of PDGF-BB on the ionic currents of these cells. To minimize disruption of intracellular contents, we used the perforated-patch configuration of the patch-clamp technique to monitor pericyte currents. We also used various imaging techniques to assess the effect of PDGF-BB on the level of calcium within pericytes and on the lumen diameter of pericyte-containing vessels. Because these assays are not currently practical in vivo, we studied microvessels that were freshly isolated from the adult rat retina.

We report that PDGF-BB can activate three types of ion channels in retinal pericytes. Metabolic conditions determined which of these pericyte channels were activated and thereby whether the membrane potential depolarized or hyperpolarized, the intracellular calcium level increased or decreased, and the vascular lumen narrowed or widened in response to PDGF-BB. By serving as a vasoconstrictor or vasodilator, PDGF-BB...
may facilitate the fine-tuning of capillary blood flow to meet local metabolic demand in the retina.

METHODS

Microvessel Isolation

Freshly isolated retinal microvessels were prepared using a modified “tissue-print” method.29 Animal use conformed to the guidelines of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the guidelines laid down by the University of Michigan Committee on the Use and Care of Animals. For each experiment, retinas were rapidly removed from a euthanized approximately 200-g rat (Harlan Sprague-Dawley, Inc., Indianapolis, IN) and incubated in 2.5 ml Earle’s balanced salt solution (Life Technologies, Grand Island, NY) supplemented with 0.5 mM EDTA, 1.5 mM CaCl₂, 1 mM MgSO₄, 20 mM glucose, 26 mM sodium bicarbonate, 2 mM cysteine, 15 U papañ (Worthington Biochemicals, Freehold, NJ), and 0.04% DNase for 30 minutes at 30°C, while 95% oxygen-5% CO₂ was bubbled through to maintain pH and oxygenation. After transfer to solution A (140 mM NaCl, 3 mM KCl, 1.8 mM CaCl₂, 0.8 mM MgCl₂, 10 mM Na-HEPES, 15 mM mannitol, and 5 mM glucose at pH 7.4 with osmolarity adjusted to 310 mOsm), each retina was gently sandwiched between two glass coverslips (diameter, 15 mm; Warner Instrument Corp., Hamden, CT). Vessels adhered to the coverslip that contacted the vitreal surface of the retina. This coverslip was placed in a recording chamber containing solution A. Pericytes were identified by their characteristic location on the abluminal wall of microvessels having outer diameters of less than 7 μm.12,29

Electrophysiology

Experiments were performed at room temperature within 3 hours after microvessel isolation. The pipette solution consisted of 50 mM KCl, 65 mM K₂SO₄, 6 mM MgCl₂, 10 mM K-HEPES, 240 μg/ml amphotericin B, and 240 μg/ml nystatin at pH 7.4 with the osmolarity adjusted to 280 mOsm. The pipettes, which had resistances of approximately 5 MΩ, were mounted in the holder of a patch-clamp amplifier (model 3900; Dagan, Minneapolis, MN) and sealed to the cell bodies of pericytes. Capacitative compensation was applied through circuits within the amplifier. We did not correct for series resistance. Correction for the calculated30 liquid junction potential was made after data collection. Currents were evoked by voltage-step protocols or by ramping membrane voltage (66 mV/sec) from negative to positive. A computer equipped with pClamp (Axon Instruments, Burlingame, CA) was used to control voltage protocols, sample currents, and analyze data. Currents were filtered at 1 kHz and digitally sampled at 400 μsec for steps and 1 msec for ramps. Membrane potentials were determined from current-clamp recordings. As detailed elsewhere,29 the nonspecific cation (NSC) conductance was measured at −103 mV, which is the equilibrium potential for potassium (E_K); the potassium current was measured at 0 mV, which is close to E_K. The net charge transfer associated with the transient chloride currents was measured at −58 mV, as described previously.29

Calcium Imaging

Freshly isolated microvessels, which adhered to a glass coverslip that formed the bottom of a recording chamber, were exposed for approximately 45 minutes to the acetoxymethyl ester form of fluo-4 (Fluo-4AM, 5 μM). Digital imaging of fluorescence was performed using a ×60 (1.4 numeric aperture [NA], oil immersion) objective on an inverted microscope (Diaphot 200; Nikon, Tokyo, Japan) with a confocal scanner (Oz; Noran Instrument Co., Middleton, WI). Fluo-4 fluorescence was monitored with 488 nm argon excitation and a 515-mm long-pass barrier filter. A software system (Intervision; Indy R5000 workstation; Silicon Graphics, Mountain View, CA) facilitated the data acquisition and analysis.

Capillary Lumen Measurements

Lumens of freshly isolated microvessels adhering to a glass coverslip that formed the base of a perfusion chamber were viewed by differential interference contrast optics using a ×60 (1.2 NA, water immersion) objective and an inverted microscope (Diaphot 200; Nikon) equipped with a transmitted light detector (Noran). A software system (Intervision; Indy R5000; Silicon Graphics) controlled image acquisition and facilitated image analysis. At 10-second intervals, 128 digitized images were acquired at 50 Hz, averaged, and stored. Lumen diameters were quantified by plotting gray-scale intensity along cross sections of capillaries. The lumen diameters were then identical in each vessel image placed along an axis perpendicular to the capillary lumen and passing through a pericyte soma. Software permitted objective measurement in each of the images of the width of the “valley” of decreased intensity that corresponded to the vessel’s lumen.

RESULTS

Using a recently developed method to isolate retinal microvessels from the adult rat retina,29 we compared the responses of pericytes to PDGF-BB under normal and metabolically compromised conditions. To monitor the membrane potential and ion channel activity of these cells, we used the perforated-patch configuration of the patch-clamp technique. In a control group of pericytes located on isolated microvessels, exposure to PDGF-BB (1.7 nM) significantly depolarized the membrane potential from −55 ± 1 to −46 ± 3 mV (P < 0.001; Fig. 1). This decrease in voltage contrast was the effect of PDGF-BB on pericytes maintained under conditions of chemical isch-
increased markedly to BB, the membrane potential of depolarized ischemic pericytes showed that exposure to PDGF-BB more than doubled the conductances by methods detailed previous-ly.29 We know that fresh pericytes have sustained nonspecific cation and potassium conductances, as well a transiently occurring chloride current. Quantification of these conductances by methods detailed previously29 showed that exposure to PDGF-BB more than doubled the activity of the nonspecific cation channels \((P = 0.007)\) and the chloride channels \((P = 0.017)\), both of which induce depolarization of retinal pericytes.29 However, the potassium currents were not affected significantly \((P = 0.177)\) by PDGF-BB. Specifically, in the control group \((n = 39)\) the nonspecific cation current, potassium current, and chloride charge transfer were \(-39 \pm 8 \text{ pA}, 74 \pm 17 \text{ pA}, \text{ and } -11 \pm 2 \text{ pC} \), respectively, and in PDGF-BB \((n = 10)\) these values were \(-87 \pm 12 \text{ pA}, 122 \pm 20 \text{ pA}, \text{ and } -23 \pm 6 \text{ pC} \), respectively. Thus, under normal metabolic conditions PDGF-BB depolarized pericytes by activating nonspecific cation channels and chloride channels.

In ischemic pericytes, the effect of PDGF-BB was markedly different. PDGF-BB activated neither the nonspecific cation channels nor the chloride channels \((P > 0.2; n = 12)\) in pericytes maintained under conditions of chemical ischemia. Rather, PDGF-BB induced a hyperpolarizing conductance in ischemic pericytes whose currents were markedly attenuated because of inhibition of ATP synthesis (Figs. 2B, 2C). The PDGF-induced current reversed near the equilibrium potential for potassium and showed modest inward rectification (Fig. 2D). Tolbutamide, a blocker of ATP-sensitive potassium \((K_{ATP})\) channels, inhibited a similar current and caused PDGF-treated ischemic pericytes \((n = 4)\) to depolarize (Figs. 2E, 2F). These experiments indicate that PDGF-BB activates \(K_{ATP}\) channels in ischemic pericytes. Because of the high membrane resistance of the depolarized ischemic pericytes, a relatively modest efflux of potassium through PDGF-activated \(K_{ATP}\) channels exerted a potent hyperpolarizing effect.

What are the physiological implications of our finding that PDGF-BB depolarized pericytes under normal conditions but hyperpolarized these cells when they were ischemic? One possible consequence is that depolarization increases calcium influx through the voltage-sensitive calcium channels that are expressed by retinal pericytes.29 Conversely, hyperpolarization tends to close these channels. To test this idea, we used the calcium indicator fluo-4 to monitor changes in the intracellular calcium concentration \((\text{Ca}^{2+})\) of pericytes located on freshly isolated microvessels. Confocal microscopy permitted selective viewing of fluo-4–loaded pericytes. We detected a PDGF-induced increase in fluo-4 fluorescence in six of nine pericytes sampled under normal metabolic conditions. The typical re-
The influx of calcium during an ischemia-induced depolarization could be reduced by exposure of pericytes to PDGF-BB. Specifically, we observed that the fluo-4 fluorescence of ischemic pericytes decreased by 41% ± 14% (*P < 0.001, *n = 9) during a 3-minute exposure to 1.7 nM PDGF-BB (Fig. 3C). We conclude that the hyperpolarization induced in ischemic pericytes by PDGF-BB causes calcium channels to close and cell calcium levels to decline. Taken together, our experiments show that the metabolic status of a pericyte determines whether PDGF-BB causes a depolarization-induced calcium influx or a hyperpolarization-induced decrease in [Ca\textsuperscript{2+}].

Because changes in intracellular calcium are thought to affect pericyte contractility\textsuperscript{6,10-12} and thereby lumen size,\textsuperscript{11,13} we assessed the effect of PDGF-BB on the diameters of freshly isolated retinal capillaries (Fig. 4). Although these vessels were partially collapsed due to the absence of intraluminal perfusion, we were able to detect changes in lumen diameters as others have done in preparations of nonperfused microvessels.\textsuperscript{11,34,35} Under normal metabolic conditions, we found that PDGF-BB (1.7 nM) induced a 40% ± 12% (*n = 4, *P = 0.018) decrease in lumen diameter within 3 minutes. In contrast, this concentration of PDGF-BB significantly (*P < 0.001) increased the diameters of ischemic microvessels (*n = 4). These constricting and dilating effects were reversible. Thus, when ATP synthesis is compromised, PDGF-BB can serve as a vasodilator. In contrast, this molecule becomes a vasoconstrictor when energy supplies are ample.

**DISCUSSION**

The results show that the response of retinal pericytes to PDGF-BB is metabolically modulated. Under normal metabolic conditions, nonspecific cation and chloride channels were activated, the pericyte membrane potential decreased, cell calcium levels increased, and microvascular lumens narrowed during exposure to PDGF-BB. In contrast, chemical ischemia prevented the activation of the nonspecific cation and chloride channels. Rather, a PDGF-induced activation of K\textsubscript{ATP} channels in ischemic pericytes resulted in a 26-mV hyperpolarization, a decrease in the intracellular calcium concentration, and a widening of the lumens. Thus, in the retinal microvasculature it appears that PDGF-BB may serve as a vasoconstrictor or a vasodilator, depending on the local metabolic status.

Our experiments suggest that the PDGF-induced linkage of pericyte function to metabolism is based on the regulation of the activity of the voltage-sensitive calcium channels that are expressed by pericytes.\textsuperscript{29} Thus, the increase in cell calcium in ischemic pericytes is likely to be due to the opening of voltage-sensitive calcium channels.

**FIGURE 5.** Schematic outline of the dual-action vasoactive effects of PDGF-BB on pericyte-containing microvessels of the retina. As detailed in the text, PDGF-BB causes pericyte membrane voltage to decrease, cell calcium levels to increase, and vessel lumens to constrict when energy supplies are ample. Opposite effects occur when the energy supply is inadequate. Dotted arrows: potentially lethal pathways that may be avoided by exposure to PDGF-BB. This illustration omits the actions of vasoactive molecules other than PDGF-BB. By serving as both a vasoconstrictor and vasodilator, PDGF-BB may enhance the ability of the retinal microvasculature to respond adaptively to physiological and pathophysiological changes. *V\textsubscript{m}*, membrane potential.
expressed by these cells. For example, when PDGF-BB caused pericytes maintained at normal metabolic conditions to depolarize from −55 to −46 mV, the membrane potential entered the “window of current” for these calcium channels. At voltages within this window (i.e., −50 to −20 mV), there is enough depolarization to activate the voltage-sensitive calcium channels, but not enough to cause complete inactivation. However, during exposure of ischemic pericytes to PDGF-BB, the membrane potential exited this window of current as the cell was hyperpolarized from −41 to −67 mV. In agreement with a role for voltage-sensitive calcium channels, we observed that PDGF-BB induced an influx of calcium under normal conditions, but, during chemical ischemia, reversed the nifedipine-sensitive increase in intracellular calcium levels.

A likely response of pericytes to the activation of their voltage-sensitive calcium channels is cellular contraction. This seems to be a reasonable possibility, because pericytes express calcium-sensitive contractile proteins and are capable of contraction, at least in culture. Although PDGF-induced contraction of pericytes remains to be definitively demonstrated, our observations are consistent with a contractile response, in that PDGF-BB caused an increase in pericyte calcium levels and a decrease in the lumen diameter of isolated microvessels maintained under normal metabolic conditions. In contrast, under ischemic conditions, the lumen of a pericyte-containing vessel enlarged as PDGF induced hyperpolarization and caused intracellular calcium levels to decrease.

Because technical challenges appear to preclude an in vivo application of the electrophysiological and imaging methods used in this study, it remains to be demonstrated that the physiological effects of PDGF-BB observed in isolated microvessels also occur in the retina in vivo. In addition, the responses of pericytes to chemical inhibition of ATP synthesis may differ from those caused by ischemia in vivo. Consequently, it remains to be established that endogenously released PDGF-BB modulates pericyte function and thereby lumen diameter and capillary blood flow in vivo under normal and compromised metabolic conditions. In addition, the concentration of PDGF-BB adjacent to pericytes in vivo is unknown. However, this concentration may be substantial, because endothelium-derived molecules are likely to be confined to the relatively small ablumenal space delimited by endothelial cells, pericytes, and the glial cell processes that ensheathe the retinal vasculature. Removal of this glial covering probably contributed to our inability to detect effects of endogenous PDGF-BB in isolated microvessels, because molecules released by the endothelium would be washed away by our perfusate. Despite these limitations, use of microvessels freshly isolated from the rat retina provides a preparation that should facilitate future studies on the mechanisms by which PDGF-BB regulates the function of retinal pericytes.

Based on reports that PDGF-BB is expressed in the vasculature of the adult retina and our findings that this molecule affects pericyte physiology, we postulate that PDGF-BB is an endothelium-to-pericyte signal. However, because it appears that PDGF-BB is not expressed in mature capillaries of the intracranial portion of the brain, the proposed function of locally synthesized PDGF-BB may be unique to the retina. This would be consistent with other specialized features of the retinal vasculature, such as being exclusively autoregulated, having the highest density of pericytes, and showing particular vulnerability to diabetic damage. Our observation that PDGF-BB activates KATP channels in ischemic pericytes raises the theoretical possibility that systemic administration of a KATP channel blocker, such as tolbutamide, may limit the adaptive response of retinal microvessels during periods of metabolic compromise.

In summary, our experiments on isolated pericyte-containing microvessels suggest that PDGF-BB is a dual-action vasoactive signal that may provide a mechanism to couple pericyte function with metabolism in the retina (Fig. 5). The basis for this coupling is the metabolically modulated effects of PDGF-BB on ion channel activity. By this mechanism, changes in metabolism regulate the membrane potential of pericytes and thereby the influx of calcium, which in turn is likely to control pericyte contraction, lumen diameter, and capillary perfusion. Although it remains to be determined in vivo, PDGF-BB may serve as a vasconstrictor when energy supplies are ample and a vasodilator under ischemic conditions. This bifunctional capability facilitates an efficient adjustment of the retinal microcirculation to match local metabolic needs. Future studies may reveal that, in addition to PDGF-BB, there are other dual-action vasoactive molecules that regulate the function of the retinal microvasculature.

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References


