Research Paper

Journal of Vascular Research

J Vasc Res 2012;49:43–49
DOI: 10.1159/000329821

Received: April 8, 2011
Accepted after revision: May 28, 2011
Published online: October 6, 2011

Endothelial-Derived Hyperpolarization Factor (EDHF) Contributes to PlGF-Induced Dilation of Mesenteric Resistance Arteries from Pregnant Rats

Maurizio Mandalà[a, b] Natalia Gokina[a] Carolyn Barron[a] George Osol[a]

[a]Department of Obstetrics, Gynecology and Reproductive Sciences, University of Vermont, College of Medicine, Burlington, Vt., USA; [b]Department of Cell Biology, University of Calabria, Arcavacata di Rende (CS), Italy

Key Words
PlGF · Mesenteric artery · Endothelial-derived hyperpolarization factor · Potassium channels

Abstract
The aim of this study was to investigate the cellular mechanism involved in the potent vasodilatory action of PlGF on mesenteric resistance arteries from pregnant rats. PlGF (3 nM) induced a vasodilation of 64 ± 3.8% that was completely abolished by endothelial denudation. Significant dilation (28 ± 4.0%) remained, however, in the presence of nitric oxide synthase and cyclooxygenase inhibition, and was associated with significant reductions in vascular smooth muscle cell calcium. Absence of dilation in potassium-depolarizing solution (30 mM) confirmed its dependence on endothelial-derived hyperpolarization factor. Subsequent studies established that vasodilation was abolished by pharmacologic inhibition of SK_{Ca} (apamin) and BK_{Ca} (iberiotoxin) but not IK_{Ca} (tram-34) potassium channels. In summary, PlGF acts through the release of a combination of endothelium-derived relaxation factors. Based on the results of potassium channel blockade, we suggest that it induces endothelial hyperpolarization via SK_{Ca} channel activation; this, in turn, leads to the release of a diffusible mediator that activates vascular smooth muscle BK_{Ca} channels, hyperpolarization and vasodilation. This is the first study to identify the mechanism for PlGF/VEGFR-1 resistance artery dilation in the pregnant state, whose attenuation likely contributes to the systemic hypertension characteristic of preeclampsia.

Introduction

PlGF is a member of the VEGF family that acts through the tyrosine kinase receptor VEGFR-1 which can be expressed as either a membrane-bound (Flt-1, Fms-related tyrosine kinase-1) or soluble form (sFlt-1). The placenta is the main source of PlGF during pregnancy, and its plasma concentrations increase more than 8 times above nonpregnant levels [1–3]. Moreover, reduced PlGF concentrations and increased sFlt-1 levels have been reported in the serum of preeclamptic women [4, 5], and overexpression of its soluble receptor (sFlt-1) induces a preeclampsia-like syndrome in rats [6].

The vasodilatory actions of PlGF have been reported in a number of vessel types, including human placental and internal mammary arteries [7, 8], piglet pulmonary

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

Accessible online at:
www.karger.com/jvr

Dr. Maurizio Mandalà
Department of Obstetrics, Gynecology and Reproductive Sciences
University of Vermont, College of Medicine
Burlington, VT 05405 (USA)
Tel. +1 802 656 1206, E-Mail maurizio.mandala@uvm.edu

vessels [9], rat aorta and renal arterioles [6, 10]. Earlier, we found PlGF to be a potent vasodilator of human and rat resistance arteries from several circulations (splanchic, uterine, subcutaneous [11]). The contribution of nitric oxide (NO) was assessed using NOS inhibition with L-NAME, and found to vary substantially with vessel type; for example, NOS inhibition virtually eliminated uterine artery dilation to PlGF, while the effects of L-NAME in mesenteric vessels were negligible.

In view of the prevalence of PlGF in pregnancy, and the accumulating evidence for the attenuation of its signaling contributing to the etiology of preeclampsia, the purpose of this study was to determine the mechanisms involved in its vasodilatory actions on isolated, pressurized third-order mesenteric arteries from pregnant rats by examining its dependence on: (1) the endothelium, (2) endothelial-derived hyperpolarization factor (EDHF) and (3) potassium channel (SK_{Ca}, IK_{Ca} and BK_{Ca}) activation. The splanchnic circulation accounts for approximately 40% of the systemic vascular resistance, and therefore contributes significantly to blood pressure regulation in both normotension and hypertension. Our working hypothesis was that PlGF dilation of mesenteric resistance arteries (MRA) was primarily endothelium dependent and mediated by EDHF through mechanisms linked to potassium channel activation.

Materials and Methods

Isolated Rat Mesenteric Artery Reactivity

Adult (12- to 14-week-old) pregnant Sprague-Dawley rats were purchased from Charles River (Canada) and shipped to the University of Vermont. All procedures were approved by the Institutional Animal Care and Use Committee. Third-order MRA were obtained from late pregnant (LP; day 20/22 of gestation, n = 32) animals following euthanasia with an injection of methohexital sodium (Brevital, 50 mg/kg, intraperitoneally) and decapitation in a small-animal guillotine. Isolated pressurized vessels were used to test the dilatory effects of PlGF. A section of the gut 5–10 cm distal to the pylorus was removed and placed in separate Petri dish containing aerated cold (4°C) physiological salt solution (PSS). Arterial segments (1–2 mm long) were dissected free from connective and adipose tissue and transferred to the chamber of a small-vessel arteriograph. One end of the vessel was tied onto a glass cannula and flushed of any luminal contents by increasing the pressure before securing the distal end onto a second cannula using a servo-null pressure system (Living Systems Instrumentation). All vessels were continuously superfused with aerated (10% O_2, 5% CO_2, 85% N_2) PSS at 37°C and initially pressurized to 50 mm Hg and equilibrated for 45–60 min before beginning experimentation. Lumen diameter was measured by transilluminating each vessel segment and using a video dimension analyzer (Living Systems Instrumentation) in conjunction with data acquisition software (IonOptix Inc.) to continuously record lumen diameter. Following equilibration, all vessels were preconstricted with phenylephrine (0.1–0.7 μM) or potassium (30 mM, only for depolarization experiments) to produce a 40–50% reduction in baseline diameter. Once constriction was achieved and stable for about 10 min, PlGF-2 (mouse; R&D Systems) was added at a concentration of 3×10^{-9} M (an intermediate concentration [11]), and the resulting dilation was recorded. Some arteries were denuded of the endothelium by air perfusion, and the effectiveness of denudation confirmed by the lack of dilation to acetylcholine (10^{-6} M).

Additional experiments using pharmacological blockade of NOS and cyclooxygenase (COX) were carried out using several inhibitors, for example Nω-nitro-L-arginine (L-NNA; 10^{-4} M) + Nω-nitro-L-arginine methyl ester (L-NAME; 10^{-4} M) for NOS and indomethacin (10^{-5} M) for COX. Finally, to better understand the ionic basis for vasodilation, we used several potassium channel inhibitors: apamin (10^{-7} M) for SK_{Ca}, TRAM-34 (10^{-5} M) for IK_{Ca}, charybdotoxin (5×10^{-8} M) for IK_{Ca} and BK_{Ca}, and iberiotoxin (10^{-7} M) for BK_{Ca} channels. Vessels were preincubated with inhibitors for 20 min before pre-constriction with phenylephrine and the addition of PlGF.

Selective Loading of Smooth Muscle Cells with Fura-2 and Measurement of Intracellular $[Ca^{2+}]_i$

Heat-polished glass cannulas were used in all experiments to prevent accidental damage of endothelium during the cannulation procedure and to avoid diffusion of fura-2 to the endothelial layer. Smooth muscle cell (SMC) loading with fura-2 was performed by extraluminal incubation of pressurized (10 mm Hg) mesenteric arteries in fura-2 AM (5 μM) solution at room temperature in the dark for 60 min. Fura-2-loaded arteries were washed 2–3 times, and then continuously superfused with aerated PSS at 37°C. Ratiometric measurements of fura-2 fluorescence from SMC were performed using a photomultiplier system (IonOptix Inc.). Experimental ratios were corrected for background fluorescence taken from each artery before loading with fura-2. Background-corrected ratios of 510-nm emission were obtained at a sampling rate of 5 Hz from arteries alternately excited at 340 and 380 nm. The arterial lumen diameter was simultaneously monitored using the IonWizard acquisition system (IonOptix Inc.).

All experimental protocols were started following an additional 15-min equilibration period at 10 mm Hg to allow intracellular de-esterification of fura-2 AM. SMC $[Ca^{2+}]_i$ was calculated using the following equation [12]: $[Ca^{2+}]_i = K_d\beta(R - R_{min})/(R_{max} - R)$, where R is an experimentally measured ratio (340/380 nm) of fluorescence intensities, R_{min} is a ratio in the absence of $[Ca^{2+}]_i$ and R_{max} is a ratio at Ca^{2+}-saturated fura-2 conditions, β is a ratio of the fluorescence intensities at 380-nm excitation wavelength at R_{min}, and $R_{max} \times R_{min}$, R_{max} and β were determined by an in situ calibration procedure in the presence of ionomycin (10 μM) and nigericin (5 μM). The K_d (the dissociation constant for fura-2) was 282 nM, as determined by in situ titration of Ca^{2+} in fura-2-loaded small arteries [13].

Drugs and Solutions

All chemicals were purchased from Sigma Chemical, including salts for physiological solution, L-NNA, L-NAME, indomethacin, phenylephrine, diltiazem, papaverine, apamin, TRAM-34, charybdotoxin and iberiotoxin. PlGF-2 (mouse) was purchased from R&D Systems.

PSS was composed of (in mM): 119 NaCl, 4.7 KCl, 24.0 $NaHCO_3$, 1.2 $MgSO_4$, 0.023 EDTA, 1.6 $CaCl_2$, 1.2 KH_2PO_4 and 11.0 glucose, pH = 7.4. Ionomycin and nigericin were obtained from Calbiochem. Fura-2-AM and pluronic acid were purchased from Invitrogen. Fura-2-AM was dissolved in dehydrated DMSO as a 1 mM stock solution, frozen in small aliquots and used within 1 week of preparation. For the fura-2 calibration procedure, we used a solution of the following composition: 140 mM KCl, 20 mM NaCl, 5 mM HEPES, 5 mM EGTA, 1 mM $MgCl_2$, 5 μM nigericin and 10 μM ionomycin, pH = 7.1.

Statistical Analysis

Relaxation to PlGF was expressed as percent of maximal diameter, which was determined at the end of each experiment by the addition of a diltiazem (10 μM) + papaverine (100 μM) cocktail. Data are expressed as means ± SEM, where n is the number of arterial segments studied. The n values refer to both number of vessels and number of animals. Differences in responses between groups were determined with one- or two-way ANOVA followed by a post hoc Bonferroni test for repeated-measures analysis. Differences were considered significant at $p \leq 0.05$.

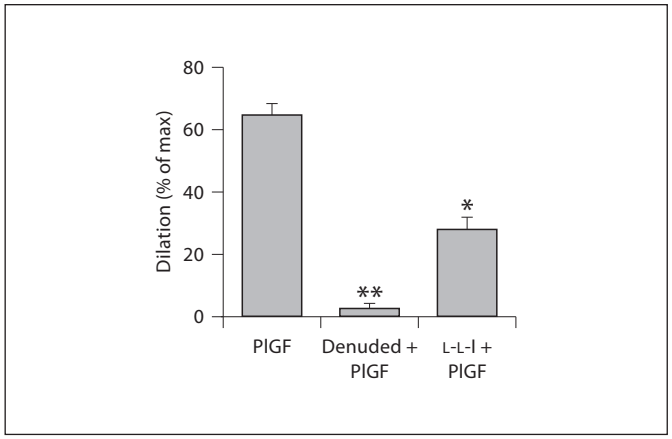

Fig. 1. PlGF vasodilation is endothelium dependent, and significant residual dilation remains in the presence of NOS + COX inhibition: intact and endothelium-denuded MRA were preconstricted with phenylephrine prior to being exposed to PlGF (3 nM). Intact arteries were used in the presence of inhibitors of NOS (L-L = L-NAME + L-NNA; each at 100 μM) and cyclooxygenase (I = indomethacin at 10 μM). Data are reported as means ± SEM; n = 4; * $p < 0.01$, ** $p < 0.001$.

Results

Based on an earlier study [11], a single intermediate concentration of PlGF (3 nM) was used to standardize the vasodilatory stimulus. As shown in figure 1, this concentration consistently produced vasodilation that averaged 64 ± 3.8% of the maximal vasodilation achieved in a relaxing solution containing diltiazem and papaverine.

PlGF-induced vasodilation was closely correlated with a reduction in vascular smooth muscle (VSM) calcium, as seen in the tracing from one vessel shown in figure 2. Note the close association between oscillations in calcium and arterial diameter, as well as the gradual reduction in both average and phasic changes in calcium induced by PlGF over a period of 8–10 min. By comparison, both calcium and diameter responses were much more rapid in response to ACh, with maximal effects observed within a minute of drug application.

In subsequent experiments, the protocol was modified slightly to include a brief (10–15 min) washout period prior to a second stimulation with the same concentration of PlGF (3 nM). As seen in figure 3, the reductions in VSM calcium and the extent of vasodilation were both significantly increased in response to the second application of PlGF. The half-time ($T_{1/2}$) of the vasodilator response was also significantly reduced from 437 ± 41 to 177 ± 20 s (n = 7); by comparison, $T_{1/2}$ for the ACh effect was only 11 ± 1 s (n = 7). Thus, in contrast to the tachyphylaxis commonly observed in response to vasoactive stimuli, pre-exposure to PlGF significantly potentiated its subsequent effect.

PlGF vasodilation was eliminated by endothelial denudation (fig. 1). Moreover, in intact vessels, preincubation with two NOS inhibitors (L-NAME, L-NNA) and a COX inhibitor (indomethacin) only reduced the extent of dilation by half, to an average of 28 ± 4.0% relative to maximal ($p < 0.01$; fig. 1).

To evaluate the involvement of the EDHF in the significant residual response, vessels were preconstricted to a comparable extent (40–50%) with a 30 mM potassium-depolarizing solution instead of phenylephrine, thereby preventing hyperpolarization. As shown in figure 4, in the presence of NOS + COX inhibition, vasodilation to PlGF was completely eliminated, pointing to EDHF/potassium channel involvement.

In the last series of experiments, preconstriction was induced with phenylephrine, and vessels preincubated with potassium channel inhibitors prior to the addition of PlGF. As shown in figure 5, the combination of apamin and charybdotoxin (SK_{Ca} and IK_{Ca}/BK_{Ca} channel inhibitors, respectively), or of apamin or iberiotoxin alone, effectively blocked PlGF vasodilation. In contrast, TRAM-34 (an IK_{Ca} potassium channel inhibitor) was without effect (fig. 5).

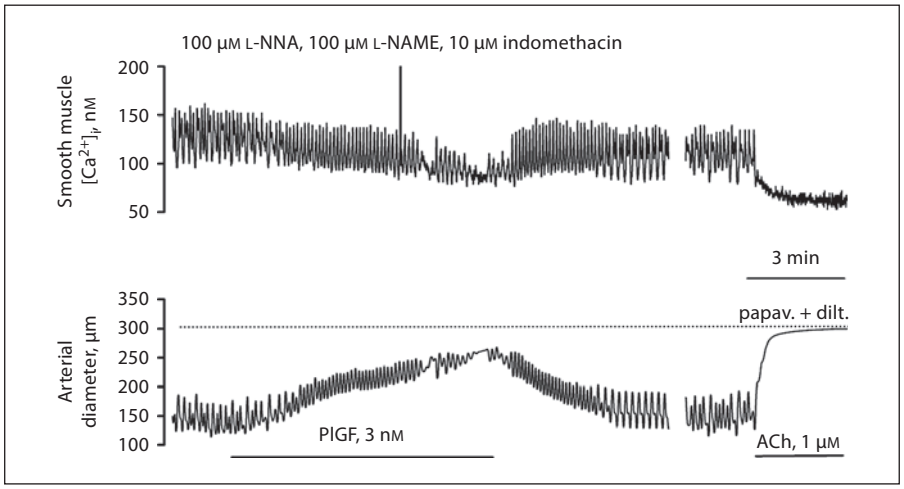

Fig. 2. PlGF vasodilation is associated with a reduction in VSM calcium: original trace demonstrating reductions in SMC $[Ca^{2+}]_i$ and dilation of MRA in response to application of 3 nM PlGF or 1 μM ACh.

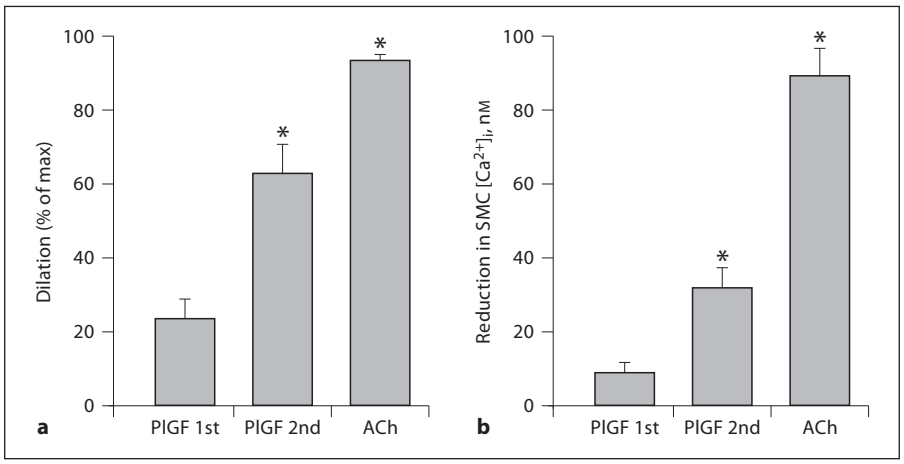

Fig. 3. Effects of repeated PlGF application on arterial VSM calcium and diameter responses: graph summarizing the vasodilatory (**a**) and SMC $[Ca^{2+}]_i$ responses (**b**) of mesenteric arteries to successive application of 3 nM PlGF. A time of 15 min was necessary to wash out the first application of PlGF prior to any readdition. The effects of 1 μM ACh are shown for comparison. Vasodilation is expressed as a percentage of maximal response obtained in papaverine and diltiazem. L-NAME, L-NNA and indomethacin were present throughout all experiments. Data are reported as mean ± SEM, n = 7. * $p < 0.05$.

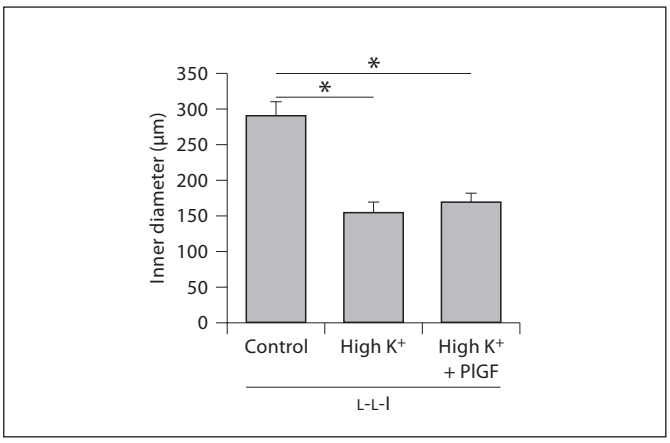

Fig. 4. Complete inhibition of PlGF vasodilation by potassium-induced depolarization: intact MRA were preconstricted with high-potassium (High K^+ = 30 mM) depolarizing solution prior to being exposed to PlGF (3 nM). L-NAME, L-NNA and indomethacin (L-L-I.) were present throughout all experiments. Data are shown as means ± SEM; n = 4; * $p < 0.05$.

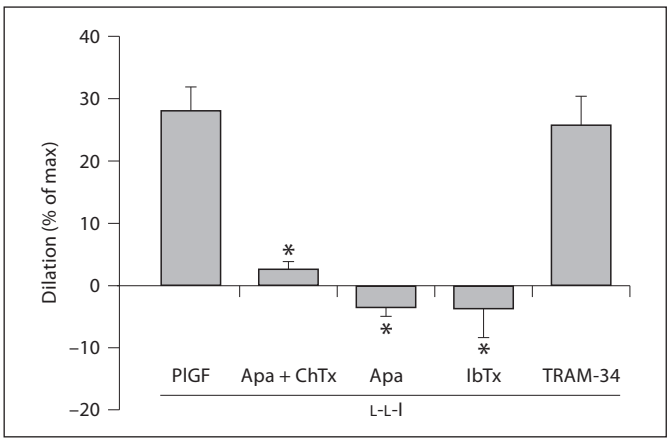

Fig. 5. PlGF vasodilation is potassium channel dependent: intact, Phe-preconstricted MRA were pre-treated with L-NNA + L-NAME (L-L, 100 μM) and indomethacin (I, 10 μM) along with combinations of potassium channel inhibitors: apamin (Apa, for SK_{Ca} channels), charybdotoxin (ChTx, for IK_{Ca} and BK_{Ca} channels), iberiotoxin (IbTx, for Bk_{Ca} channels) and TRAM-34 (for IK_{Ca} channels), prior to the application of PlGF (3 nM). Data are reported as mean ± SEM; n = 4; * $p < 0.001$.

Discussion

The main findings of this study are that (1) PlGF vasodilation of mesenteric arteries from pregnant animals is entirely endothelium dependent, (2) the significant residual vasodilatory component seen in the presence of NOX + COX inhibition is due to an EDHF mechanism, and (3) the signaling pathway involves a combination of SK_{Ca} and BK_{Ca} potassium channel activation that induces a reduction in VSM cytosolic calcium secondary to membrane hyperpolarization, most likely from a diffusible molecule derived from the endothelium.

VEGF vasodilation is mediated mainly by VEGFR-2 through varying mechanisms in different blood vessels [8, 15, 16]. Much less is known about the vasodilatory effects of PlGF, which only binds to the VEGFR-1 receptor [17], whose physiological functions are not well understood. Plasma concentrations of PlGF increase substantially during pregnancy, rising more than 8 times above control levels [1–3].

Excess soluble VEGFR-1 (sFlt-1) is associated with preeclampsia in women, and its overexpression induces a preclampsia-like syndrome in rats [6, 18, 19] that is thought to result from the reduced availability of VEGF and PlGF to tissues. We recently documented a potent vasodilatory effect of PlGF on resistance vessels from women and rats [11], including an effect on uterine vessels from both species that suggests that PlGF may augment uterine blood flow during pregnancy. These observations suggest that loss of PlGF/VEGF vasodilatory influence at the level of the vascular wall may reduce uteroplacental blood flow and impair flow-induced expansive remodeling, as has been observed in the uterine circulation under conditions of systemic NOS inhibition [20].

Because the mesenteric circulation contributes significantly to total peripheral resistance, and in view of reports associating reduced PlGF/VEGF signaling with preeclampsia [4, 5, 21, 22], we were interested in better understanding the action of PlGF on resistance arteries from the gut. The potent vasodilatory effect observed in intact MRA was lost in denuded arterial segments, demonstrating that the endothelium was both necessary and sufficient for its action. Although other studies have documented PlGF vasodilation in other types of vessels [7–9], this is the first proof of its endothelial dependence in resistance vessels, and during pregnancy.

The next series of experiments were aimed at identifying the nature of the endothelial vasodilator-derived factors that mediate the PlGF vasodilation. It is well known that pregnancy increases the expression of both NO and EDHF, the latter especially in resistance arteries [23–27]. Here, we used an intermediate concentration of PlGF (3 nM) to examine the dilatory mechanism. For reference, physiological concentrations of free PlGF in pregnant women are approximately 30 nM (500 pg/ml).

Approximately half of the overall effect remained in the presence of NOS + COX inhibition, pointing to a significant EDHF component. Complete inhibition of vasodilation by preconstriction with potassium-depolarizing solution was confirmatory in this regard.

The involvement of VSM calcium handling mechanisms can be seen in the fura-2 data, which show the close correlation between oscillations in intracellular calcium and vessel diameter, exhibited as vasomotion. Application of PlGF produced a slow decline in calcium concentrations and a gradual vasodilation that took almost 10 min to stabilize. Conversely, the action of ACh was swift, with full dilation in 30–60 s.

These kinetics likely reflect differences between tyrosine kinase and muscarinic receptor pathway activation. Interestingly, in contrast to the tachyphylaxis often seen in response to vasoactive stimuli in vitro [28], a second application of PlGF produced a greater reduction in VSM calcium and larger vasodilation. VEGF and PlGF signaling is thought to involve receptor dimerization [29], and it is possible that the initial exposure to PlGF facilitated subsequent responses via this mechanism (i.e. stimulated the formation of homodimers, and their associated submembrane signaling linkages that increase both the speed and the amplitude of the vasodilatory response to repeated stimulation). This hypothesis, while speculative, is supported by the fact that the half-time to maximal response was reduced >60% in response to the second stimulus. Although cross-talk and interactive signaling have been described for PlGF/VEGF receptors [30], it is not known whether these mechanisms pertain to vasodilatory responses.

Although the number of candidate molecules for EDHF is growing [31–33], its identity is still not established and may differ in vessel types [34]. On the other hand, it is generally accepted that endothelial SK_{Ca} and IK_{Ca} channels play a pivotal role in mediating EDHF effects in many microcirculatory vascular beds [25, 35–37].

A determination of the cellular distribution of potassium channel subtypes in MRA was beyond the scope of this study. Several recent reports [38, 39] support the localization of SK_{Ca} channels on the endothelium. The combined observations of PlGF dilation being completely endothelium dependent and of its action being abolished by apamin strongly suggests a linkage between the

VEGFR-1 receptor and the SK_{Ca} potassium channel subtype in the endothelium. Similarly, the effects of iberiotoxin point to a role for BK_{Ca} channels, whose localization is thought to be primarily on VSM rather than endothelium [38, 39]. Based on these results, we suggest that PlGF vasodilation of MRA involves its binding to the VEGFR-1 receptor on the endothelium and an activation of SK_{Ca} channels that stimulate endothelial cell hyperpolarization. The effects of iberiotoxin may be interpreted in support of the EDHF being, in this case, a diffusible molecule that activates VSM BK_{Ca} channels, leading to hyperpolarization, a reduction in cytosolic calcium and vasodilation [39].

From a pathophysiological perspective, PlGF signaling is of clinical interest, since a number of studies have shown that its attenuation leads to preeclampsia in women. Additional studies have confirmed excess soluble VEGR-1 (sFlt-1) as well as reduced free PlGF (and VEGF) levels in preeclamptics, and have correlated the magnitude of changes with severity of hypertension [6].

The signaling pathways that result from VEGFR-1 activation in the vascular wall as well as their intracellular linkages are not well understood; in some cases, it has been viewed as a decoy receptor with little physiological significance [40, 41]. In contrast, the results of this study point to an important vasoactive role for endothelial VEGFR-1 (Flt-1) during pregnancy, and one that has a significant EDHF component. In view of the upregulation of EDHF signaling noted in several studies on resistance arteries [21, 26], and of the potency of PlGF as a vasodilatory stimulus, we believe that VEGFR-1 (Flt-1 rather than sFlt-1) signaling within the vascular wall deserves closer investigation with regard to its potential to influence both systemic blood pressure and regional (for example, uteroplacental) blood flow in the pregnant state.

Acknowledgment

This study was supported by NIH HL79253 (G.O.).

References

1 Molvarec A, Szarka A, Walentin S, Szucs E, Nagy B, Rigo J Jr: Circulating angiogenic factors determined by electrochemiluminescence immunoassay in relation to the clinical features and laboratory parameters in women with pre-eclampsia. Hypertens Res 2010;33:892–898.

2 Jeevaratnam K, Nadarajah VD, Judson JP, Nalliah S, Abdullah MF: Periodic assessment of Plasma sFlt-1 and PlGF concentrations and its association with placental morphometry in gestational hypertension (GH) – a prospective follow-up study. BMC Pregnancy Childbirth 2010;10:58.

3 Woods AK, Hoffmann DS, Weydert CJ, Butler SD, Zhou Y, Sharma RV, Davisson RL: Adenoviral delivery of VEGF121 early in pregnancy prevents spontaneous development of preeclampsia in BPH/5 mice. Hypertension 2011;57:94–102.

4 Brownbill P, Mills TA, Soydemir DF, Sibley CP: Vasoactivity to and endogenous release of vascular endothelial growth factor in the in vitro perfused human Placental lobule from Pregnancies complicated by pre-eclampsia. Placenta 2008;29:950–955.

5 Savvidou MD, Noori M, Anderson JM, Hingorani AD, Nicolaides KH: Maternal endothelial function and serum concentrations of placental growth factor and soluble endoglin in women with abnormal placentation. Ultrasound Obstet Gynecol 2008;32:871–876.

6 Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP, Karumanchi SA: Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelail dysfunction, hypertension, and protenuria in preeclampsia. J Clin Invest 2003;111:649–658.

7 Szukiewicz D, Szewczyk G, Watroba M, Kurowska E, Maslinski S: Isolated placental vessel response to vascular endothelial growth factor and placental growth factor in normal and growth-restricted pregnancy. Gynecol Obstet Invest 2005;59:102–107.

8 Wei W, Jin H, Chen ZW, Zioncheck TF, Yim AP, He GW: Vascular endothelial growth factor-induced nitric oxide- and PGI2- dependent relaxation in human internal mammary arteries: a comparative study with KDR and Flt-1 selective mutants. J Cardiovasc Pharmacol 2004;44:615–621.

9 Janvier A, Nadeau S, Baribeau J, Perreault T: Role of vascular endothelial growth factor receptor 1 and vascular endothelial growth factor receptor 2 in the vasodilator response to vascular endothelial growth factor in the neonatal piglet lung. Crit Care Med 2005;33:860–866.

10 Parenti A, Brogelli L, Filippi S, Donnini S, Ledda F: Effect of hypoxia and endothelial loss on vascular smooth muscle cell responsiveness to VEGF-A: role of flt-1/VEGF-receptor-1. Cardiovasc Res 2002;55:201–212.

11 Osol G, Celia G, Gokina N, Barron C, Chien E, Mandala M, Liksha L, Kublickiene K: Placental growth factor is a potent vasodilator of rat and human resistance arteries. Am J Physiol Heart Circ Physiol 2008;294:H1381–H1387.

12 Grynkiewicz G, Poenie M, Tsien RY: A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J Biol Chem 1985;260:3440–3450.

13 Knot HJ, Nelson MT: Regulation of arterial diameter and wall $[Ca^{2+}]$ in cerebral arteries of rat by membrane potential and intravascular pressure. J Physiol (Lond) 1998;508:199–209.

14 Neufeld G, Cohen T, Gengrinovitch S, Poltorak Z: Vascular endothelial growth factor (VEGF) and its receptors. FASEB J 1999;13:9–22.

15 Grummer MA, Sullivan JA, Magness RR, Bird IM: VEGF acts through novel, pregnancy-enhanced receptor signaling pathways to stimulate endothelial nitric oxide synthase activity in uterine artery endothelial cells. Biochem J 2009;417:501–511.

16 Brownbill P, McKeeman GC, Brockelsby JC, Crocker IP, Sibley CP: Vasoactive and permeability effects of vascular endothelial growth factor-165 in the term in vitro dually perfused human placental lobule. Endocrinology 2007;148:4734–4744.

17 Park JE, Chen HH, Winer J, Houck KA, Ferrara N: Placenta growth factor. Potentiation of vascular endothelial growth factor bioactivity, in vitro and in vivo, and high affinity binding to Flt-1 but not to Flk-1/KDR. J Biol Chem 1994;269:25646–25654.
18 Sugimoto H, Hamano Y, Charytan D, Cosgrove D, Kieran M, Sudhakar A, Kalluri R: Neutralization of circulating VEGF by anti-VEGF antibodies and sFlt-1 induces proteinuria. J Biol Chem 2003;278:12605–12608.
19 Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai BM, Sukhatme VP, Karumanchi SA: Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med 2004;350:672–683.
20 Osol G, Barron C, Gokina N, Mandala M: Inhibition of nitric oxide synthases abrogates pregnancy-induced uterine vascular expansive remodeling. J Vasc Res 2009;46:478–486.
21 Lyall F, Greer IA, Boswell F, Fleming R: Suppression of serum vascular endothelial growth factor immunoreactivity in normal pregnancy and in pre-eclampsia. Br J Obstet Gynaecol 1997;104:223–228.
22 Molvarec A, Ito M, Shima T, Yoneda S, Toldi G, Stenczer B, Vásárhelyi B, Rigó J Jr, Saito S: Decreased proportion of peripheral blood vascular endothelial growth factor-expressing T and natural killer cells in preeclampsia. Am J Obstet Gynaecol 2010;203:567.e1–e8.
23 Burger NZ, Kuzina OY, Osol G, Gokina NI: Estrogen replacement enhances EDHF-mediated vasodilation of mesenteric and uterine resistance arteries: role of endothelial cell Ca^{2+}. Am J Physiol Endocrinol Metab 2009;296:E503–E512.
24 Kenny LC, Baker PN, Kendall DA, Randall MD, Dunn WR: Differential mechanisms of endothelium-dependent vasodilator responses in human myometrial small arteries in normal pregnancy and pre-eclampsia. Cli Sci (lond) 2002;103:67–73.
25 Gokina NI, Kuzina OY, Vance AM: Augmented EDHF signaling in rat uteroplacental vasculature during late pregnancy. Am J Physiol 2010;299:H1642–H1652.
26 Gerber RT, Anwar MA, Poston L: Enhanced acetylcholine induced relaxation in small mesenteric arteries from pregnant rats: an important role for endothelium-derived hyperpolarizing factor (EDHF). Br J Pharmacol 1998;125:455–460.
27 Luksha L, Agewall S, Kublickiene K: Endothelium-derived hyperpolarizing factor in vascular physiology and cardiovascular disease. Atherosclerosis 2009;202:330–344.
28 Benedito S, Prieto D, Nielsen PJ, Nyborg NC: Histamine induces endothelium-dependent relaxation of bovine retinal arteries. Invest Ophthalm Vis Sci 1991;32:32–38.
29 Rahimi N: VEGFR-1 and VEGFR-2: two non-identical twins with a unique physiognomy. Front Biosci 2006;11:818–829.
30 Autiero M, Waltenberger J, Communi D, Kranz A, Moons L, Lambrechts D, Kroll J, Plaisance S, De Mol M, bono F, Kliche S, Fellbrich G, Ballmerhofer K, Maglione D, Mayr-Beyrle U, Dewerchin M, Dombrowski S, Stanimirovic D, Van Hummelen P, Dehio C, Hicklin DJ, Persico G, Herbert JM, Communi D, Shibuya M, Collen D, Conway EM, Carmeliet P: Role of PlGF in the intra-and intermolecular cross talk between the VEGF receptors Flt1 and Flk1. Nat Med 2003;9:936–943.
31 Hecker M, Bara AT, Bauersachs J, Busse R: Characterization of EDHF as a cytochrome P450-derived arachidonic acid metabolite in mammals. J Physiol 1994;481:407–414.
32 Sandow SL, Tare M, Coleman HA, Hill CE, Parkington HC: Involvement of myoendothelial gap junctions in the actions of endothelium-derived hyperpolarizing factor. Circ Res 2002;90:1108–1113.
33 Edward G, Dora KA, Gardener MJ, Garland CJ, Weston AH: K^+ is an endothelium-derived hyperpolarizing factor in rat arteries. Nature 1998;396:269–272.
34 McNeish AJ, Nelli S, Wilson WS, Dowell FJ, Martin W: Differential effects of ascorbate on EDHF-mediated vasodilation in the bovine ciliary vascular bed and coronary artery. Br J Pharmacol 2003;138:1172–1180.
35 Busse R, Edwards G, Feletou M, Fleming I, Vanhoutte PM, Weston AH: EDHF: bringing the concepts together. Trends Pharmacol Sci 2002;23:374–380.
36 Feletou M, Vanhoutte PM: EDHF: an update. Clin Sci (Lond) 2009;117:139–155.
37 Grgic I, Kaistha BP, Hoyer J, Kohler R: Endothelial Ca^{2+}-activated K^+ channels in normal and impaired EDHF-dilator responses-relevance to cardiovascular pathologies and drug discovery. Br J Pharmacol 2009;157:509–526.
38 Jackson W: Potassium channels in the peripheral microcirculation. Microcirculation 2005;12:113–127.
39 Feletou M: Calcium-activated potassium channels and endothelial dysfunction: therapeutic options? Br J Pharmacol 2009;156:545–562.
40 Meyer RD, Rahimi N: Comparative structure-function analysis of VEGFR-1 and VEGFR-2: What have we learned from chimeric systems? Ann NY Acad Sci 2003;995:200–207.
41 Cunningham SA, Tran TM, Arrate MP, Bjercke R, Brock TA: KDR activation is crucial for VEGF165-mediated Ca^{2+} mobilization in human umbilical vein endothelial cells. Am J Physiol 1999;276:C176–C181.