HYPERTENSION IN PREGNANCY

Hypertension in Pregnancy

ISSN: 1064-1955 (Print) 1525-6065 (Online) Journal homepage: http://www.tandfonline.com/loi/ihip20

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To cite this article: Tijana Drobnjak, Sveinbjörn Gizurarson, Natalia I. Gokina, Hamutal Meiri, Maurizio Mandalá, Berthold Huppertz & George Osol (2017) Placental protein 13 (PP13)-induced vasodilation of resistance arteries from pregnant and nonpregnant rats occurs via endothelial-signaling pathways, Hypertension in Pregnancy, 36:2, 186-195, DOI: 10.1080/10641955.2017.1295052

To link to this article: http://dx.doi.org/10.1080/10641955.2017.1295052

Published online: 19 May 2017.

HYPERTENSION IN PREGNANCY
2017, VOL. 36, NO. 3, 186–195
https://doi.org/10.1080/10641955.2017.1295052

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Placental protein 13 (PP13)-induced vasodilation of resistance arteries from pregnant and nonpregnant rats occurs via endothelial-signaling pathways

Tijana Drobnjak[a], Sveinbjörn Gizurarson[a], Natalia I. Gokina[b], Hamutal Meiri[c], Maurizio Mandalá[d], Berthold Huppertz[e], and George Osol[b]

[a]Faculty of Pharmaceutical Sciences, School of Health Science, University of Iceland, Reykjavik, Iceland; [b]Department of Obstetrics, Gynecology and Reproductive Sciences, University of Vermont, Burlington, Vermont, USA; [c]Hy Laboratories, Rehovot, and TeleMarpe, Tel Aviv, Israel; [d]Department of Biology, Ecology and Earth Sciences, University of Calabria, Rende, Italy; [e]Institute of Cell Biology, Histology and Embryology, Medical University of Graz, Graz, Austria

ABSTRACT

Placental protein 13 (PP13) induces hypotension in rats. This study aims to evaluate PP13 effects on isolated uterine arteries from nonpregnant and mid-pregnant rats. Vessels were isolated, cannulated, and pressurized to 50 mmHg within an arteriograph, preconstricted and exposed to increasing PP13 concentrations (10^{-13}–10^{-8} M). PP13 elicited 38–50% arterial vasodilation with half-maximum response (EC_{50}) = 1 pM. The relaxation was mediated by activating the endothelial-signaling pathways of prostaglandin and nitric oxide (NO). Accordingly, these results encourage evaluation of PP13 as a possible therapy for gestational diseases characterized by insufficient uteroplacental blood flow and/or maternal hypertension.

ARTICLE HISTORY
Received 19 November 2016
Revised 2 January 2017
Accepted 8 February 2017

KEYWORDS
eNOS; prostacyclin (PGI_2); uterine vasculature; endothelial layers; preeclampsia

Introduction

During mammalian pregnancy, the uterine circulation undergoes significant and expansive remodeling necessary for normal pregnancy outcome. Systemic and uterine vascular resistance decreases with advancing gestational age, which makes structural adaptation of the maternal vasculature, especially the uteroplacental vasculature, mandatory for accommodating the changes, and ensuring appropriate blood supply for normal fetal growth. Placental protein 13 (PP13) is a member of the galectin family. There are several galectins (like galectins 1, 3, 4) that have very broad tissue and species expression. However, PP13 is quite a nonconventional galectin, as it is only expressed in the placenta of humans and some anthropoid apes with deep placentation and long gestation. PP13 was found to confer immunoregulatory functions to enable deep placentation and it is galectins with putative immune and membrane (carbohydrate tethering) effects (1). Several studies in humans have shown that low PP13 levels early in human pregnancy are associated with the subsequent development of preeclampsia, suggesting its possible utility as a biomarker for predicting the disorder (2, 3). Its administration to rats induces hypotension, and a vasodilatory effect on isolated small arteries was recently reported by Gizurarson et al. (4, 5).

The intent of this follow-up study was to further elucidate the cellular and molecular mechanism of action of PP13 on resistance-sized arteries. Specifically, we were interested in (1) determining whether there was any regional specificity in its action on uterine versus systemic (mesenteric) vessels in terms of vasodilator reactivity (efficacy, sensitivity), (2) identifying its principal cellular target (s) within the vascular wall (endothelium, vascular smooth muscle), and (3) understanding the molecular signaling responsible for induction of PP13-induced vasodilation.

Materials and methods

Animals and ethics approvals

The animal studies were approved by the Institutional Animal Care and Use Committee at the University of Vermont, USA, and carried out in accordance with the US NIH guidelines for the care and use of laboratory animals (6). Feed and water were provided *ad libitum*. All efforts were undertaken according to the "3R principles" (www.nc3rs.org.uk) to reduce the number of animals used in this study and optimize experimental protocols for obtaining maximum data from each tested animal. Pregnant and age-matched nonpregnant female Sprague-Dawley rats (12–14 weeks of age) were

CONTACT Sveinbjörn Gizurarson sveinbj@hi.is Faculty of Pharmaceutical Sciences, School of Health Science, University of Iceland, Hofsvallagata 53, 107 Reykjavik, Iceland.

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purchased from Charles River Laboratories International (St-Constant, Quebec, CA) and housed at the animal care facility at the University of Vermont, College of Medicine, Burlington, VT, USA. The animals were allowed to acclimatize for at least 72 h prior to use, and pregnant animals were used during mid-pregnancy (day 15 of a 22-day gestation).

Vessel preparation

Uterine arcuate arteries (UAAs) and third-order mesenteric arteries (MAs) having similar resting diameters (150–250 μm) were dissected free of surrounding adipose and connective tissue, cannulated in the chamber of a small artery arteriograph (Instrumentation and Model Facility, University of Vermont, Burlington, VT, USA), and pressurized to 50 mmHg using a pressure-servo system (Living Systems Instrumentation, St. Albans City, VT). Lumen diameters were measured under a microscope (Zeiss, Jena, Germany) by using a video-dimension analyzer (Living Systems Instrumentation, St Albans City, VT, USA) and recorded on WinDaq software, DATAQ Instruments.

In some vessels, removal of endothelium was accomplished via intraluminal air perfusion and confirmed by a complete loss of acetylcholine (ACh 1 μM)-induced dilation in vessels pre-constricted with phenylephrine (Phe 0.1–0.3 mM).

Evaluation of vasodilator reactivity

Vessels were equilibrated for 40 min in oxygenated physiological salt solution (PSS) at 37°C and pH 7.4. All experiments were performed at an intraluminal pressure of 50 mmHg, a pressure that approximates *in vivo* conditions (7). Arteries were pre-constricted by the addition of Phe or a synthetic thromboxane analog (U46619) to the superfusate at concentrations sufficient to produce a 40–60% reduction in lumen diameter, as described in earlier studies (8). Once constriction stabilized, PP13 was added to the superfusate in increasing concentrations (10^{-13}–10^{-8} M), and the resulting changes in diameter were recorded, allowing sufficient time (typically 10–15 min) for diameter to stabilize at each concentration. The preconstriction with Phe was allowed to stabilize for at least 15–20 min prior to initiating the PP13 concentration response. In cases when this stabilization was difficult to achieve, Phe was washed out and the synthetic thromboxane analog U46619 used instead. The level of preconstriction was 50% on the average for either agonist.

Vascular reactivity was evaluated by determining both sensitivity and efficacy: *sensitivity* was defined as the concentration of PP13 required to produce half-maximal dilation (EC_{50}) and *efficacy* was defined as the maximal extent of dilation induced by PP13 relative to complete dilation. The latter was determined at the end of each experiment in a solution containing a mixture of a phosphodiesterase inhibitor (papaverine, 100 μM) and an L-type Ca^{2+} channel blocker (diltiazem, 1 μM).

Nitric oxide and prostaglandin involvement in PP13-induced vasodilation

UA were pretreated for 30 min with a combination of Nω-nitro-L-arginine (L-NNA) (100 μM) and Nω-nitro-L-arginine-methyl-ester (L-NAME) (100 μM) to inhibit NO production before PP13 was administered. The combination of inhibitors has been shown to be more effective than either alone for inhibitiing NO generation (9). To inhibit the production of prostaglandins (PGs), arteries were pretreated with 10 μM indomethacin (a cyclooxygenase [COX] 1/2 inhibitor) for 30 min prior to Phe administration. A prostacyclin (IP) receptor inhibitor, RO1138452, was used at a concentration of 10 μM to block the downstream effects of PGI_2 production (10).

Endothelial cell calcium measurements

The role of PP13 in calcium mobilization was previously presented in BeWo cells and in cultured trophoblasts (11–13). To examine the PP13 effect on endothelial cell (EC) cytosolic calcium levels, UA ECs were loaded with Ca^{2+} sensitive dye fura-2 by intraluminal perfusion with 5 μM fura-2-AM-containing solution for 5 min at room temperature, as previously described (14, 15). To remove excess fura-2, the vessels were additionally perfused with PSS for 10 min at 20 mmHg intraluminal pressure. Measurement of EC fura-2 fluorescence was performed using a photomultiplier system (IonOptix Inc., Milton, MA, USA). Background fluorescence was defined for each artery before loading with fura-2-AM. The background-corrected ratio of 510 nm emission was obtained at a sampling rate of 5 Hz from arteries alternately excited at 340 and 380 nm. After dye loading, the vessels were superfused for 10 min with PSS containing 10^{-8} M PP13 (maximally effective concentration for eliciting vasodilation). In these experiments, PP13 was tested without pre-constriction with Phe, and at an intraluminal pressure of 50 mmHg to eliminate movement artifact. Finally, ACh (10 μM) was applied as a positive control, as it is known to elicit rapid, significant increases in calcium (16).

Intracellular endothelial calcium ($[Ca^{2+}]_i$) of the EC was calculated using the following equation:

$$[Ca^{2+}]_i = K_d \beta \frac{R - R_{min}}{R_{max} - R}$$

R is the experimentally measured ratio (340/380 nm) of fluorescence intensities (14, 17), R_{min} is the ratio when $[Ca^{2+}]_i$ is not present, and R_{max} is the 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}. R_{min}, R_{max}, and β were determined by an *in situ* calibration procedure from the arteries treated with the ionophores ionomycin (10 μM) and nigericin (5 μM) (14).

Solutions and drugs

The PSS contained 119 mM NaCl, 4.7 mM KCl, 24.0 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 1.6 mM $CaCl_2$, 1.2 mM $MgSO_4$, 0.023 mM EDTA, and 11.0 mM glucose, pH = 7.4. 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. Fura-2-AM and pluronic acid were purchased from Invitrogen (Carlsbad, CA); ionomycin and nigericin were obtained from Calbiochem (La Jolla, CA, USA).

Placental protein 13

Recombinant PP13 was prepared by Hy-Laboratories (Hylabs, Rehovot, Israel) without a histidine-tag from the clone of the constructed recombinant wild type PP13 construct. The sequence of the PP13 construct was validated by Sanger sequencing according to the published National Center for Biotechnology Information (USA) website (http://www.ncbi.nlm.nih.gov/), and transfected and expressed in *Escherichia coli*. The protein was harvested and affinity-purified, and the molecular weight and purity were verified by SDS-PAGE, HPLC, and immunoblots with PP13-specific monoclonal antibodies. The concentration was determined using a PP13 ELISA as previously described (18).

Statistical analysis

The data are presented as mean ± SEM. The efficacy of PP13 (% dilation relative to complete dilation induced by a relaxing solution papaverine/diltiazem) was obtained by adding PP13 until no further dilation could be observed. Sensitivity (EC_{50} values) was obtained using sigmoid logistic curves (Prism, v.6; Graph Pad Software, San Diego, CA, USA), and extrapolating the concentration of drug required to produce half-maximal relaxation in each vessel. Differences in responses to PP13 in denuded vessels and control vessels were determined by an unpaired *t*-test. Two-way repeated measurements ANOVA were followed by the Holm–Sidak approach to multiple comparisons. p Values ≤0.05 were considered significantly different.

Results

Arterial dimensions and vasodilatory effects of PP13

At a transmural pressure of 50 mmHg, initial lumen diameters of UAA from nonpregnant rats were 172 ± 21.7 μm (n = 5) versus 230 ± 17.0 μm (n = 9) in mid-pregnant animals (p = 0.002); lumen diameters of MA were similar in nonpregnant versus mid-pregnant animals, averaging 213 ± 17.8 versus 211 ± 22.6 μm, respectively (p = 0.26). The former reflects the expansive remodeling characteristic of maternal uterine vascular adaptation during gestation, as documented in earlier studies (19, 20).

Following approximately half-maximal (40–60%) preconstriction with Phe or U46619, the addition of PP13 in increasing concentrations (10^{-13}–10^{-8} M) induced progressive vasodilation in all intact vessels. An example showing the diameter and time pattern of a UAA response to one concentration (10^{-9} M) of PP13 is shown in Figure 1.

Concentration–response curves are depicted in Figures 2 and 3. Efficacies were 45 ± 4.8% and 51 ± 7.0%, respectively, in UA and MA from nonpregnant rats; in mid-pregnant animals, they were 50 ± 5.9% (UAA) and 38 ± 5.9% (MA). There were no significant differences in efficacy between vessel types (p = 0.76) or groups (p = 0.25). EC_{50} values were also not different between vessel types (p = 0.98), or treatment groups (p = 0.56), and ranged from 0.035 to 0.063 PM.

Dilation to the highest concentration of PP13 (10^{-8} M) was seen in UAA (Figure 2) from mid-pregnant animals (p < 0.05) with a similar trend (p > 0.05) evident in MA (Figure 3). In view of PP13 having potential therapeutic promise in bolstering uteroplacental perfusion, the remaining experiments were restricted to UAA from mid-pregnant animals.

The role of the endothelium in uterine artery relaxation

Vasodilation to PP13 was completely abolished by endothelial denudation (data not shown), indicating that the endothelium (rather than vascular smooth muscle) is its sole target. Since PP13 was administered in the superfusate bathing the vessel (rather than perfusate), this effect cannot be attributed to an inability of PP13 to access smooth muscle, as could be the case *in vivo*. To better define the

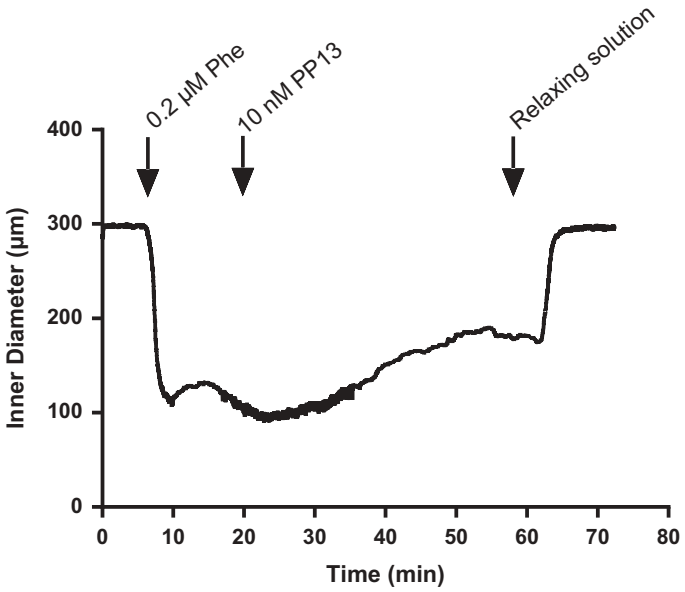

Figure 1. Reactivity of a pressurized rat uterine arcute artery to PP13. Tracing of a concentration–response to PP13 in a single uterine arcuate MP artery. Note initial constriction to Phe, and dilation to a single 10 nM concentration of PP13, prior to inducing complete vasodilation with a relaxing solution containing diltiazem and papaverine. The pressure was kept constant at 50 mmHg, and the recording has been made over 2 h and 5 min (7412 s).

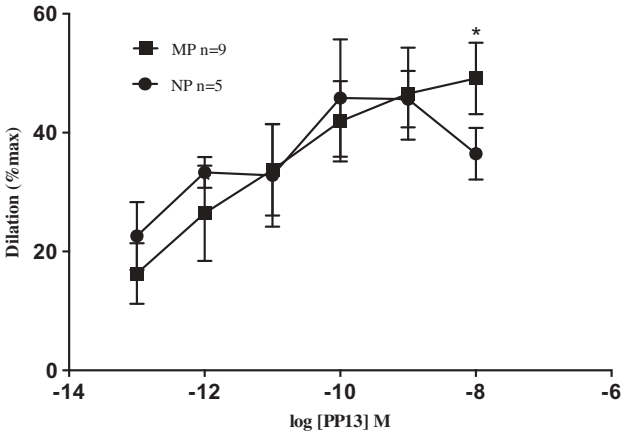

Figure 2. Concentration-response of isolated pressurized uterine arcuate arteries from mid-pregnant (MP) and nonpregnant (NP) rats to PP13. Responses were similar between treatment groups; the only difference was noted at 10^{-8} M PP13 due to constriction of vessels from NP animals. Data are reported as mean ± SEM, n = number of experiments, *$p < 0.05$.

endothelial signals responsible for PP13-induced vasodilation, we next evaluated the effects of NOS and COX 1/2 inhibition with L-NAME/L-NNA (0.2 mM) and indomethacin (INDO) (10 µM), respectively, on PP13-induced vasodilation.

Effects of blocking NOS and COX pathways

Combined pretreatment with NOS and COX 1/2 inhibitors completely abolished PP13-dependent relaxation ($p < 0.001$). Pretreatment with either INDO or L-N^G-nitroarginine methyl ester (L-NAME)/N^G-nitro-L-arginine (L-NNA) had intermediate effects, suppressing the maximal vasodilatory effect by 28% and 34%, respectively (Figure 4). Due to the significant effect of INDO, we hypothesized that PP13 vasodilation was mediated by signaling through the IP prostacyclin (PGI_2) receptor.

Effects of IP-receptor inhibition with RO1138452

UAA were preincubated for 30 min in a 10 µM solution of the specific IP receptor antagonist RO1138452. Compared with the control group, no effect was observed on the dilation caused by the application of PP13 (Figure 5). These findings do not support the hypothesis that PP13 causes dilation through the IP prostanoid receptors.

Endothelial Ca^{2+} measurement

As depicted in Figure 6, there were no detectable changes in endothelial Ca^{2+} in response to application of 10 mM PP13 ($n = 3$). However, a significant rise of endothelial Ca^{2+} was observed after subsequent administration of ACh that was used as a positive control.

Discussion

The major findings of this study are as follows: (1) PP13 is a potent vasodilator of resistance arteries

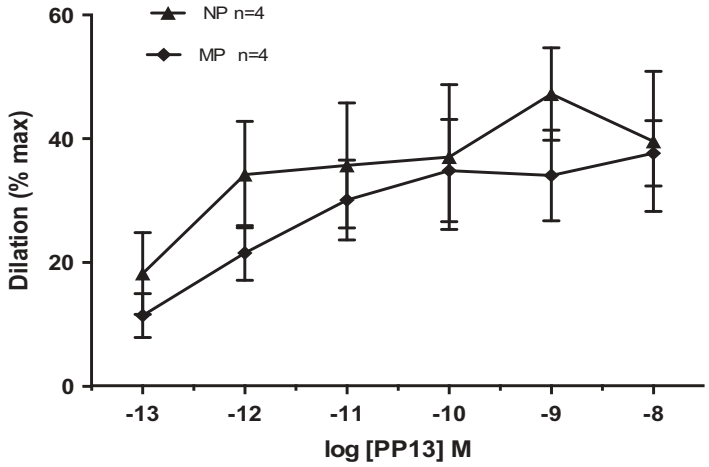

Figure 3. Concentration–response curve of PP13 effect on uterine mesentery artery in nonpregnant and mid-pregnant rats. PP13 induces vasodilation of third-order mesenteric arteries from mid-pregnant (MP) and nonpregnant (NP) rats. Data are reported as mean ± SEM, *n* = number of experiments.

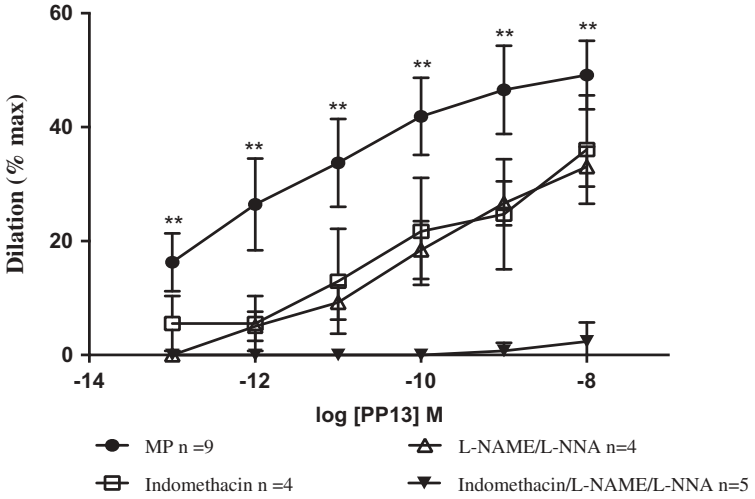

Figure 4. Effects of NOS and/or COX 1/2 inhibition on PP13-induced vasodilation of uterine arcuate arteries from mid-pregnant rats. Vasodilation was measured relative to untreated vessels. The vasodilation was significantly reduced by pretreatment with indomethacin (10 μM) or L-NAME/L-NNA (2 × 100 μM), administered separately or in combination. Data are reported as mean ± SEM, *n* = number of experiments, *$p < 0.001$.

from both the uterine and systemic (mesenteric) circulations with similar efficacy and sensitivity, (2) gestation does not significantly modify its effect, (3) the vasodilatory action of PP13 in uterine vessels is endothelium-dependent, and (4) effected by a combination of eNOS (NO) and COX 1/2 (prostanoid)-derived products that play an important role in inducing the effects. Finally, (5) PP13 vasodilation does not involve the prostacyclin (IP) receptor and (6) is not associated with an overall increase in EC calcium.

While hypertension is a major symptom of preeclampsia, women who subsequently develop preeclampsia already have an approximately 10 mmHg increase in mean arterial pressure during the first trimester, long before the development of clinical symptoms (21, 22). Vasodilation is considered to be one of the early hallmarks of pregnancy, a condition associated with increased levels of both prostacyclin and NO regulating systemic and, specifically uteroplacental blood flow (23, 24). Our previously published studies have shown that intraperitoneal administration of PP13 lowers blood pressure in pregnant rats (5, 25). The present results indicate that this effect may be due to a systemic (nonspecific) peripheral vasodilatory influence. Based on its ability to dilate mesenteric resistance arteries in rats, PP13 could contribute to blood pressure reduction that normally occurs during human pregnancy.

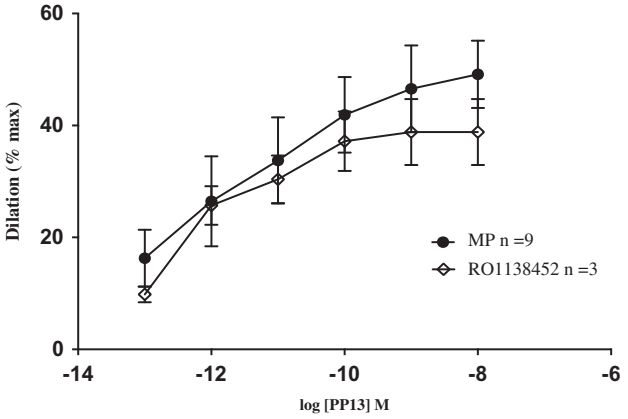

Figure 5. Vasodilation by PP13 with IP receptor inhibition. IP receptor inhibition with RO1138452 (10 μM) did not affect PP13 vasodilation on uterine arcuate arteries from mid-pregnant rats. Data are reported as mean ± SEM, *n* = number of experiments.

Unlike other galectins (galectin 1 and galectin 3, and to a lesser extent galectins 4, 7, and 9), PP13 is only expressed in the placenta of humans and of some arthropoid apes (1). In women undergoing normal pregnancy, its levels average 200–300 pg/ml during the first trimester, and continue to rise during pregnancy, reaching 400–500 pg/ml by term (3). In preeclamptic pregnancies, however, PP13 levels are significantly reduced during the first trimester (to approximately 50–100 pg/ml) but then rise sharply after 20 weeks of gestation, increasing to 600–700 pg/ml before delivery. Furthermore, the steepness of the slope of PP13 appears to indicate the severity of the disorder (3). As discussed below, galectins bind to sugar residues of various polypeptides and exhibit diverse biological activities. Although in the classical sense galectins have no "receptors," cross-linkage studies have shown high affinity to certain molecules. Indeed, it has been shown that out of many beta galactoside proteins, PP13 has very high affinity for annexin IIA, actin beta and gamma, and the B blood group antigen (12, 26).

Our current and earlier findings (5) suggest that low PP13 levels in early pregnancy may reduce systemic vasodilation and possibly uteroplacental perfusion, increasing the risk of developing preeclampsia later in pregnancy. Notably, the PP13 effect in rats is pharmacological rather than physiological, since rodents lack the LGALS13 gene encoding for the protein, which is specific to primates (1, 26). At the same time, there are other galectins in rats (including galectin 1 and 3) that share 50% sequence homology with PP13 (1, 12, 26).

Meta-analyses of PP13 across various human studies have indicated a major decrease in the circulating concentrations during early pregnancy related to disease severity, ethnic origin, influence of climate, and genetic polymorphism in the protein primary sequence, especially among women of an African ethnic origin (27–30). Studies have also shown that the PP13 carbohydrate region is involved in the acquired immune tolerance to pregnancy (31). Than et al. (1) have shown induction of apoptosis of white blood cells mediated through the carbohydrate-binding domain of PP13, and Kliman et al. (32) showed PP13-induced apoptosis of various types of maternal white blood cells in the placenta. Also, in humans, the PP13 carbohydrate region may affect the bioavailability of PP13, or the amount secreted into blood and, therefore, the susceptibility to developing preeclampsia (33).

While the complete spectrum of immunological, ethnic, or environmental aspects of the PP13 response remains to

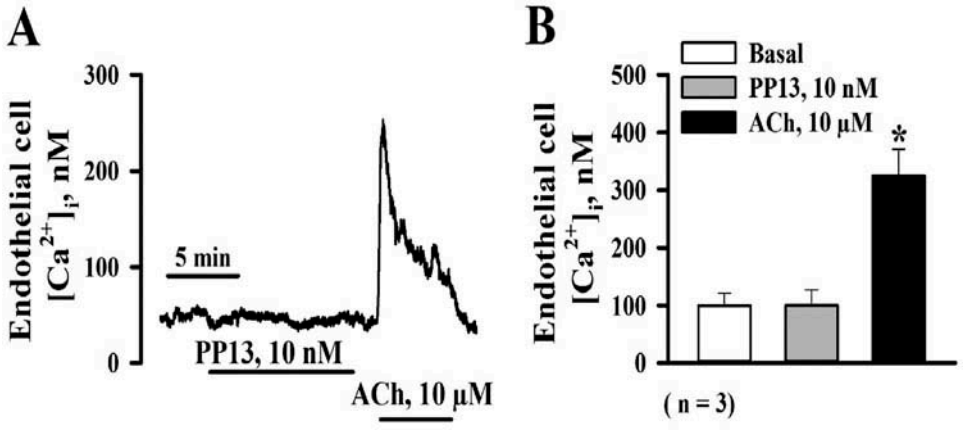

Figure 6. (A) Tracing of endothelial calcium (EC $[Ca^{2+}]_i$) in an isolated, pressurized uterine arcuate artery from a mid-pregnant rat showing that a high concentration of PP13 (10 nM) did not alter basal calcium levels, while subsequent addition of ACh (10 μM) induced rapid, significant $[Ca^{2+}]_i$ elevation. (B) A summary graph demonstrating lack of changes in EC $[Ca^{2+}]_i$ to application of PP13. In the same arteries, ACh induced a marked increase in EC $[Ca^{2+}]_i$. Data are reported as mean ± SEM, *n* = number of experiments. *$p < 0.05$.

be fully elucidated, our group has now shown in a series of four publications that the protein has a substantial impact on the uteroplacental vasculature during pregnancy causing increased circumferential remodeling of rat uterine veins, and vasodilation of small arteries and veins (2, 5, 18, 34). Thus, PP13 may favor hypotension during pregnancy and increase uteroplacental blood flow to the placenta secondary to maternal uterine artery vasodilation. It should be noted that a PP13 concentration of 300 pg/ml corresponds to approximately 9×10^{-12} M, which is close to the threshold for dilation observed in this study (~10^{-13} M). As already mentioned, rats do not express PP13, so it is not possible to directly relate arterial sensitivity in these animals to humans.

PP13-induced relaxation was abolished in endothelium-denuded vessels of mid-pregnant rats, supporting an entirely endothelium-dependent mechanism of action. This holds therapeutic promise, since pharmacologic supplementation of PP13 would allow PP13 to contact its target tissue via the intravascular route. This is something that cannot be presumed for vascular smooth muscle, whose contact to circulating proteins is largely restricted due to endothelial barrier properties.

There are three known main signaling molecules that are involved in endothelium-induced vasodilation: NO, PGI_2, and endothelium-dependent hyperpolarizing factor (EDHF). In this study, both inhibition of NOS and COX 1/2 reduced the dilatory effects of PP13 and, when combined in the same solution, abolished it completely (Figure 4). Two related findings, however, deserve note: first, there was no effect of RO1138452, a highly selective IP receptor antagonist, suggesting that a vasodilatory prostanoid other than prostacyclin may be responsible (35, 36). Second, in spite of it having a primary action on the endothelium, PP13 did not produce any detectable increases in endothelial cytosolic calcium. This was unexpected, as calcium entry into the endothelium is commonly a prerequisite for endothelial vasodilator release (37) and thus suggests a Ca^{2+}-independent mechanism (Figure 7).

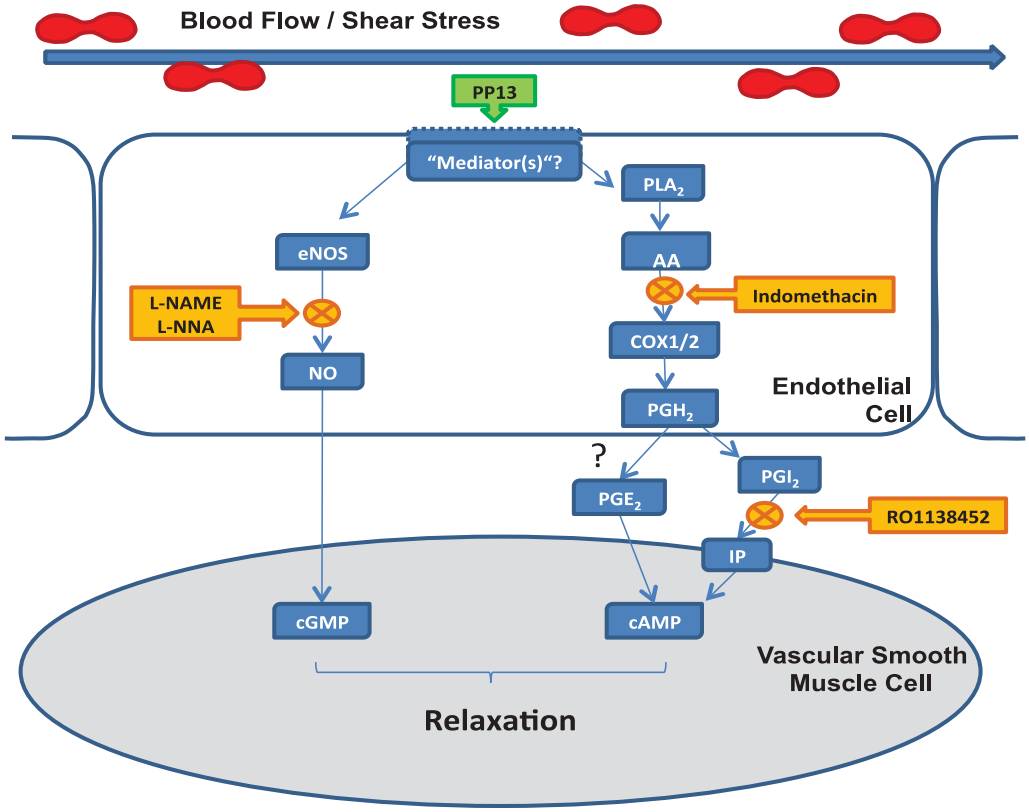

Figure 7. Signaling pathways affected by PP13. Endothelium-dependent vascular relaxation in response to placental protein 13 (PP13) occurs through an as-yet unidentified response element whose activation results in stimulation of nitric oxide (NO) production via endothelial nitric oxide synthase (eNOS), as well as metabolism of arachidonic acid (AA) to prostaglandins (PG) via COX1/2 enzymes. NO and PG normally elicit relaxation of vascular smooth muscle cells through cGMP and cAMP, respectively. Note that in the context of blood vessel relaxation, PP13 does not alter endothelial cytosolic Ca^{2+} levels, and that the IP (prostacyclin) receptor is not involved, suggesting that another prostaglandin, e.g., prostaglandin E2 (PGE2) may be responsible. cAMP: Cyclic adenosine monophosphate; cGMP: cyclic guanosine monophosphate, PLA_2: phospholipase A2; L-NAME: Nω-nitro-L-arginine-methyl-ester; L-NNA (Nω-nitro-L-arginine; PGH2: prostaglandin H2; RO1138452: 4,5-dihydro-1*H*-imidazol-2-yl)-[4-(4-isopropoxy-benzyl)-phenyl]-amine.

The effects of galectins are thought to be mediated through their ability to bind sugar residues of other proteins (18). Such sugars (like *N*-acetyl galactose amine) are common to many molecules and thus it is quite hard to verify a unique "receptor" for a given galectin in the classical sense (1, 34, 41). However, research revealed that each galectin, has a preference for sugar residues of certain proteins, that may be related to the three-dimensional configuration of the protein, and the level its respective sugar residues (34, 41). In the case of PP13, it has been shown by members of our team that it has high affinity for sugar residues of annexin IIA, actin beta and gamma (26, 34) that are expressed in the syncytiotrophoblast and the decidua (38), and to the erythrocytes B group antigen (26).

PP13 has also been reported to interact with lysophospholipase A, and to induce the release of PGs from cultured trophoblasts (11). Its mechanism of action on the endothelium, at least in terms of activating eNOS and COX enzymes and thereby stimulating vasodilation, is demonstrated in this study for the first time. COX enzymes may be localized to the endoplasmic reticulum and/or nuclear envelope (39), while eNOS is not only concentrated in caveolae membranes but also repeatedly found intracellularly near the Golgi apparatus (40). Galectins are not only known to cross-link membrane components but also cross the cell membrane and enter the cytoplasm (41). The complete chain of actions in the functional pathway by which PP13 stimulates these endothelial enzymes remains to be further discovered.

One limitation of this study is that the LGALS13 gene, expressing PP13, is only expressed in primates and not in the rat model used. However, the mechanisms involved in blood pressure regulation and vasodilation in rats are quite similar to those of humans in terms of primary endothelial involvement. To assess the relevance to human pregnancy, isolated human uterine arteries need to be studied directly to confirm its vasodilator effects. At the same time, this study has shown a novel action of PP13 on the ECs of UAAs, which contribute significantly to uterine vascular resistance and are important in the regulation of uteroplacental perfusion (19). Its potency encourages future studies of this galectin as a potential therapeutic agent for lowering blood pressure and improving uteroplacental perfusion in women with gestational disease. Studies of PP13 construct expressed in human placental-derived cell line – BeWo cells – have shown that there is no difference between the recombinant PP13 expressed in human to the one used here that is expressed in *E. coli* (2, 12, 18, 34). The carbohydrate content of PP13 is the lowest of the placental proteins, 0.6% (11, 12, 34), and thus, for any analytical purpose, it is very unlikely that PP13 glycosylation is critical for its function.

Conclusion

The results of his study provide the mechanistic basis for the vasodilation of resistance arteries to PP13 by demonstrating its endothelial specificity and activation of both NO and PG-signaling pathways in this process. The potent vasodilatory effects of PP13 in small mesenteric arteries support earlier findings documenting its hypotensive effect and, with regard to its effects on uterine arteries and veins, its augmentation of pup and placental weights. This peptide deserves to be evaluated as a potential therapeutic candidate for improving blood flow and outcome in pregnancies complicated by hypertension and/or preeclampsia.

Acknowledgment

Hylabs is thanked for providing the PP13 for this study.

Declaration of interest

HM and SG hold a patent for the potential therapeutic use of PP13 in pregnancy complications. All other authors declare no conflicts of interest.

Funding

This study was mainly sponsored by the European Union (FP7) through the ASPRE project (601852) and Hananja ehf, and the Icelandic Centre for Research. We also appreciate financial support from the Cardiovascular Research Institute of the University of Vermont.

Author contribution

The study was designed by all authors. GO, MM, and NG supervised the experimental work of TD, who conducted all experiments. NG developed the calcium measurement model and the respective experiments and was involved with GO, SG, and TD in the designing and conducting the study. The pharmacological aspects of the study were managed by TD, SG, GO, and NG. HM and BH were involved in the design of PP13 experiments, and in the provision of calibrated PP13 and its verification. All authors were involved in writing the manuscript, data analysis, and discussions.

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