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Vasoreactivity of Chorionic Plate Arteries in Response to Vasoconstrictors Produced by Preeclamptic Placentas☆

C. Benoit[a,c], **J. Zavecz**[b], and **Y. Wang**[a,c,*]

[a] Department of Molecular and Cellular Physiology, Louisiana State University Health Sciences Center, PO Box 33932, 1501 Kings Highway, Shreveport, LA 71130, USA

[b] Department of Pharmacology, Toxicology and Nueroscience, Louisiana State University Health Sciences Center, PO Box 33932, 1501 Kings Highway, Shreveport, LA 71130, USA

[c] Department of Obstetrics and Gynecology, Louisiana State University Health Sciences Center, PO Box 33932, 1501 Kings Highway, Shreveport, LA 71130, USA

Abstract

Inadequate blood flow and increased vasoconstriction of the placenta contribute to pregnancy associated disorders such as preeclampsia (PE). Because placental vessels lack autonomic innervation, humoral effects of the placenta must play critical roles in regulation of fetal-placental vascular contractility. In this study, we examined the nature of humoral factors produced by PE trophoblasts on placental vessel contractility using an organ bath perfusion model. Vasomotor responses were studied in vitro using placental chorionic plate arteries. Vessel rings from third branch chorionic plate arteries were dissected from human placentas following normal or PE delivery. The arterial rings were equilibrated in Krebs Henseleit buffer and exposed to placental conditioned medium, which was prepared by culture of villous tissue from PE placentas. Receptor antagonists for angiotensin II (ANG II), thromboxane (TX), and endothelin (ET) were used to determine which humoral factor produced by placental tissue (trophoblasts) was more effective in promoting vasoconstriction. The role of angiotensin converting enzyme (ACE) and non-ACE ANG II generating enzymes in regulation of placental vasomotor tone were also investigated. A total of 80 arterial rings from 48 placentas were studied. Our results showed: 1) enhanced vasomotor tone in arteries from PE placentas compared to those from normal placentas; 2) PE-CM induced vaso-constrictive activity could be partially attenuated by receptor antagonists for TX, ANG II and ET, respectively; and 3) chymostatin (a chymase inhibitor) produced a stronger inhibitory effect than captopril (ACE inhibitor) on PE conditioned medium induced vasoconstriction. Our data demonstrate increased vasocontractility in PE placentas and suggest that the non-ACE pathway is probably a major source of ANG II produced in the human placenta.

Keywords

Placenta; Vasoactivity; ANG II; ET; TX; Preeclampsia

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* Corresponding author. Tel.: +1 318 675 5379; fax: +1 318 675 4671. ywang1@lsuhsc.edu (Y. Wang)..

1. Introduction

Preeclampsia (PE) is a multiple system disorder unique to human pregnancy. Although the etiology remains unclear, increased vasoconstriction is one of the major underlying pathophysiological events in this pregnancy disorder. During normal pregnancy, vascular resistance is reduced, allowing adequate maternal blood perfusion into the placenta to support fetal development. However, in PE there is increased vascular resistance leading to maternal hypertension and reduced blood flow in the placenta. The placenta is a key component in the pathophysiology of PE particularly those with reduced placental perfusion. Many investigators believe that preeclampsia is the result of vasoactive and inflammatory mediators secreted by the placenta acting on the vascular endothelium leading to vasoconstriction of the smooth muscle [1,2]. These mediators are likely secreted from trophoblast cells (TCs) in response to placental hypoxia/ischemia and shallow cytotrophoblast invasion acting on vascular endothelium [3].

It has been demonstrated that multiple mechanisms contribute to the increased vascular resistance during PE, including increased vascular sensitivity to angiotensin II (ANG II) [4,5], the presence of angiotensin II receptor-1 (AT_1) autoantibody [2,6], an imbalance between the vasoconstrictor thromboxane (TX) and decreased vasodilator prostacyclin (PGI2) [7,8], and altered endothelin (ET) [9,10] and nitric oxide (NO) [11] levels in both the maternal circulation and in the placenta in women with PE. These alterations may result in a detrimental positive feedback system that promotes inflammation and further vasoconstriction. Since the human placenta lacks autonomic innervation, its vascular tone must be controlled by circulating or locally released vasoactive substances. Therefore, vasoconstrictors produced by placental trophoblast cells may play a critical role in regulating vasomotor tone in the placental vasculature.

Past studies have described that placental tissues produce ANG II [12-16], TX [17,18] and ET [19-21] and they are major contributors to the vasoconstriction in PE. Receptors for ANG II [2,6,22-25], TX [26,27], and ET [28] are present in placental trophoblasts and vasculature. However, the differential effects of each vasoconstrictor in the regulation of placental vasomotor tone have not been simultaneously studied. In the present study we examined vasomotor responses using placental chorionic plate artery organ bath perfusion as a testing model. Receptor antagonists for ANG II, TX, ET were used and the role of ACE and non-ACE ANG II generating systems in the regulation of placental vasomotor tone were also investigated. The purpose of this study was to determine if placenta trophoblast-produced vasoactivate agents could induce a vasomotor response in placental chorionic arteries and what soluble vasoconstrictor agents produced by preeclamptic placentas were involved.

2. Materials and methods

2.1. Placenta and patient information

Placentas were obtained immediately after delivery from normal pregnant women and from women with PE. Normal pregnancies are defined as mothers with normal blood pressure (<140/90 mmHg), absence of proteinuria, and without medical and obstetrical complications. Preeclampsia was defined as gestational blood pressure of greater than 140/90 mmHg on two occasions measured 6 h apart and with urinary protein >300 mg over a 24 h period. This study was approved by the Institutional Review Board for Human Research at LSUHSC-Shreveport, LA. A total of 48 placentas were used, 43 from normal and 5 from PE pregnancies. In the PE group, the mean gestation age was 34^{+5} weeks with 4 out of 5 delivered by cesarean section. In the normal group, the mean gestation age was 39^{+1} weeks with 5 out of 43 delivered by cesarean section.

2.2. Conditioned medium preparation

Placental conditioned media (CM) were prepared from villous tissue explant culture from the placentas of preeclamptic patients as previously described [29,30]. Briefly, placentas were obtained immediately after delivery from preeclamptic pregnancies. Placental villous tissue, excluding chorionic basal plates, was removed from these placentas under sterile conditions. Villous tissues were washed with phosphate buffered saline (PBS) containing 1% penicillin (v/v 10,000 units/ml, streptomycin 10,000 μg/ml) and placed in 6 well plates with 500 mg tissue/well in 7 ml serum free Dulbecco's Modified Eagles Medium (DMEM, Sigma Chemical Inc., St Louis, MO). The plates were then placed in a cell culture incubator (5% CO2 in air) for 48 h culture. At the end of incubation, the culture medium was collected and stored at −70 °C until use. Before use, the cultured medium was thawed and centrifuged for 5 min at 3000 to remove cellular debris in the conditioned medium.

2.3. Chorionic artery myography

Placental vessel contractility was examined by placing placental chorionic arterial rings in an organ bath apparatus. Rings were tested with placental conditioned media derived from PE villous tissue culture (PE-CM), which was used to examine the humoral effects of trophoblasts on placental vascular contractility. Chorionic plate arteries chosen were tertiary branches of the umbilical arteries just before they submerge into the chorionic plate, with an internal diameter of 1 mm. The vessels were gently dissected then immediately placed in cold Krebs buffer. The vessels were then transferred into warm Krebs-Henseleit (KH) physiologic saline solution (37 °C, pH 7.4) continuously aerated with a gas mixture consisting of 95% O_2 and 5% CO_2. The formulation of the KH buffer was as follows (in mmol/L): 118 NaCl; 4.8 KCl; 2.5 $MgSO_4$.7H2O; $1KH_2PO_4$; 27.2 $NaHCO_3$; and 2 g/L of glucose [31].

The vessels were cut into 3 mm long rings and threaded onto wire clips attached to isometric force transducers via silk thread. The size of the vessel rings were selected based on previously published works [32-35]. In general, two sister vessels from the same placenta were studied in parallel in each experiment. The chorionic plate artery rings were then placed in organ baths with U-shaped chambers, and the clips attached to the isometric force-displacement transducers, which were in turn connected to a Grass Model 7 D polygraph recorder.

Vessels were equilibrated without passive tension for 60 min. Thereafter, 1 g of tension was applied to the arterial rings for a 1.5 h period followed with periodic resetting of the 1 g passive tension until they could maintain consistent tone. Before, between and after each application of placental conditioned media and inhibitors/blockers, the vessel rings were washed with Krebs buffer for at least 90 min. This procedure ensured that the vessels return to their baseline tension and that all media or antagonist effects were washed out.

In each experiment, a KH buffer with a concentration of 100 mmol KCl was used to determine the maximal contraction capable of being produced by the vessel [32]. The vessels were exposed to 100 mmol KCl until the response plateaued and maximal contraction was achieved. The vessels were then washed with KH until the vessels relaxed to the baseline level. Vessels incapable of producing 500 mg tension by 100 mmol KCl were considered to be unacceptable for data collection. After a wash period the vessels were perfused with PE-CM. This was used to examine the humoral effects of trophoblasts secretions on placental vascular contractility. This also acted as a comparative control against media mixed with receptor antagonists or enzyme inhibitors. Fig. 1 (upper panel) displays the means of two sister vessels from a normal placenta in response to either 100 mmol KCl or PE-CM. The responses of the vessels to PE-CM with or without a selective

receptor antagonist were also determined. DMEM and all inhibitors/receptor antagonists in KH buffer were perfused alone for fifteen minutes prior to addition of PE-CM. Neither DMEM alone nor receptor antagonist or inhibitor alone affected baseline vasomotor tone in tested vessels (Fig. 1 lower panel). In general, a 90 min wash between CM and CM plus a selected receptor antagonist was applied in all experiments. At the end of each experiment the tissues were again exposed to 100 mmol KCl to determine the contractile activity of the artery over the course of the experiment.

2.4. Chemicals

The receptor antagonists and inhibitors used in this study included the selective thromboxane receptor (TP) antagonist SQ-29548 [Cayman Chemicals; Ann Arbor, MI], a specific AT_1 receptor antagonist losartan [a generous gift from Dr. Neil Granger; LSUHSC-S], a selective non-peptide AT_2 receptor antagonist PD-123,319 [Sigma Chemical Inc. St. Louis, MO.], an ACE inhibitor captopril [ICN biochemicals Costa Mesa, CA], the chymase inhibitor chymostatin [Sigma Chemical Inc.], a selective ET_A and B receptor antagonists PD-151,242 and BQ-788 [Sigma Chemical Inc.], respectively.

2.5. Statistical analysis

Data were expressed as the mean (±SE) of % maximum contraction elicited by 100 mmol/L KCl and expressed as % contraction normalized to mg tension during CM perfusion. Statistical analyses were performed with non-parametric Mann–Whitney test or a paired *t*-test. A computer software program StatView (Cary, NC) was used. A probability level of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Vasoconstrictor response by vessels from normal and preeclamptic placenta

The maximum vasoconstrictive response to 100 mmol KCl by artery rings from normal and PE placentas are shown in Fig. 2. For this data, 40 vessels rings from normal placentas and 7 vessel rings from PE placentas were used. Our data showed that the vasoconstrictive response to KCl was greater in PE vessels than those of normal vessels, indicating that vessels from PE placentas are more sensitive to KCl stimulation than normal vessels ($p < 0.05$).

3.2. Placentas release vasoconstrictors

Organ bath myography was then used to determine if vasoactive agents produced by PE placentas could elicit vasoconstrictive effects on the placenta vessels. To study this, placental conditioned media (CM) derived from PE placental explant culture (PE-CM) was used. As shown in Fig. 3, compared to 100 mmol KCl stimulation as 100% of maximal contraction, PE-CM perfusion of placental vessel rings could induce 50 ± 5% of maximal contraction ($P < 0.01$).

3.3. Role of angiotensin converting enzymes in the vasomotor responses to placental derived vasoactivators

To determine if ANG II was released from PE placentas and to determine which pathway was responsible for the production of ANG II, artery rings were exposed to the ACE inhibitor captopril at a concentration of 10^{-5} M or the chymase inhibitor chymostatin at a concentration of 10^{-4} M prior to perfusion of the vessel rings to PE-CM. The concentrations for captopril and chymostatin were selected based on organ bath perfusion studies [36,37]. The results illustrated in Fig. 4 showed that the treatment with the inhibitor captopril or chymostatin resulted in significantly less vasoconstrictive effects of PE-CM with a 20 ± 4%

attenuation of PE-CM by captopril, and a 40% attenuation by chymostatin. These data suggest that chymase may play a more significant role than ACE in ANG II generation.

3.4. The involvement of ANG II AT_1 and AT_2 receptors in the vasoconstrictve response to placental derived vasoactivators

To further investigate the involvement of ANG II pathway in the vasoconstrictor response produced by PE placentas, losartan (a selective AT1 blocker) and PD-123319 (a selective AT2 blocker) were used. Losartan (10^{-5} M) significantly attenuated the contractile response to PE-CM by 30 ± 8% ($P < 0.05$), while the AT_2 receptor antagonist PD-123319 (10^{-6}M) only reduced the contractile response of the vessels to PE-CM by 16 ± 8% (Fig. 5).

3.5. The involvement of endothelin-1 in the vasoconstrictive response to placental derived vasoactivators

The ET_A antagonist PD-151242 and the ET_B antagonist BQ788 were used to study the effects of endothelin on placental vasoconstriction. PD-151242 (10^{-5} M) attenuated the contractile response of PE-CM significantly (28 ± 6%, $P < 0.05$), whereas, BQ788 (10^{-6} M) produced a non-significant degree of attenuation (Fig. 6).

3.6. The involvement of thromboxane in the vasoconstrictive response to placental derived vasoactivators

In this experiment, the TXA2 receptor antagonist, SQ-29548 at a concentration of 10^{-6} M was used. SQ-29548 significantly attenuated the contractile response induced by PE-CM (Fig. 7). The mean contraction of chorionic vessels with PE-CM ± SQ29548 was reduced by approximately 50 ± 6% of that compared to CM alone ($P < 0.01$).

4. Discussion

In the present study, the vasomotor responses of chorionic plate arteries from placentas delivered by normal pregnant women and by pregnant women complicated by preeclampsia were examined. One of the most important findings in our study was that chorionic plate arteries from preeclamptic placentas produced a significantly stronger contractile response to 100 mmol KCl challenge than the arteries from normal placentas, which demonstrated that increased vasocontractile activity is present in the placental vasculature in preeclampsia. The finding of increased vasomotor response in preeclamptic vessels is consistent with the current view that increased vasoconstriction occurs in the placental vasculature during preeclampsia. Our results agree with those of Bertrand et al. where they found that chorionic plate arteries and veins developed a greater active tension than those from normal placentas [32]. The ability of preeclamptic vessels to develop a greater tension than normal vessels suggests that there are physical and biochemical differences in vasomotor responses to contractile stimuli between the vessels of normal placentas and those of preeclamptic placentas.

Since the placenta lacks autonomic innervation, it is believed that placental vascular tone must be regulated by circulating or locally released vasoactive substances. To study whether placental trophoblast-derived vasoactivators could be responsible for the increased vasomotor tone observed in preeclamptic placental vessels, chorionic plate artery rings from normal placentas were perfused with conditioned media, which was prepared by culture of villous tissue from preeclamptic placentas. Our results showed that when normal vessels were perfused with preeclamptic placental conditioned media, they could produce approximately 50% of maximum tension of that produced by 100 mmol KCl. These data suggest that placental tissue-produced vasoactivators could, at least in part, be responsible for the increased vasomotor activity in the placental vasculature during preeclampsia.

The placenta produces ANG II [12-16], TX [17,18] and ET [19-21]. To study which vasoactivators and their pathways produced by PE placenta were more responsible for regulating the vasomotor response in the placental vasculature, selected receptor antagonists or inhibitors of three major vasoconstrictors, ANG II, TX and ET-1, were used. Our results showed that all these inhibitors or receptor blockers produced a more or less suppressive effect on PE-CM induced vasomotor response with TX receptor blocker as the most potent one in chronic plate artery vessels.

Our data indicated that TX is probably the strongest vasoconstrictor produced by the preeclamptic placenta since the thromboxane receptor blocker, SQ29548, elicited the greatest inhibitory effects on vasoconstriction induced by PE-CM. SQ29548 was used at a concentration of 10^{-6} M, as based on previously published works [20,38]. This concentration was shown to be able to completely block TXB induced vasoconstriction stimulated by bradykinin in a perfused placental cotyledon study [38].

During normal pregnancy, the placenta produces equivalent amounts of TXA2 and PGI2, and thus their activities are balanced. In PE women, TXA2 production is significantly increased while PGI2 and PGE2 production are significantly decreased and results in vasoconstriction [17]. It has been reported that placental trophoblast cells are the major source of TX production in placental tissue and that trophoblast cells from PE placentas produce significantly more TX than normal placentas [8,17], although the possibility exists that vasoconstrictors may or may not exert their actions through TXA receptor [20,38].

Several studies have demonstrated that ANG I [13] and ANG II [39] are present in the placenta and the uteroplacental unit possesses all components of the RAS necessary for their generation [12]; renin [14], angiotensin [40] and ACE [15]. However, the impact of the altered RAS on the fetal side of the placenta is not well defined [41,42]. Most of the vasoactive effects of ANG II are produced depending on which angiotensin II receptor is expressed in the smooth muscle of the particular tissue of interest. AT_1 receptor activation produces vasoconstriction and AT_2 receptor activation produces vasoconstriction or dilation depending on the cell type. It is likely that ANG II mainly binds to AT_1 receptor and elicits downstream vasocontrictor effects in the placenta. Li X et al. [23] examined AT_1 and AT_2 receptor expression in PE and normal placental trophoblast cells and found that both receptors are present in the placenta and that AT_1 receptor predominates [23]. However, Li and colleagues observed that in placentas from PE and intrauterine growth restriction pregnancies the AT_1 receptor expression is decreased compared to normal placental expression. Our data demonstrated that the AT_1 receptor antagonist, losartan, inhibited the effect of PE-CM induced vascular vasocontractility by 30% (Fig. 5), while the AT_2 antagonist, PD123319, was less effective. This suggests that AT_1 receptor exerts predominate function in the placental arteries. In addition, our results confirm the observation made by MaasenVanDenBrink et al., that the AT_2 receptor antagonist, PD123319, had no effect on ANG II induced contraction in human coronary artery (HCA) rings [37].

Interestingly, our results showed that chymostatin had a stronger inhibitory effect on PE-CM induced vascontraction. Chymostatin is a specific inhibitor for chymase which has been demonstrated to be a major non-ACE angiotensin II generating enzyme in the human heart [43]. Our results indicate that ANG II can be generated by both ACE and non-ACE systems in the placenta. These non-ACE and ACE inhibitory results imply that the strong vasoconstrictive effects produced by preeclamptic placentas may be regulated via the ANG II pathway and that the non-ACE generating system may be more dominant in regulation of vasoactivity in the placental vasculature during PE. The observations of enhanced chymase

expression and activity in PE placental tissue than that in normal placental tissue supports this notion [44].

The importance of endothelin in the vascular consequences of PE was examined pharmacologically with endothelin ET_A and ET_B receptor antagonists. We found that the ET_A receptor antagonist produced a significant reduction in the response of chorionic tertiary level arteries to PE-CM. Sand et al. also studied endothelin induced contraction in placental arteries [45]. They found that block of the ET_A receptor can attenuate approximately 75% of ET-1 induced vasoconstriction, whereas ET_B receptor block produced a 58% decrease in the effect of ET-1. Using the same concentrations of ET_A and ET_B receptor blockers showed that the ET_B receptor does not significantly alter the vasoconstriction produced by PE-CM, and only 27.5% of the vasoconstrictor response to PE-CM was altered with the ET_A blocker PD-151242 (Fig. 6), indicating that ET-1 induced vasoconstriction is not the major component produced by placentas in the vascoconstriction seen in PE.

Placental trophoblast cells release TX, ANG II and ET into intervillous space. These vasoactivators could enter the maternal circulation and affect vascular function. Although at the present time there is no information available regarding basolateral release of vasoactivators by placental trophoblast cells, an in vitro cell culture study by Larry Guilbert group did show that more than 90% of MMP2 and MMP9 produced by trophoblast cells were released from the basolateral surface compared to epithelial surface by cultured human placental syncytiotrophoblasts [46]. Our published works using cell co-culture model: co-culture of endothelial cells and trophoblast cells [47,48] also support the idea that trophoblast-produced bioactive agents could be released not only into the epithelial direction (inter-villous space) but also into the basolateral direction (villous stromal compartment). This concept is also supported by the fact that the maternal circulation is separated from the fetal circulation in the placenta, so vasoactivity of placental vasomotor tone must be regulated by locally produced vasoactive agents or come from the maternal circulation shuttled across (diffuse) the trophoblast layer to villous core fetal vessels. Results from the present study suggest that paracrine regulation of trophoblast-derived vasoactivators might play an important role in control of placental vessel motor tension and contractile function. Further studies on paracrine regulation of trophoblast-derived vasoactivitors would provide insight into mechanisms in vessel motor tone of placental vasculature.

In summary, in the present study using organ bath perfusion model, we tested ANG II, TX, ET-1 receptors and ACE and non-ACE angiotensin generating systems in the regulation of chorionic plate artery vasoactivity as altered by preeclampsia. We found that: 1) PE placental vessels exert an increased vasocontractile activity; 2) placental tissues (trophoblasts) release vasoconstrictors; 3) thromboxane might be the strongest vasoconstrictor produced by placental tissue in PE; and 4) the most significant finding of the study is that the non-ACE angiotensin II generating system is dominate in the placenta and may play a critical role in regulation of placental vascular contractile function. Further study is needed to determine the interplay of these vasoconstrictors in the regulation of vasomotor tone of the placental vasculature in normal pregnancy and in preeclampsia.

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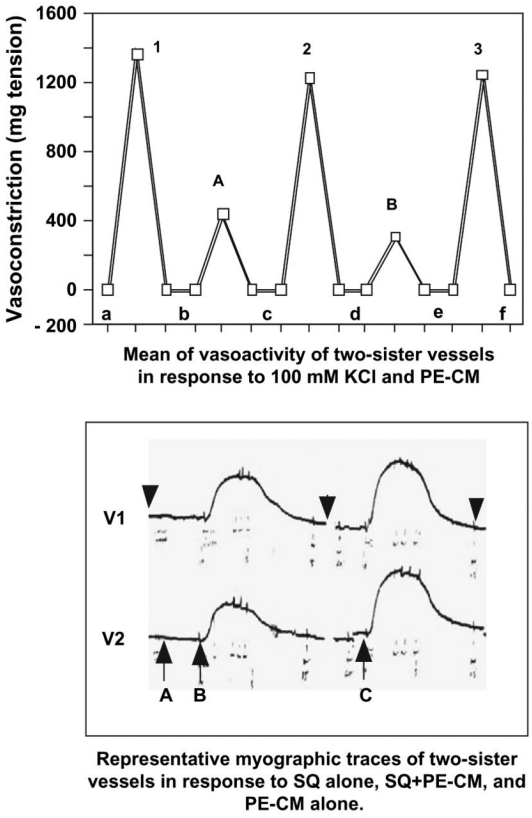

Fig. 1.

Upper Panel: Representative vasomotor responses of two sister vessels from a normal placenta during the duration of a 6 h organ bath perfusion experiment in response to 100 mmol KCl and PE-CM stimulation. Data is expressed in milligram tension. This data illustrate that over a 6 h period, the vessels are still healthy and responsive to stimulation. 1–3: maximum contraction induced by 100 mmol KCl; A and B: maximum contraction induced by PE-CM; and a–f: baseline tension of vessels before, between and after 100 mmol KCl and PE-CM stimulation. **Lower Panel**: Myographic traces of two sister vessels from the same placenta: V1 and V2. A: SQ29548 alone for 15 min; B: SQ29548 + PE-CM; and C: PE-CM alone. Arrowheads: wash period before, between, and after each perfusion with inhibitor or PE-CM, respectively.

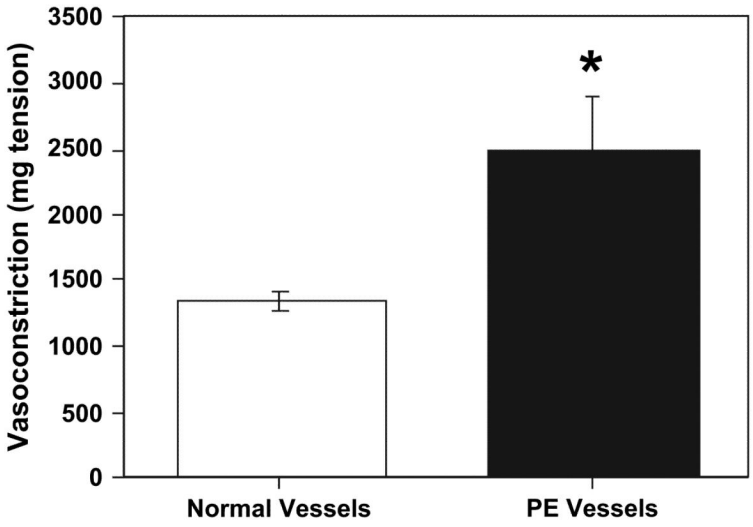

Fig. 2.

Maximal contraction of vessel rings from normal and PE placentas in response to 100 mmol KCl. Data are expressed as mean ±SE of mg tension developed. Vasoconstriction was significantly increased in vessel rings 7 from PE placentas compared to 40 from normal placentas, $*p < 0.05$.

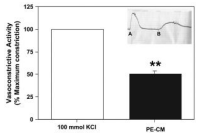

Fig. 3.

Percent of maximal contraction of chorionic vessel rings from normal placentas in response to PE-CM perfusion compared to maximal contraction induced by 100 mmol KCl perfusion (vessel rings, $n = 20$). PE-CM induced contraction of normal placental vessels, which indicate placentas from preeclampsia release vasoconstrictors. ** Significantly different compared to 100 mmol KCl, $p < 0.01$. (Insert: Example of an organ bath trace, A: 100 mmol KCl; B: PE-CM).

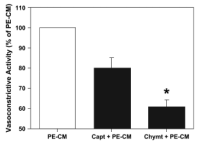

Fig. 4.

Effect of captopril and chymostatin on PE-CM induced vasoconstriction of placental vessels. Captopril: ACE inhibitor (capt; $n = 6$); chymostatin: chymase inhibitor (chymt; $n = 5$). Chymase is a non-ACE ANG II generating enzyme. Data is presented as mean % ± SE of PE-CM induced contraction, $*p < 0.05$.

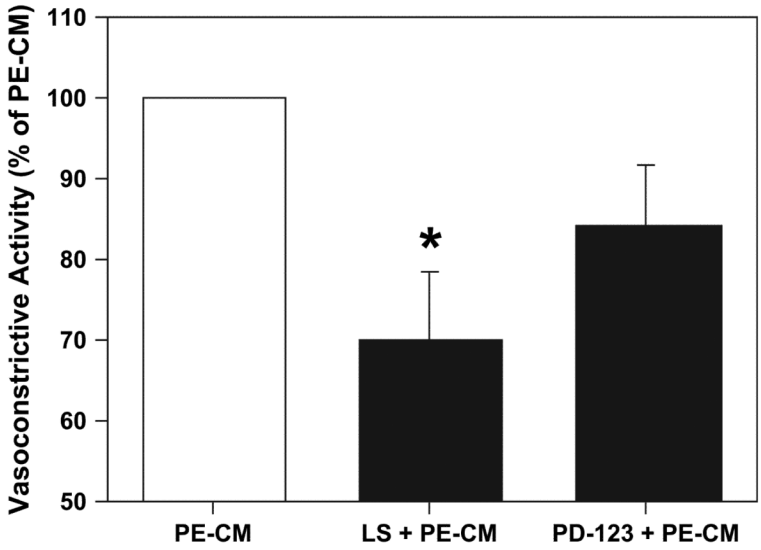

Fig. 5.

Blockade of angiotensin AT_1 or AT_2 receptors with losartan (LS) and PD123319 (PD-123) respectively attenuates PE-CM induced vasoconstriction in chorionic vessels ($n = 6$). Data is presented as mean % ± SE of PE-CM induced contraction, $*p < 0.05$.

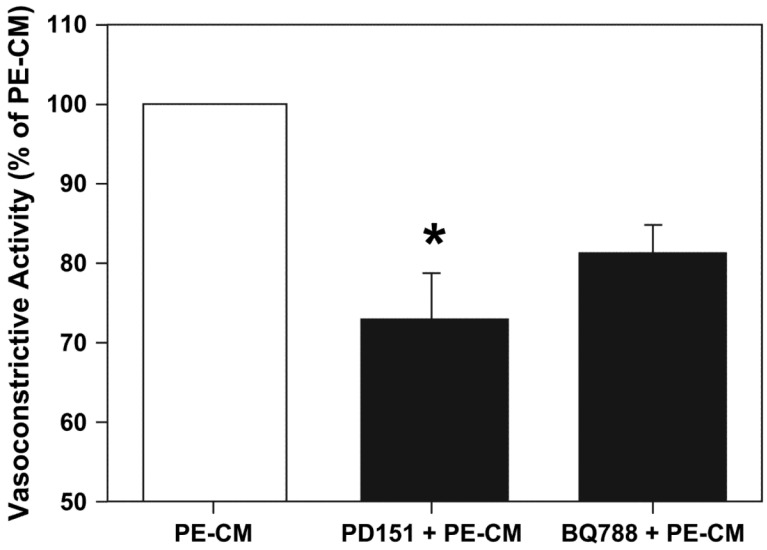

Fig. 6.

Blocking of endothelin ET_A and ET_B receptors with PD151242 and BQ788 respectively attenuates PE-CM induced vasoconstriction in chorionic vessels ($n = 6$). PD151242 (PD151) = ET_A receptor antagonist; BQ788 = ET_B receptor antagonist. Data is presented as mean % ± SE of PE-CM induced contraction, $*p < 0.05$.

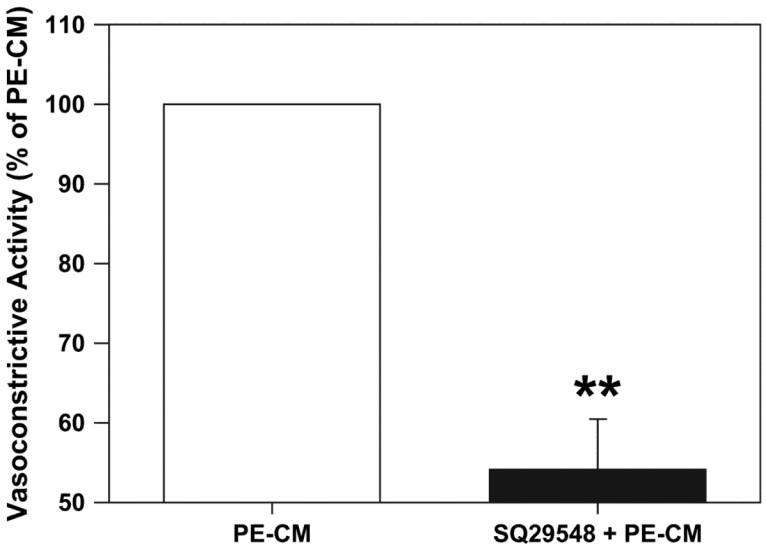

Fig. 7.

Thromboxane receptor antagonist SQ29548 greatly attenuates the chorionic vessel vasocontractility produced by PE-CM perfusion in the organ bath (vessel rings, $n = 6$). Data is presented as mean % ± SE of PE-CM induced contraction, $^{**}p < 0.01$.