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Sensitivity to the thromboxane A_2 analog U46619 varies with inner diameter in human stem villous arteries

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ABSTRACT

Introduction: The vascular resistance of stem villous arteries is determined by the balance between different contractile and relaxant agents and in the utero-placental circulation. Thromboxane A_2 (TxA_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and endothelin-1 (ET-1) are considered to be among the most important contractile factors. However, it is not known if their contractile effects are consistent along the villous tree. We hypothesized that the sensitivity to different agonists could be influenced by artery diameter and thus that their contribution to placental vascular resistance may differ.
Methods: Using an isometric wire myograph, the contractility and sensitivity (pD_2) to the thromboxane A_2 mimetic U46619, $PGF_{2\alpha}$ and ET-1 were investigated in isolated human stem villous arteries and human uterine fundus and isthmus arteries obtained from healthy, pregnant women who had experienced uncomplicated pregnancy.
Results: In fetal arteries, the pD_2 values for U46619 correlated positively with arterial diameter with no such dependence observed for ET-1 and $PGF_{2\alpha}$. In maternal arteries, pD_2 remained constant for all the agonists tested despite highly variable vessel diameter.
Discussion: A selective decrease in sensitivity to TxA_2 receptor stimulation was observed with decreasing vascular diameter in human stem villous arteries. The contractile factors $PGF_{2\alpha}$ and ET-1 show no such relationship.

1. Introduction

Adequate placental perfusion is critical for the normal progression of pregnancy and fetal development. As the placental vascular bed is devoid of autonomous innervation [1], vascular resistance relies upon autoregulation as well as locally generated and circulating vasoactive factors [2,3]. Of these, eicosanoids are attributed a major role. Vasoconstriction of placental arteries occurs with the eicosanoid prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) [4] as well as with the other major eicosanoid thromboxane A_2 (TxA_2). TxA_2 is actively produced in the placenta [5,6] where it potently constricts placental vessels, and local TxA_2 release is also stimulated by other vasoconstrictors (e.g. ET-1) thereby potentiating vasoconstriction [7,8]. Placental TxA_2 production increases under hypoxic conditions and in preeclampsia [5,6] where increased levels of TxA_2 metabolites are present in both maternal and fetal blood [9]. Thus TxA_2-induced placental vasoconstriction apparently contributes to the pathogenic process of preeclampsia and fetal distress [10–12]. Endothelin-1 (ET-1) is also attributed a key regulatory role in placental arteries [13] and in preeclampsia and intrauterine growth restriction [14,15]. ET-1 has been reported to have similar potency across the fetoplacental vascular tree [13].

In the placenta, the resistance vessels considered most important for fetal perfusion pressure are presumed to be those of the stem villous [2,16]. It is not known whether the effectiveness of

Abbreviations: CCRC, Cumulative concentration–response curve; ET-1, Endothelin-1; $PGF_{2\alpha}$, Prostaglandin $F_{2\alpha}$; SVA, Stem villous arteries; TxA_2, Thromboxane A_2.

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vasocontractile agents varies within the stem villi. We hypothesized that stem villous artery (SVA) diameter-dependent specialization could alter the sensitivity to different contractile agonists and we investigate this in the present study using the TxA_2 mimetic U46619, $PGF_{2\alpha}$ and ET-1 in human placental SVA of varying size in comparison with maternal intramyometrial arteries obtained from pregnant women.

2. Methods

2.1. Ethical approval

This study was approved by the Regional Committee for Research Ethics (#20100229) and the Danish Data Protection Agency. All experiments were performed in accordance with the ethical standards of the national ethical committee and with the Helsinki Declaration. All women who met the inclusion criteria participated voluntarily and with informed consent. Maternal arteries were obtained as described previously [17]. In the instance of placental material, exclusion criteria included maternal and fetal disease, smoking during pregnancy, and any maternal medication (excluding that in relation to Caesarean section). Both primiparous and multiparous women participated.

2.2. Tissue collection

All experiments were performed at the Department of Obstetrics and Gynecology, Aarhus University Hospital, Skejby, Denmark. The collection, dissection and experimental handling of myometrial tissue (isthmus and fundus) have been described previously [17]. Term placentas (gestational age 38 + 0–41 + 6) were obtained from normal and uncomplicated pregnancies; preferentially elective Caesarean section (90%) although vaginal deliveries were also included as previous work has demonstrated contractility is unaffected by mode of delivery (23). From placentas, macroscopically normal cotyledons were excised and the tissue subsequently placed in 4 °C Krebs buffer (for composition, see below). Cotyledons were submerged in buffer with the maternal side oriented upwards. Under a stereomicroscope (Olympus KYOWA SDZ/SDS) villous tissue was removed using forceps and scissors until a main villous branch appeared. To distinguish stem villous arteries (SVA) from corresponding veins, the chorionic artery feeding into the stem villous was cannulated and flushed with buffer: the SVA was then positively identified by the exit of buffer from the cut surface of the villous tree. The vein was cut away and SVA of 1–2 mm in length were isolated and all surrounding perivascular tissue removed.

2.3. Isometric force measurement

The arteries were mounted in wire myographs (DMT, Denmark). Typically four arteries were harvested from a single placenta and examined in parallel in different protocols: only one artery from each placenta was used for each protocol and SVA were included for analysis if internal diameter was ≤600 μm. Vessels were normalized to 0.9 × $L_{13.3kPa}$ [18,19]. Using Laplace's law (and post-normalization baseline tension and diameter values) an average equivalent transmural pressure of 21.7 ± 1.6 mmHg (≈2.9 kPa; $n = 12$) is derived: normalization was thus appropriate for the expected pressure range of the placental circulation. Vessel viability was assessed by repeated exposure to high-potassium containing Krebs solution, until the contractions differed less than 10%. The average value of these K^+-constrictions was defined as K_{max}. Isometric force was continuously recorded and printed on chart paper (ASEA Brown Boveri SE120 or Epson LX-400).

2.4. Solutions

Krebs solution (in mM) consists of 135.2 Na^+, 4.6 K^+, 1.2 Mg^{2+}, 1.5 Ca^{2+}, 124.4 Cl^-, 15 HCO_3^-, 1.2 $H_2PO_4^-$ and 11 glucose. In high-potassium Krebs solution, all NaCl (119 mM) was iso-osmotically substituted with KCl. All Krebs solutions were continuously bubbled with 5% CO_2 and 95% O_2. The compounds U46619, $PGF_{2\alpha}$ and ET-1 as well as all salts were of analytical quality and purchased from Sigma–Aldrich (Denmark). Stock solutions were prepared by dissolving in sterile distilled water and stored in aliquots at −20 °C until use.

2.5. Data analysis and statistics

Vasoconstrictive responses were transformed into wall tension (mN/mm) by dividing force (mN) with two times the vessel length (mm). Internal diameter was derived from the normalized inner circumference, and the maximum response to an agonist was determined as the highest force measured. In cumulative concentration–response curves (CCRC) half-log increments of agonist were added to bath. Maternal arteries were exposed to: U46619 (1×10^{-9}–3×10^{-6} M), $PGF_{2\alpha}$ (1×10^{-8}–3×10^{-4} M), and ET-1 (1×10^{-11}–3×10^{-8} M). Uterine isthmus and fundus arteries did not demonstrate any differences in contractility or sensitivity and were therefore pooled and referred to hereafter as maternal arteries. SVA were challenged with U46619 (3×10^{-10}–3×10^{-6} M), $PGF_{2\alpha}$ (1×10^{-7}–3×10^{-4} M), and ET-1 (1×10^{-12}–3×10^{-7} M). Vessels were exposed to each concentration until a stable contraction was attained: time to attain plateau was highly variable therefore no fixed time interval was adhered to, however, steps generally were 6–10 min in duration. The average tension during the last 2 min was analyzed. While not commonly observed, a few arteries oscillated after agonist stimulation. In these few cases, oscillations were permitted to continue for at least 2 min, and then the average force over those 2 min was derived for CCRC analysis. CCRC values were subsequently fitted to a sigmoidal function with variable slope:

$$Y = bottom + (top + bottom)/(1 + 10\hat{}((log(EC_{50}) - X) * HillSlope))$$

The pD_2 was calculated as −log (EC_{50}) and plotted against normalized inner circumference and a linear regression was applied:

$$Y = a * X + b$$

All statistical analyses were performed with Prism v.4 software (GraphPad). Statistical analyses were performed using N (number of placentas/patients) although n (number of vessels) is also reported here for clarity. All data were normally distributed thus statistical comparisons were made using unpaired Students t-test or one-way ANOVA with Tukey's post-test, as appropriate. Data are presented as mean ± standard error of the mean (SEM). A probability (P) value of $P < 0.05$ was considered significant.

3. Results

Potassium-induced constriction was compared between stem villous arteries (SVA) and maternal arteries with inner diameters <600 μm (Table 1). This comparison demonstrated that the contractile capacity of myometrial resistance arteries is greater than SVA of comparative size.

Maximal contractile response in fetal arteries (Table 2) did not differ significantly between $PGF_{2\alpha}$, ET-1 and U46619 ($P = 0.987$;