Rho A/Rho kinase: human umbilical artery mRNA expression in normal and pre eclamptic pregnancies and functional role in isoprostane-induced vasoconstriction

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Abstract

Pre eclampsia represents a state of increased or prolonged vasoconstriction, partially linked to the potent vasocontractile effect of isoprostanes. The process of Rho A-mediated calcium sensitisation is inherent to a state of prolonged contractility in many smooth muscle types. The aim of this study was (1) to investigate mRNA expression levels of Rho A and Rho kinase isoforms (I and II) in the umbilical artery from normotensive and pre eclamptic women and (2) to determine whether the effects of two isoprostanes, 8-iso prostaglandin F2α (8-iso PGF2α) and 8-iso prostaglandin E2 (8-iso PGE2), on umbilical artery tone, were mediated via the Rho kinase pathway. Real-time RT-PCR using primers for Rho A, ROCK I and ROCK II was performed on total RNA isolated from umbilical artery specimens obtained from normotensive and pre eclamptic women. The effects of both isoprostanes (n = 6) (in the absence and presence of the specific Rho kinase inhibitor Y-27632), on umbilical artery tone were measured, and compared with control recordings. Rho A mRNA expression levels were significantly lower in umbilical artery samples obtained from pre eclamptic women (n = 4) in comparison to those from normotensive women (n = 6) (P < 0.05). ROCK I and ROCK II mRNA levels were similar in both vessel types (P > 0.05). Both isoprostanes exerted a significant concentration-dependent vasocontractile effect (n = 7) (P < 0.001) on umbilical artery. For 8-iso PGE2, this effect was antagonised by Y-27632 (n = 6) (P < 0.01). The significant reduction of Rho A mRNA levels in umbilical arteries from pregnancies complicated by pre eclampsia may serve to counteract the diminished perfusion associated with the pathophysiology of pre eclampsia. The vasocontractile effect of 8-iso PGE2 in pre eclampsia may in part be mediated via the Rho kinase pathway.

Introduction

Pre eclampsia is a hypertensive disorder affecting 3–5% of all pregnancies and is a leading cause of maternal and foetal morbidity and mortality (Walker 2000). It is associated with foetal growth restriction, premature birth and low birth-weight babies (Walker 2000, Byrne & Morrison 2001). Pre eclampsia is characterised by intense and prolonged vasospasm. This ultimately leads to elevated systemic vascular resistance and the clinical manifestation of maternal hypertension, which may result in decreased perfusion to organs including the kidney, uterus, placenta, liver and brain (Roberts & Cooper 2001). Central to this condition are mechanisms that regulate vascular smooth muscle contractility, namely signalling pathways that regulate vasoconstriction in the systemic circulation.

Research has indicated that the process of calcium sensitisation (increase in smooth muscle tension and/or phosphorylation of myosin light chains at a constant [Ca2+]i, by inhibition of myosin light chain phosphatase (MLCP)), is of major importance in regulating the state of vasoconstriction of vascular smooth muscle (Somlyo & Somlyo 2000). It is now apparent that the small G protein, Rho A is associated with inhibition of MLCP (Uehata et al. 1997, Kunihiko et al. 1999). Although the precise mechanism of action is unknown, two target proteins of Rho A, ROCK I and its isoform ROCK II, which are collectively known as Rho kinases, have a major role in Rho A-mediated calcium sensitisation. Upon activation, they enhance Rho-mediated calcium sensitisation and hence smooth muscle contractility. It is now clear that this Rho kinase pathway plays a central role in the pathogenesis of hypertension in animal
models, in humans and in various situations of increased peripheral vascular resistance observed in hypertensive disorders (Chitaley et al. 2001) and the prolonged enhanced arterial vasoconstriction in heart failure (Hisaoa et al. 2001). There is no information pertaining to the role of the Rho pathway in foeto-placental vasculature during normal pregnancy or in pregnancies complicated by pre eclampsia. The foeto-placental unit is apparently not innervated (Fox & Khong 1990) and hence the regulation of blood flow to the placenta must depend on structural changes, the influence of vaso-active factors and local signalling mechanisms.

It is known that isoprostanes, metabolites of arachidonic acid, are closely linked to the severe vasoconstriction associated with pre eclampsia (Walsh et al. 2000) and can exert their action in part via the Rho kinase pathway (Janssen et al. 2001). Isoprostanes are implicated in the pathogenesis of a wide variety of human disorders and are used extensively as markers of oxidative stress (Roberts & Morrow 2000), with markedly increased levels reported in disorders associated with increased vascular constriction such as in angina (Cipollone et al. 2000), heart failure (Mallat et al. 1998), pulmonary hypertension (Christman 1998) and pre eclampsia (Barden et al. 1996, Staff et al. 1999, Walsh et al. 2000). To date, there are minimal data outlining the potential role of RhoA/Rho kinase in foeto-placental vasculature. First in normal pregnancies and pregnancies complicated by pre eclampsia, and secondly in the vasoconstrictor actions of isoprostanes. Therefore, the aim of this study was twofold. First, to investigate the mRNA expression levels of Rho A, ROCK I and ROCK II in human umbilical artery in normal pregnancies and pregnancies complicated by pre eclampsia. Secondly, to investigate the effects of two isoprostanes, 8-iso PGF2α and 8-iso PGE2, on human umbilical artery tone and to determine if their effects were mediated via the rho kinase pathway.

Materials and Methods

Tissue collection

Patient recruitment took place in the Department of Obstetrics and Gynaecology, University College Hospital, Galway, Ireland. Ethical Committee approval for tissue collection was obtained from the Research Ethics Committee at University College Hospital Galway and patient recruitment was by written informed consent. For tissue collection, sections of umbilical cord were excised from the proximal segment of the cord (i.e. nearest placental attachment) immediately after vaginal delivery or elective caesarean section at term, from normotensive pregnancies and pregnancies complicated by pre eclampsia. Umbilical artery was dissected free of Warton’s jelly, immediately snap frozen in liquid nitrogen and stored at −80°C. The normotensive group were non-proteinuric patients with uncomplicated pregnancies. The criteria for pre eclampsia were as follows: at least two separate blood pressure readings > 140/90 mmHg, and the presence of +1 protein or more, by dipstick analysis on more than one occasion (Fleming et al. 2000). Women with known pre-existing cardiac or renal disease were excluded from the study. For organ tissue bath studies, sections of umbilical cord excised from the proximal segment of the cord immediately after elective caesarean section were placed in Krebs–Henseleit physiologic salt solution, pH 7.4, containing: 4.7 mmol l−1 KCl, 118 mmol l−1 NaCl, 1.2 mmol l−1 MgSO4, 1.2 mmol l−1 CaCl2, 1.2 mmol l−1 KPO4, 25 mmol l−1 NaHCO3 and 11 mmol l−1 glucose. Indomethacin (10 μmol l−1) was also added to the Krebs–Henseleit solution to prevent generation of cyclo-oxygenase metabolites of arachidonic acid. Cord was stored at 4°C and used within 12 h of collection.

RNA extraction and RT

Total RNA was isolated using TRIzol reagent (Life Technologies) (Chomczynski 1993). All RNA samples were DNA-free treated (Ambion Inc., Austin, TX, USA) and checked by standard RT-PCR to ensure that RNA used for real-time fluorescence RT-PCR contained no contaminating genomic DNA. One microgram of RNA (DNA-free treated) (Ambion Inc.) was reverse transcribed into cDNA for use as a template for PCR. The RNA samples were then denatured at 65°C for 10 min. RT was performed at 42°C for 60 min in a reaction volume of 20 μl containing the following: oligo dT primer (500 ng), Moloney murine leukaemia virus (M-MLV) RT buffer (50 mmol l−1 Tris–HCl pH 8.3, 75 mmol l−1 KCl, 3 mmol l−1 MgCl2, 10 mmol l−1 dithiothreitol (DTT) (Promega), diethylpyrocarbonate (DEPC)-treated water (BDH, Dorset, England), dNTPs (0.2 mmol l−1) (Promega) and 200 U M-MLV reverse transcriptase (Promega). Reverse transcriptase activity was stopped by heating samples at 65°C for 10 min. Control RNA samples, in which no RT enzyme was added, were included to confirm that no genomic DNA contamination was present.

PCR

Five microlitres of the RT reaction mixture was then used in the subsequent PCR. PCR was performed in a final volume of 50 μl containing 1.5 mmol l−1 MgCl2, 20 mmol l−1 Tris–HCl, 50 mmol l−1 KCl pH 8.3 (Life Technologies), 1.25 U Taq DNA polymerase (Life Technologies), 40 μmol l−1 dNTPs (Promega) and 0.2 pmol l−1 each sense and antisense primer. cDNA amplification was carried out by an initial denaturation step of 5 min at 95°C followed by 45 cycles of denaturation at 94°C for 20 s, annealing at 55°C for 45 s and elongation at 72°C for 45 s. Five microlitres of
each PCR product were then separated by gel electrophoresis on a 1.5% agarose gel. Products were separated alongside a 2-log DNA molecular weight ladder for sizing. Primers used were designed to published DNA and mRNA sequences from GenBank as previously reported (Moran et al. 2002, Friel et al. 2005) (Table 1).

One step real-time fluorescence RT-PCR

One step RT-PCR using specific primers for Rho A, ROCK I and ROCK II was performed on total RNA isolated from umbilical artery using the LightCycler (Roche Diagnostics). Reagents from the RNA Amplification kit SYBR Green I (Roche Diagnostics) were used throughout the experiment. Standard curves containing a certain number of cDNA copies were generated for each of Rho A (1 × 10⁹ cDNA copies, 1 × 10⁷ cDNA copies, 1 × 10⁶ cDNA copies), ROCK I (1 × 10⁹ cDNA copies, 1 × 10⁶ cDNA copies) and ROCK II (1 × 10⁶ cDNA copies, 1 × 10⁵ cDNA copies) genes. Copy number/μl cDNA was calculated according to the following formula, available from the Roche Lightcycler website (Curley et al. 2004):

\[
6 \times 10^{23} \text{ [copies/mol]} \times \text{concentration [g/μl]} = \text{amount [copies/μl]} \times \text{molecular weight [g/mol]}
\]

500 ng of the DNA-free treated RNA samples, in which no genomic contamination was present, were used in the subsequent one step real-time fluorescence RT-PCR. This reaction was performed in a final volume of 20 μl containing 6 mmol MgCl₂ l⁻¹, 0.4 μl enzyme mix, 4 μl reaction mix, 2 μl resolution solution, (Roche Diagnostics GmbH), and 0.3 μl 1⁻¹ of each sense and antisense primer. The final volume of 20 μl was achieved using sterile water (Roche Diagnostics GmbH). RT was carried out at 55 °C for 30 min. cDNA amplification was carried out by an initial denaturation step at 95 °C for 30 s, followed by 45 cycles of denaturation at 95 °C with a 5 s hold time, annealing at 55 °C with a 10 s hold time and elongation at 72 °C with a 15 s hold time. The temperature transition rate for the elongation step was 2 °C/s. The temperature transition rate for each step was 20 °C/s unless otherwise stated. Fluorescence data was acquired at the end of each PCR cycle, as previously described (Friel et al. 2005). The LightCycler Software version 3 (fit-points method, Roche), calculated cDNA copy numbers for each gene, generated from their respective amplification curve crossing points (point at which exponential amplification begins) and generated standard curve. This point is equivalent to fluorescence data plotted on the logarithmic scale. Generated cDNA copy numbers for Rho A, ROCK I and ROCK II were then normalised to the housekeeping gene β-actin. Melting curve analysis was performed by an initial denaturation step of 95 °C, cooling to 65 °C for 10 s and finally gradually increasing the temperature to 95 °C. Fluorescence was measured continually during the melting curve cycle.

10 μl of each PCR product were then separated by gel electrophoresis on a 1.5% (w/v) agarose gel. Products were separated alongside a 2-log DNA molecular weight ladder for sizing, cDNA copy numbers for Rho A, ROCK I and ROCK II generated automatically via the LightCycler from their respective standard curves were normalised to the housekeeping gene β-actin.

Umbilical artery tissue bath experiments

Human umbilical artery was dissected free of Warton’s jelly and cut into transverse rings, approximately 3–5 mm in length. Rings were suspended on stainless-steel hooks and mounted in organ tissue baths under 2 g tension as previously described (Dennedy et al. 2002, Ravikumar et al. 2004). The tissue baths contained 10 ml of Krebs–Henseleit physiologic salt solution maintained at 37 °C, pH 7.4 and gassed continuously with 95%O₂/5%CO₂. Individual rings were allowed to equilibrate for at least 90 min, during which time the Krebs–Henseleit physiologic salt solution was changed every 15 min. After the equilibration period, rings were challenged with 60 mM KCl. Once the maximum response to KCl was achieved, rings were washed and allowed to equilibrate for 20 min, to allow base-line to be reached again. The KCl challenge was repeated three times. After 40 min of the final KCl washout, either 8-iso PGF₂α or 8-iso PGF₄ was added in a cumulative manner, at 20 min intervals, at concentrations of 1, 10 and 100 nmol l⁻¹ and 1 and 10 μmol l⁻¹. The mechanical response of tissues was measured by calculation of the

<table>
<thead>
<tr>
<th>RT-PCR Primers</th>
<th>Primer Sequence</th>
<th>Accession Code</th>
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<tbody>
<tr>
<td>Human Rho A</td>
<td>Sense 5'-CTCATAGTCCTTCAAGGACCAGTTF-3'</td>
<td>L25080</td>
</tr>
<tr>
<td>Human ROCK I</td>
<td>Antisense 5'-ATCATCCGAGATTCTTATTT-3'</td>
<td>XM_008814</td>
</tr>
<tr>
<td>Human ROCK II</td>
<td>Sense 5'-GAAGAAGAGAAGGTCCAGAGAAGG-3'</td>
<td>XM_002676</td>
</tr>
<tr>
<td>Human β-actin</td>
<td>Antisense 5'-ATCTTGTAGCTCCCGCATCT-3'</td>
<td>M10277</td>
</tr>
</tbody>
</table>

Table 1 Primers used for standard RT-PCR and real-time fluorescence RT-PCR.
mean amplitude of contraction for 20-min periods using the PowerLab hardware unit and Chart version 3.6 software (AD Instruments, Hastings, UK). The mean amplitude of contraction for the first 20 min (following the 40-min period after the final KCl washout) was calculated and this value served as a control. Antagonism of the effects of 8-iso PGF2α and 8-iso PGE2 were investigated by addition of the rho kinase inhibitor, Y-27632 (10 μmol l⁻¹) 30 min prior to the addition of 8-iso PGF2α or 8-iso PGE2. Control strips were simultaneously run with bath exposure to vehicle, but without addition of drug. The effects of 8-iso PGF2α, 8-iso PGE2 alone and with Y-27632 were expressed in terms of g tension generated.

**Drugs and solutions**

All the chemicals were purchased from Sigma-Aldrich, unless otherwise stated. 8-iso PGF2α and 8-iso PGE2 were obtained from Cayman Chemical, Ann Arbor, MI, USA. A stock solution (10 mmol l⁻¹) of 8-iso PGF2α or 8-iso PGE2 was prepared in dimethylsulphoxide (DMSO). Series of dilutions were made with Krebs–Henseleit physiologic salt solution on the day of experimentation and maintained at room temperature for the duration of the experiment. Y-27632 was kindly donated by Welfide Corporation, Osaka, Japan. A stock solution (10 mmol l⁻¹) of Y-27632 was made with deionised water. Series of dilutions were made with Krebs–Henseleit physiologic salt solution on the day of experimentation. A stock solution (100 mmol l⁻¹) of indomethacin was made in DMSO. Fresh Krebs-Henseleit physiologic salt solution was made daily.

**Statistical analysis**

For the mRNA expression study, normalised cDNA copy numbers for each transcript, between both vessel types, were compared using the Student’s t-test. For the organ tissue bath study, calculated mean g tension for control rings and rings exposed to either 8-iso PGF2α (alone or with Y-27632) and 8-iso PGE2 (alone or with Y-27632) were compared using Student’s t test. A P value of < 0.05 for the Student’s t test was considered to be statistically significant. Comparisons of g tension, for each bath concentration of 8-iso PGF2α (alone or with Y-27632) and 8-iso PGE2 (alone or with Y-27632) were performed using ANOVA followed by Sheffe post hoc comparison where appropriate. The statistical package SPSS for Windows version 11 (SPSS Inc., Chicago, Illinois, USA) was used for these statistical calculations. The concentration of drug resulting in half the maximal effect (i.e. the EC50) was measured and represented in pharmacological terms as its appropriate − log10 value (i.e. − log10 EC50), which is also known as the pD2 value. The mean maximum contractile (MMC) effect is the maximum contractile effect produced by the highest concentration of drug (i.e. 10 μmol l⁻¹). Curve fitting was performed with the package Prism (Graphpad Software, San Diego, USA).

**Results**

**Tissue samples**

For the mRNA expression study, umbilical cords were obtained from six normotensive women and four pre eclamptic women after delivery. All the six normotensive women had elective caesarean sections. The reasons for elective caesarean section were previous caesarean section (n = 5) and breech presentation (n = 1). The mean patient age (years) ± S.E.M. was 35.67 ± 2.06; median gestation 39 weeks (range 38–40); parity 0 (n = 1), 1 (n = 4), 3 (n = 1). Among the four pre eclamptic women, one had an elective caesarean section. The reason for the caesarean section was breech presentation. The mean patient age (years) ± S.E.M. was 32.25 ± 3.90; median gestation 37.5 weeks (range 36–39); parity 0 (n = 2), 1 (n = 1), 2 (n = 1).

For organ tissue bath studies umbilical arteries were obtained from a total of 12 women following delivery. Among these 12 women, eight underwent elective caesarean section. The reasons for elective caesarean section included previous caesarean section (n = 2), breech presentation (n = 3), patient request (n = 1), high head (n = 1) and macrosomia (n = 1). The mean patient age (years) ± S.E.M. was 33.50 ± 2.08; median gestation 39.5 weeks (range 38–41); parity 0 (n = 6), 1 (n = 4), 2 (n = 1), 3 (n = 1).

**Standard RT-PCR**

β-actin, Rho A, ROCK I and ROCK II mRNA expression was detected in all samples (Fig. 1). Amplification of umbilical artery cDNA with the β-actin primer set yielded a 377 bp PCR product. Amplification with the Rho A primer set resulted in a 309 bp PCR product and amplification with ROCK I and ROCK II primers yielded 369 and 390 bp products. These products were

![Figure 1](image-url)
sequenced (MWG-Biotech Ltd, London UK) and results verified that they were the appropriate parts of the β-actin, Rho A, ROCK I and ROCK II gene sequences. PCR of the reverse transcriptase negative controls (RT-) showed no amplification confirming the absence of significant genomic DNA contamination. Similarly, the PCR negative control (no cDNA template) showed no amplification. Therefore, RNA in which no genomic contamination was present was used for subsequent quantitative real-time fluorescence RT-PCR.

One-step fluorescence RT-PCR

To compensate for any undue experimental error, analyses of each gene, for both vessel types, were performed in triplicate. The mean values of these experiments were used for statistical analysis. The four primer sets yielded RT-PCR products of the expected sizes (data not shown). All the patients showed expression of β-actin, Rho A, ROCK I and ROCK II mRNA. Standard curves generated for each of the genes under investigation were used to determine their respective transcript number, per 0.5 μg total RNA, in both vessel types studied. Using the LightCycler Software version 3 (fit-points method), calculated cDNA copy numbers for each gene were generated from their respective amplification curve crossing points (point at which exponential amplification begins) as previously described (Friel et al. 2005). A representative recording of fluorescence plotted on the logarithmic scale corresponding to Rho A amplification in umbilical artery is shown in Fig. 2. The melting peak analyses of Rho A, ROCK I and ROCK II showed specificity of product amplification (data not shown).

β-actin mRNA expression did not significantly differ between normotensive and pre eclamptic umbilical arteries (Table 2), which indicated that β-actin was suitable as a housekeeping gene for this vessel type. Therefore, cDNA copy numbers for Rho A, ROCK I and ROCK II were normalised to the β-actin gene for the determination of their absolute cDNA copy numbers per 0.5 μg total RNA. Comparisons of cDNA copy numbers, between both groups, for Rho A, revealed that Rho A mRNA expression was significantly down-regulated in artery obtained from pre eclamptic women in comparison to that measured in artery obtained from normotensive women (P < 0.05). The cDNA copy numbers (per 0.5 μg total RNA) ± the s.e.m. for Rho A were: (normal) 7.0 × 10^7 ± 7.6 × 10^6 (n = 6) and (pre eclamptic) 4.8 × 10^7 ± 4.5 × 10^6 (n = 6) (Fig. 3). The mRNA expression levels of ROCK I and ROCK II were not significantly different between the two vessel types analysed (P > 0.05). The cDNA copy numbers for ROCK I were: (normal) 1.3 × 10^7 ± 8.7 × 10^5 (n = 6);

![Figure 2](image-url)

**Figure 2** Quantitative real-time fluorescence RT-PCR amplification curve for Rho A mRNA expression in human umbilical artery (both normal and pre eclamptic). Fluorescence is plotted on the y-axis and PCR cycle number on the x-axis. Continuous lines represent the Rho A cDNA standards (1 × 10^5 and 1 × 10^7 cDNA copy numbers). Closed circles represent normal samples (n = 6), open circles represent pre eclamptic samples (n = 4) and closed squares represent the water control.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Normal</th>
<th>Pre eclamptic</th>
</tr>
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<tbody>
<tr>
<td>Rho A</td>
<td>7.0 × 10^7 ± 7.6 × 10^6</td>
<td>4.8 × 10^7 ± 4.5 × 10^6</td>
</tr>
<tr>
<td>ROCK I</td>
<td>1.3 × 10^7 ± 8.7 × 10^5</td>
<td>1.0 × 10^7 ± 1.9 × 10^5</td>
</tr>
<tr>
<td>ROCK II</td>
<td>5.2 × 10^7 ± 1.1 × 10^7</td>
<td>3.0 × 10^7 ± 6.7 × 10^6</td>
</tr>
<tr>
<td>β-actin</td>
<td>2.8 × 10^7 ± 4.5 × 10^5</td>
<td>4.1 × 10^4 ± 9.6 × 10^7</td>
</tr>
</tbody>
</table>

*Values presented are means ± S.E.M.

**Table 2** cDNA copy numbers ± S.E.M. for Rho A, ROCK I, ROCK II and β-actin in human umbilical artery (both normal and pre eclamptic).

![Figure 3](image-url)

**Figure 3** Rho A, ROCK I, ROCK II and β-actin mRNA expression in human umbilical artery from normal pregnancies (N; n = 6) and pre eclamptic pregnancies (PET; n = 4) by real-time fluorescence RT-PCR. cDNA copy numbers are shown on the y-axis and the genes investigated on the x-axis. The histogram depicts Rho A, ROCK I and ROCK II cDNA copy numbers normalised to the housekeeping gene β-actin. Grey columns represent normal samples. Columns with diagonal grey stripes represent pre eclamptic samples. Vertical error bars represent standard error of the mean (S.E.M.). *N vs PET P < 0.05.
Effects of isoprostanes on umbilical artery

Both 8-isoprostane PGF2α and 8-isoprostane PGE2 exerted a significant concentration-dependent vasocontractile effect on human umbilical artery. This is graphically represented as a histogram in Fig. 4 for 8-isoprostane PGF2α, and in Fig. 5 for 8-isoprostane PGE2. The MMC effect (in g tension) and the pD2 values (± S.E.M.) are detailed in Table 3. Calculated increases in g tension for control rings and rings exposed to 8-isoprostane PGF2α were compared by Student’s t-test. Analysis revealed a significant contractile effect at increasing 8-isoprostane PGF2α concentrations of 1 μmol l⁻¹ (P<0.01) and 10 μmol l⁻¹ (P<0.001). Similarly, calculated increases in g tension for control rings and rings exposed to 8-isoprostane PGE2 were compared by Student’s t-test. Again, analysis revealed a significant contractile effect at increasing 8-isoprostane PGE2 concentrations of 1 (P<0.001) and 10 μmol l⁻¹ (P<0.001). 8-isoprostane PGE2 induced vasoconstructions were significantly greater than those induced by 8-isoprostane PGF2α (P<0.05). There was no significant difference between pD2 (P>0.05) values for both compounds.

Effects of rho kinase antagonism on umbilical artery

8-isoprostane PGE2 induced contractions were significantly antagonised by the specific rho kinase inhibitor Y-27632 (P<0.01). This is demonstrated graphically in Fig. 5. 8-isoprostane PGF2α induced contractions were not significantly antagonised (P>0.05) (Fig. 4). The MMC and pD2 values (± S.E.M.) for antagonised 8-isoprostane PGF2α and 8-isoprostane PGE2 are detailed in Table 3. There was no significant difference in pD2 values for antagonised 8-isoprostane PGF2α and 8-isoprostane PGE2 in comparison to 8-isoprostane PGF2α (P>0.05) and 8-isoprostane PGE2 (P>0.05) alone.

Discussion

Pre eclampsia is one of the major disorders of obstetrics practice, which contributes to maternal and perinatal morbidity and mortality. An understanding of the biological processes that result in the adverse maternal and foetal consequence is lacking. The factors regulating the foeto-placental vasculature during normal pregnancy, and in pre ecleampsia, are poorly understood. The Rho A/Rho kinase system is closely linked to prolonged states of smooth muscle contraction, or vasoconstriction, and is closely linked to hypertensive disorders in animal and human models. For these reasons, we hypothesised that the Rho A/Rho kinase system may be linked to normal foeto-placental system.

Table 3 Effects of 8-isoprostane PGE2 and 8-isoprostane PGF2α alone and antagonised by Y-27632 on human umbilical arterial tone.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Contractility (g tension)</th>
<th>pD2</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-isoprostane PGE2</td>
<td>2.91 ± 0.14 (n=7)</td>
<td>6.77 ± 0.13</td>
</tr>
<tr>
<td>8-isoprostane PGF2α</td>
<td>1.42 ± 0.28* (n=6)</td>
<td>6.65 ± 0.49</td>
</tr>
<tr>
<td>8-isoprostane PGE2 + Y-27632</td>
<td>1.99 ± 0.27 (n=7)</td>
<td>6.07 ± 0.35</td>
</tr>
<tr>
<td>8-isoprostane PGF2α + Y-27632</td>
<td>1.68 ± 0.40 (n=6)</td>
<td>5.73 ± 0.18</td>
</tr>
</tbody>
</table>

Values presented are MMC mean ± S.E.M.
*P<0.01 vs 8-isoprostane PGE2 alone.
circulatory regulation and the changes that occur in pre eclampsia.

We have demonstrated that the mRNA expression of Rho A appears to be down regulated in umbilical arteries in association with pre eclampsia. An obvious interpretation of this finding is that there is reduced expression, with presumably reduced activity of the Rho A/Rho kinase pathway in these vessels, in association with pre eclampsia, which may facilitate greater vasodilatation or enhanced foetal blood flow. Therefore, these findings imply that Rho A/ROCK does not influence the increased vasoconstriction seen in association with PET. These data are preliminary, and there are limitations in concluding from these findings. The total RNA for these results was extracted from total human umbilical artery preparations, and hence includes the endothelium and the vascular smooth muscle layer. This was the deliberate design of the experiments, as it would have been technically difficult to denude these vessels, and these samples were all snap frozen in the operating theatre from women with pre eclampsia or normal pregnancy. Further attempts to explore this issue, i.e. to evaluate and quantify Rho A/Rho kinase pathway expression or activity in the vascular smooth muscle, would require methods that are not as accurate in terms of quantitation, such as immunohistochemical techniques. The other issue, which needs to be addressed, is that of the protein expression and that would require Western Blotting experiments. However, as a preliminary finding, it is apparent from our experiments that Rho A is downregulated at the mRNA level in total umbilical artery vessels from women with pre eclampsia in comparison to control women with normal pregnancies.

It is evident that isoprostanes contribute significantly to the prolonged vasoconstriction that occurs in pre eclampsia. Using umbilical artery ring preparations, with standard in vitro techniques, we have demonstrated that the two isoprostanes 8-iso PGE2 and 8-iso PGF2α, both exert a potent vasoconstrictor effect as has been demonstrated previously (Oliveira et al. 2000). By preincubation with a specific Rho kinase inhibitor, it is clear from our experiments that 8-iso PGE2 is unable to elicit the same response after Rho kinase inhibition, indicative of the fact that the Rho kinase pathway is involved in the vasoconstrictor effect of 8-iso PGE2. These results were not found for the vasoconstrictor of 8-iso PGF2α. There is no obvious reason why the effects of 8-iso PGF2α are apparently different to those of 8-iso PGE2, but it is evident that a different mechanism for 8-iso PGE2 exists, which operates at least in part via the Rho kinase pathway. On speculation, this difference observed in relation to antagonism with Y-27632 can only be due to a relative difference in potencies observed in tissues, whereby 8-iso-PGE2 is more potent (Oliveira et al. 2000, Tazzeo et al. 2003). There are no signalling pathways to our knowledge, known to operate via 8-iso-PGE2 and 8-iso-PGF2α directly. Finally, a further limitation in interpreting these data relate to the fact that, while the cyclooxygenase pathway was blocked, the lipoxygenase pathway was not.

In summary, these findings highlight the potential importance of the Rho A/Rho kinase pathway in the umbilical artery circulation in normal pregnancy, and raise the question of reduced expression at the mRNA level for Rho A in pre eclampsia. The factors regulating these potential changes require further investigation. Future studies include the assessment of the protein expression of the various components of the Rho A/Rho kinase pathway in normal pregnancy and in pregnancies complicated by pre eclampsia. Finally, from a functional point of view, the vasocontractile effect of 8-iso PGE2, a potent isoprostane linked to pre eclampsia appears to be mediated at least in part via the Rho kinase pathway.

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