Chronic Glucocorticoid Exposure Potentiates Placental Chorionic Plate Artery Constriction: Implications for Aberrant Fetoplacental Vascular Resistance in Fetal Growth Restriction


Maternal and Fetal Health Research Centre (J.L.N., M.W., V.P., C.P.S., R.L.J.) and Endocrine Sciences Research Group (D.W.R., S.N.F.), Institute of Human Development, Manchester Academic Health Science Centre, University of Manchester, Manchester M13 9WL, United Kingdom; and Liggins Institute (P.N.B.), University of Auckland, 1142 Auckland, New Zealand

Fetal growth restriction (FGR) is a serious pregnancy complication, resulting in significant perinatal morbidity and mortality. Increased vascular resistance in the fetoplacental circulation is a hallmark of FGR and is associated with enhanced vasoconstriction of the resistance arteries in the placenta, the chorionic plate arteries (CPAs). Although the cause is unknown, FGR is associated with excess exposure to glucocorticoids (GCs), key mediators of vascular resistance in the systemic circulation. We hypothesized that GCs alter CPA reactivity, thereby contributing to the altered blood flow dynamics seen in FGR. We aimed to examine the acute and chronic effects of GCs on CPA reactivity and the operational mechanisms. Glucocorticoid receptors were highly expressed by CPA. 11β-Hydroxysteroid isoenzyme type 2 was detected within the endothelium, whereas 11β-hydroxysteroid isoenzyme type 1 was absent. Acute GC treatment significantly attenuated U46619-induced constriction. This effect was reversed by cotreatment with mifepristone or an endothelial NOS inhibitor. In contrast, chronic GC treatment potentiated U46619 constriction in a dose-dependent manner, which was partially abolished by mifepristone cotreatment. Similar effects were observed using a novel nonsteroidal glucocorticoid receptor-specific agonist. Chronic treatment with GCs altered the expression of several vasoactive factors, including thromboxane and bradykinin receptors, prokineticin-1, cyclooxygenase-2, and endothelial NOS. In summary, acute and chronic GC treatment exerts contrasting effects on CPA vasoactivity. These opposing effects are consistent with temporal actions in other vascular beds and reflect activation of distinct nongenomic and genomic pathways. Chronic exposure to elevated GCs may contribute to the raised vascular resistance observed in the fetoplacental circulation in FGR. (Endocrinology 154: 876–887, 2013)
nervated and therefore fetoplacental blood flow is regulated primarily by local environmental and humoral factors (8). Umbilical arteries branch on the fetal surface of the placenta to form chorionic plate arteries (CPAs); these arteries exhibit the characteristics of resistance arteries (<300 μm internal diameter) and are therefore likely to contribute significantly to modulating vascular tone in the fetoplacental circulation (9). CPA reactivity has previously been shown to be altered in placentas from pregnancies complicated by FGR (10), with increased constriction to the thromboxane-mimetic, U46619, and increased relaxation to the nitric oxide (NO) donor, sodium nitroprusside (SNP), suggesting aberrant control of vascular tone contributes towards the increased vascular resistance.

Glucocorticoids (GCs) have well-documented actions in the regulation of systemic vascular reactivity, both via genomic and nongenomic pathways (11). Glucocorticoid receptors (GRs) are expressed in both smooth muscle and endothelial cells (12, 13) together with both 11β-hydroxysteroid (11β-HSD) isoforms, indicating a possible role for prereceptor GC metabolism (14). The placenta and developing fetus are partially protected from maternal cortisol in pregnancy by the activity of 11β-HSD2 in the placental syncytiotrophoblast, in which this enzyme metabolizes cortisol to its inactive metabolite cortisone (15). However, in pregnancies complicated by FGR, 11β-HSD2 expression and activity is reduced (16). It has been speculated that the ensuing elevated exposure of the fetoplacental unit to cortisol contributes to the pathogenesis of FGR, a hypothesis supported by reduced birth weight in animal models after maternal GC administration (17-19) or gene ablation of 11β-HSD2 (20). Given the established roles for GCs in modulating vascular tone, GC excess may contribute to the increased CPA vasoconstriction and raised vascular resistance observed in placentas from pregnancies complicated by FGR.

The in vitro effects of acute exposure to GCs on the fetoplacental circulation have been investigated previously. In perfused placental cotyledons, dexamethasone infusion significantly reduced basal arterial pressure and induced vasodilatation following a submaximal KCl-induced vasoconstriction (21). Hydrocortisone, dexamethasone, and betamethasone also had an acute vasodilatory effect on rings of human umbilical arteries, preconstricted with 5-hydroxytryptamine (22). These vasodilatory effects are consistent with previous observations of acute actions of GCs in systemic blood vessels. However, the longer-term actions of GCs on placental arteries have not been examined.

We hypothesized that GCs alter CPA reactivity, thereby contributing to the altered blood flow dynamics seen in pregnancies complicated by FGR. The aims of this study were to determine the following: 1) whether CPAs express the proteins involved in the cellular actions of GCs and 2) the acute and chronic actions of GCs on the vascular reactivity of CPAs.

Materials and Methods

All drugs and chemicals were obtained from Sigma-Aldrich (Dorset, UK) unless stated otherwise.

Placental collection

The Central Manchester and North West Regional Ethics Committees gave approval for this work at St Mary’s Hospital, Manchester (08/H1010/55). Written informed consent was obtained for all samples used. Term placentas were obtained after vaginal delivery or elective cesarean section from women who had otherwise uncomplicated pregnancies. All deliveries were between 37 and 42 weeks of gestation and had an individualized birth weight centile 10th or greater and 90th or less centile. Women with preexisting medical or obstetric disorders were excluded.

Localization of GR and 11β-HSD isoforms in CPA

Chorionic plate biopsies were fixed in 10% neutral-buffered formalin at 4°C, prior to routine wax embedding. Immunohistochemical staining for vascular markers α-smooth muscle actin (α-SMA; vascular smooth muscle cells [VSMCs]) and CD31 (endothelial cells) was performed on 5-μm tissue sections to identify CPA as previously described (23) using monoclonal antibodies: anti-CD31 and anti-α-SMA (Dako, Glostrup, Denmark) applied at 1.33 and 5 μg/mL, respectively, followed by standard immune-peroxidase detection with diaminobenzidine chromogen and hematoxylin counterstaining. A similar protocol was utilized to localize GR, 11β-HSD1, and 11β-HSD2 within CPA. For GR, a mouse monoclonal antibody (Novocastra, Leica Biosystems, Milton Keynes, United Kingdom) was applied at 2.4 μg/mL. For 11β-HSD1, a rabbit polyclonal antibody raised against human 11β-HSD1 (Cayman Europe, Tallinn, Estonia) was applied at 2 μg/mL. For 11β-HSD2, a sheep polyclonal antihuman 11β-HSD2 (kind gift from M. Kilby and P. Stewart, University of Birmingham, Birmingham, UK) antibody was applied at 9 μg/mL. In all cases, a serial section was incubated with nonimmunized IgG from the appropriate species at a matching concentration to the primary antibody as a negative control. Immunohistochemically stained chorionic plate section images were obtained using an Olympus BX41 microscope with QI-cam video capture (Olympus, Southend-on-Sea, United Kingdom), and ImagePro Plus 7.0 Software (Media Cybernetics, Marlow, United Kingdom). CPAs were identified by anti-CD31 and anti-α-SMA; vascular smooth muscle cells (VSMCs)

Acute effects of GCs on chorionic plate vascular reactivity

Chorionic plate biopsies were taken within 30 minutes of delivery and placed into ice-cold physiological salt solution (PSS) [in millimoles: NaCl 119; NaHCO3 25; KCl 4.69; MgSO4 2.4;
CaCl₂ 1.6; KH₂PO₄ 1.18; glucose 6.05; EDTA 0.034, pH 7.4). Under a stereomicroscope, small CPAs were identified and dissected. Four arterial segments, 2-3 mm in length, were mounted on a Danish Myotechnologies M610 wire myograph (Aarhus, Denmark). Each bath contained 6 mL of PSS, warmed to 37°C, and gassed with 5% O₂/5% CO₂/N₂ (BOC Special Gases, Worsley, United Kingdom). CPAs were normalized to 0.9 of L₅.1 kPa as described previously (9). After normalization, vessels were equilibrated (20 minutes) prior to the commencement of the experiment. Vessel viability was assessed using 120 mM KCl PSS (KPSS), and vessels were excluded if they did not constrict more than 1.995 kPa.

Effect of acute GC exposure on vasodilatation

CPAs were preconstricted with U46619 (10⁻⁷ M; approximate EC₉₀) and relaxation measured in response to incremental doses of hydrocortisone (10⁻¹⁰ to 10⁻⁵ M) or dexamethasone (10⁻¹⁰ to 10⁻⁵ M at 2 minute intervals). Vessel segments were also incubated with carbenoxolone (an 11β-HSD2 inhibitor, 10⁻⁷ M) for 15 minutes prior to preconstriction with U46619 (10⁻⁷ M). Vessel relaxation was then measured in response to incremental doses of hydrocortisone (10⁻¹⁰ to 10⁻⁵ M at 2 minute intervals). Time controls were exposed to an equivalent volume of diluent and run in parallel.

Effect of acute GC exposure on basal tone and subsequent vasoconstriction

CPAs were incubated with hydrocortisone (10⁻⁶ M) in the presence and absence of carbenoxolone (10⁻⁷ M) or dexamethasone (10⁻⁶ M) for 60 minutes. Basal tone was measured at time 0 and 60 minutes after the treatment. Vessels were then exposed to increasing doses of U46619 (10⁻¹⁰ to 2 × 10⁻⁶ M). Time controls were exposed to an equivalent volume of diluent and performed in parallel. To investigate potential effectors of GC effects, a subset of CPA was treated with dexamethasone (10⁻⁶ M) in the presence and absence of either mifepristone (a GR antagonist, 10⁻⁵ M) or N⁶(ω)-nitro-L-arginine (LNNa; nitric oxide synthase (NOS) inhibitor, 10⁻⁵ M) for 60 minutes. Basal tone was assessed before and after the treatment, and the effect of increasing doses of U46619 was assessed as above.

Effect of long-term GC-exposure on CPA gene expression

A further set of cultured vessels was fixed in 10% neutral buffered formalin, wax embedded, and immunostained for vascular markers as described above. Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) staining was also performed to assess vascular cell viability using the in-situ cell death detection kit with peroxidase detection (Roche Diagnostics, Mannheim, Germany) in accordance with the manufacturer’s instructions with minor modifications. The reaction mix comprised terminal deoxynucleotidyl transferase (prediluted 1:10 with TUNEL dilution buffer), and fluorescein isothiocyanate-labeled deoxyuridine 5-triphosphate (prediluted 1:2), at a ratio of 1:10. For negative controls, fluorescein isothiocyanate-deoxyuridine 5-triphosphate was applied alone.
degrees of freedom are quoted for all significant effects. The EC50 (Figure 1I). Neither the endothelial cells or VSMCs were consistent with the intense staining detected in the syncytiotrophoblast of villos placenta included as a positive control (Figure 1I). Neither the endothelial cells or VSMCs were immunostained for 11β-HSD1 (Figure 1J), whereas strong staining was detected in decidual cells in fetal membranes included as a positive control (Figure 1K). All negative controls exhibited no staining (Figure 1, C, I inset, and L).

Statistical analysis

Vessel tension production was expressed as a percentage of the initial maximal constriction to KPSS or as active wall tension (mN per millimeter) and transformed to active effective pressure (kilopascals) to standardize for variations in vessel diameter. For statistical analysis of U46619 and SNP dose-response curves, data were log transformed and compared by repeated-measures, 2-way ANOVA with Bonferroni post hoc tests using GraphPad Prism (GraphPad, San Diego, California). Sensitivity and maximal effect data were compared using the Mann-Whitney U test or Kruskall-Wallis with Dunn’s post hoc test. All data are expressed as mean ± SEM or median interquartile range for n placentas; 3 or 4 vessels were studied and averaged per placenta. A value of P < .05 was indicative of statistical significance.

Results

Localization of GR and 11β-HSD1 and -2 in CPA

Immunoreactivity for CD31 and α-SMA was used to identify the endothelial and vascular smooth muscle layers in the walls of CPA (Figure 1, A and B). Both the endothelium and vascular smooth muscle showed strong nuclear immunoreactivity for GR (Figure 1, D and E). Villous placenta was included as a positive control (24) and showed strong nuclear immunoreactivity in the stromal compartment; no staining was detected in the negative control (Figure 1F and inset). Faint immunoreactivity for 11β-HSD2 was detected in the endothelial, but not smooth muscle, cell layer (Figure 1, G and H) in comparison with the intense staining detected in the syncytiotrophoblast of villos placenta included as a positive control (Figure 1I). Neither the endothelial cells or VSMCs were

Table 1. RT-QPCR Primer Details.

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Acute effects of GCs on CPA vascular reactivity

Effect of acute GC exposure on vasorelaxation

Dexamethasone (10−10 to 10−5 M), or hydrocortisone (10−10 to 10−5 M) in the presence and absence of carbenoxolone (10−7 M), did not promote relaxation of CPAs preconstricted with U46619 (n = 16 placentas, P > .05, 2 way ANOVA, data not shown).

Effect of acute GC exposure on basal tone and subsequent vasoconstriction

Basal tone was unaltered in CPAs treated with dexamethasone (10−6 M) or hydrocortisone (10−6 M) in the presence of carbenoxolone (10−7 M) for 1 hour (data not shown). U46619-induced contraction was significantly attenuated with dexamethasone (10−6 M) pretreatment compared with controls (P < .01, F = 7.83, Df = 1, 2 way ANOVA; Figure 2A); however, sensitivity (EC50) and maximum constriction to U46619 were not significantly different (P > .05, Mann-Whitney U test, data not shown). Hydrocortisone (10−6 M) pretreatment in the absence or presence of carbenoxolone (10−7 M) or carbenoxolone (10−7 M) alone did not significantly modify U46619-induced contraction of CPAs (P < .05, 2 way ANOVA; data not shown).

The attenuation in constriction to U46619 in CPAs after treatment with dexamethasone appeared to be abolished by cotreatment with mifepristone, but this failed to
reach statistical significance ($P = .07, F = 3.60, Df = 1, 2$ way ANOVA; Figure 2B). Cotreatment with dexamethasone and LNNA inhibited the attenuation in U46619-induced constriction ($P < .05, F = 5.59, Df = 1$, compared with dexamethasone treatment, 2 way ANOVA; Figure 2C).

**Chronic effect of GCs on CPA vascular reactivity**

**Determination of optimal conditions for CPA culture**

Preliminary experiments demonstrated a significant reduction in KPSS- and U46619-induced contraction of

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**Figure 1.** Immunolocalization of GR, $11\beta$-HSD1, and $11\beta$-HSD2 in CPAs. Immunoreactivity for endothelial marker CD31 (A) and $\alpha$-SMA (B), with representative negative control (C) is shown. D–F, GR immunostaining. D and E, GR localized to CPA endothelial (EN) and VSMCs (examples indicated with arrowheads). F, Term villous placenta was included as a positive control and exhibited strong immunostaining in villous stroma (STR) cells (arrows). The negative control is shown in inset. G–I, Immunostaining for $11\beta$-HSD2 in endothelial cells of CPA (G and H, arrowheads) and syncytiotrophoblast (ST) (I) of term placenta as positive control; inset is negative control. J–L, $11\beta$-HSD1 immunostaining. Staining was absent in CPA (J) but in intensely localized to decidual stromal cells (DEC) in term fetal membranes (K). L, Negative control for $11\beta$-HSD1 in fetal membranes. M–O, Representative images of cultured CPAs stained for endothelial and apoptotic markers. M, Disrupted endothelium in cultured CPAs identified with immunostaining for CD31 (arrowhead). N, Apoptotic endothelial cells in cultured CPAs identified with TUNEL staining (arrowheads). O, TUNEL-negative control in cultured CPAs. Scale bars, 100 $\mu$m (A–D, G, J–M) and 25 $\mu$m (E, F, H, I, N, and O).
CPAs cultured for 24 hours in DMEM- or CMRL-based media using 20% O₂ when compared with fresh CPAs (data not shown). CPAs cultured in DMEM for 24 hours at 5% O₂ produced similar contractile function compared with fresh vessels from the same placenta; in CMRL, vessel reactivity was significantly attenuated (n = 6, P < .05, Mann-Whitney U test; Figure 3, A and B). Culture in DMEM for 24 hours at 5% O₂ did not significantly modify SNP-induced relaxation of CPAs preconstricted with an EC₈₀ dose of U46619 up to a dose of 10⁻⁵ M (Figure 3C); with 10⁻⁸ M, SNP-cultured CPAs remained in a relaxed state, whereas fresh CPAs reconstituted (P < .05, F = 4.99, Df = 1, 2 way ANOVA; Figure 3, A and B). Culture in 5% O₂ produced similar contractile function compared with fresh vessels from the same placenta; in CMRL, vessel reactivity was significantly attenuated (n = 6, P < .05, Mann-Whitney U test; Figure 3, A and B). Culture in DMEM for 24 hours at 5% O₂ did not significantly modify SNP-induced relaxation of CPAs preconstricted with an EC₈₀ dose of U46619 up to a dose of 10⁻⁵ M (Figure 3C); with 10⁻⁸ M, SNP-cultured CPAs remained in a relaxed state, whereas fresh CPAs reconstituted (P < .05, F = 4.99, Df = 1, 2 way ANOVA; Figure 3, A and B).

Figure 2. Effect of acute dexamethasone (Dex) treatment on CPA vasoconstriction. A, The dose-response curve to thromboxane mimetic U46619 was significantly attenuated in CPAs pretreated with Dex (10⁻⁶ M) for 1 hour compared with diluent control. **P < .01 (2 way ANOVA). All data are mean ± SEM (n = 22). The attenuation in constriction to U46619 by dexamethasone was partially inhibited by cotreatment with mifepristone (B) (P = .076) and abolished by cotreatment with LNNA (C). *P < .05. All data are mean ± SEM (n = 8, 2 way ANOVA with Bonferroni post hoc test).

Chronic effect of GCs on CPA vasoconstriction and dilatation

The DMEM-based culture system (DMEM; 24 hours; 5% O₂) was used to assess the effect of chronic effect of GCs on CPA function. Dexamethasone (10⁻⁸ to 10⁻⁶ M) significantly increased U46619-induced constriction in a dose-dependent manner [n = 12; 10⁻⁸ M (P = .05, F = 3.99, Df = 1); 10⁻⁷ M (P < .01, F = 8.90, Df = 1); 10⁻⁶ M (P < .001, F = 13.31, Df = 1) vs vehicle control; 2 way ANOVA; Figure 4, A–C]. Post hoc tests showed significantly enhanced constriction in CPA treated with dexamethasone (10⁻⁶ M) at concentrations of U46619 of 10⁻⁸ M and above. Sensitivity to U46619 (EC₅₀) was not significantly altered (P < .05, Kruskal-Wallis, Figure 4D), but dexamethasone (10⁻⁶ M) induced a significantly elevated maximal constriction to U46619 (P < .05, Kruskal-Wallis with Dunn’s post hoc test, Figure 4E).

Hydrocortisone incubation (24 hours; 10⁻⁶ M) significantly increased U46619-induced vasoconstriction (n = 12, P < .0001, F = 18.15, Df = 1; 2 way ANOVA; Figure 5A). Sensitivity to U46619 (EC₅₀) was significantly increased (P < .01, Mann Whitney, Figure 5B), without significant alterations in maximum constriction (not shown). The effect of hydrocortisone was dose dependent (10⁻⁷ and 10⁻⁸ M had no effect on U46619 constriction; n = 6, data not shown) and was abolished in the presence of carbenoxolone (P > .05, 2 way ANOVA; Figure 5C). Culture of CPAs with carbenoxolone alone had no effect on U46619-induced constriction (Figure 5D).
To determine the mechanism underlying GC-potentiated vasoconstriction, CPAs were treated with mifepristone (GR antagonist, 24 hours, $10^{-5}$ M), in the presence and absence of dexamethasone ($10^{-6}$ M), together with untreated time controls (Figure 5E). The previously observed potentiation of vasoconstriction was observed with dexamethasone treatment ($10^{-6}$ M; $P = .0014$, $F = 10.81$, $Df = 1$ compared with control CPA, 2 way ANOVA). Cotreatment with dexamethasone and mifepristone partially abolished this effect, although this did not reach statistical significance ($P = .14$, $F = 2.22$, $Df = 1$, compared with dexamethasone alone, 2 way ANOVA). Mifepristone treatment alone had a marginal, but not significant, stimulatory effect on vasoconstriction compared with control vessels, suggesting a slight agonist response to mifepristone in this model ($P = .09$, $F = 2.86$, $Df = 1$).

Further confirmation of an involvement of GR in the potentiation of U46619-induced vasoconstriction was obtained by treating CPAs with a nonsteroidal GR agonist (GSK47869A at $10^{-6}$ M; 24 hours). In the continued presence of GSK47869A, U46619-induced vasoconstriction was significantly enhanced, confirming that the effect is mediated through the GR ($P = .012$, $F = 6.54$, $Df = 1$, 2 way ANOVA; Figure 5F).

Dexamethasone ($10^{-8}$ to $10^{-6}$ M) did not significantly modify SNP-induced relaxation of CPAs preconstricted with an EC$_{80}$ dose of U46619 ($P > .05$, 2 way ANOVA; data not shown).

Effect of GCs on CPA gene expression

To investigate the potential molecular mechanisms involved in GC-enhanced vasoconstriction, expression of 6 genes [thromboxane receptor (TBX-R), prokineticin-1 (PROK1), angiotensin II receptor type 1 (AGTR1), bradykinin receptor, cyclooxygenase (COX)-2, and endothelial nitric oxide synthase (eNOS), known to regulate CPA tone] were amplified using real-time PCR in CPAs cultured with dexamethasone or hydrocortisone ($10^{-6}$ M), compared with vehicle control. Exponential amplification curves were obtained, with no amplification in the negative control. TBP (housekeeping gene) mRNA was not significantly altered by GCs; thus, all data are normalized for TBP expression levels. TBX-R mRNA expression was significantly up-regulated by dexamethasone ($P < .001$) and hydrocortisone ($P < .01$), whereas PROK1 was up-regulated by hydrocortisone only ($P < .05$) and AGTR1 was unchanged by GC treatment (Figure 6). Bradykinin receptor mRNA expression was significantly reduced by dexamethasone or hydrocortisone ($P < .001$), as was COX-2 ($P < .01$). Unexpectedly, eNOS mRNA expression was significantly elevated by dexamethasone or hydrocortisone ($P < .05$ for both).
The aim of this study was to determine whether GCs affect CPA vascular reactivity and hence have the potential to contribute to the aberrant vascular function observed in pregnancies complicated by FGR. Strong nuclear immunoreactivity for GR was identified in CPA endothelial and vascular smooth muscle cells, supporting that GCs may influence fetoplacental vascular tone via actions on both cell types. Acute exposure to dexamethasone induced a small, but significant, reduction in vasoconstriction, whereas chronic exposure to dexamethasone or hydrocortisone exerted a dose-dependent augmentation of vasoconstriction. These 2 opposing effects are consistent with temporal actions in other vascular beds and reflect downstream activation of distinct nongenomic and genomic pathways.

Acute dexamethasone exposure attenuated U46619-induced vasoconstriction of CPAs by approximately 15%. A reduction of this magnitude would be expected to significantly affect spatial blood flow within the placenta due to the key role of CPA as placental resistance arteries (8). However, in contrast to previous observations using the intact placental lobule perfusion technique (21), dexamethasone did not induce dilatation in unstimulated or preconstricted CPAs. This is likely due to differences in the methodologies used; placental perfusion models incorporate a number of blood vessel subtypes and nonvascular cell types in the villous tissue, which may respond to experimental conditions by release of vasoactive factors (eg, thromboxane, prostaglandins) (25). The current study delineates the contribution of altered CPA tone to the overall effect and suggests that acute GCs induce additional vasorelaxatory effects in the distal portions of the vascular tree.

The importance of GR to the acute responses to dexamethasone is underlined by the abolition of the effect with mifepristone. Furthermore, the inhibition/attenuation of the dexamethasone effect by the eNOS inhibitor LNNA suggests that short-term dexamethasone treatment blunted CPA vasoconstriction through a local up-regulation of NO synthesis stimulated by nongenomic GR activation. This mechanism is consistent with previous reports of the nongenomic actions of GCs in human saphenous vein endothelial cells (26).

The observations after acute exposure of CPAs to GCs may explain the acute improvement in umbilical artery Doppler waveforms after corticosteroid administration in pregnancies complicated by severe FGR (27, 28). However, the chronic effects of GCs on the fetoplacental vasculature are clinically more relevant in deciphering the

![Figure 4](https://academic.oup.com/endo/article-abstract/154/2/876/2423646/883)
Figure 5. Effects of chronic exposure to glucocorticoid agonists and antagonists on CPA vasoconstriction. A, Constriction to U46619 was potentiated after culture with hydrocortisone (HC; $10^{-6}$ M) compared with diluent control. ** $P < .001$ (2 way ANOVA). B, Hydrocortisone increased sensitivity to U46619, demonstrated by a significantly lower EC$_{50}$. ** $P < .01$, Mann-Whitney test (data shown as box and whisker plots). Cotreatment with HC and carbenoxolone (CBX; $10^{-7}$ M) abolished the potentiating effect of HC on CPA constriction (C), whereas carbenoxolone alone had no significant effect on U46619-induced constriction (D). Data in A, C, and D are mean ± SEM (n = 12). E, The previously observed potentiation of vasoconstriction induced by Dex (** $P < .01$, 2 way ANOVA; †† $P < .01$, † $P < .05$, Bonferroni post hoc test) was abolished by cotreatment with mifepristone (** $P < .001$, compared with Dex treatment, 2 way ANOVA). F, Exposure to nonsteroidal glucocorticoid receptor agonist GSK47869A significantly potentiated constriction to U46619 compared with control (** $P < .01$). Data in E and F are mean ± SEM (n = 7-10).

potential mechanistic link between placental exposure to elevated GCs and the increased vascular resistance in FGR placentas.

Initial studies to elucidate the mechanisms involved used the GR antagonist mifepristone. Cotreatment with mifepristone partially abolished the dexamethasone-induced potentiation of vasoconstriction, suggesting the chronic effects of glucocorticoids are mediated via GR. To add support to this hypothesis, we made use of a new series of highly GR-specific agonists, which lack any detectable mineralocorticoid receptor binding activity. One of these, GSK47869A, produced a near identical effect to both dexamethasone and hydrocortisone, thereby strongly supporting the conclusion that this response is mediated through GR.

Mifepristone treatment alone produced a slight potentiation of vasoconstriction to dexamethasone. Although this was not statistically significant, it may reflect a GR agonist effect, as previously described in cells with high receptor density (30, 31). Alternatively, it may reflect blockade of progesterone receptors by mifepristone; PRs are abundantly expressed by CPA, and progesterone exerts potent vasodilatory effects on these vessels (32, 33). Thus, the antagonism of progesterone present in the culture medium (supplemented with fetal calf serum) may contribute to the small increase in CPA constriction induced by mifepristone.

GR is expressed in both endothelial and vascular smooth muscle cells in other human blood vessels. GCs are thought to modulate vascular tone through actions in both cell types: GCs inhibit vasodilator release (NO, prostacyclin) (34, 35) from endothelial cells and increase vasoconstrictive effects of catecholamines and angiotensin II by up-regulating a1B-adrenergic and AGTR1, respectively, in vascular smooth muscle cells (36, 37). Our PCR analyses provided evidence that dexamethasone and hydrocortisone alter the balance of vasoconstrictor and vasodilatory action in favor of constriction by enhancing CPA
TBX-R expression and decreasing bradykinin receptor and prostaglandin production via reduced COX-2 expression. Moreover, PROK1, a potent stimulator of smooth muscle constriction (38), is also enhanced, whereas AGRT1 was unaffected. These data suggest GCs induce highly selective effects on vasoconstrictive pathways in CPAs. TBX-R up-regulation is particularly pertinent to the current study because GCs potentiated constriction in response to U46619, a thromboxane-mimetic, and the TBX-R contains a GRE in its promoter region (39). Thromboxane A2 has been implicated in the pathogenesis of FGR because transgenic mice overexpressing TBX-R deliver growth-restricted pups (40). It is unknown whether there is an increase in TBX-R expression in CPAs from human pregnancies complicated by FGR.

In the current study, dexamethasone did not significantly alter SNP-induced vasorelaxation of CPAs, despite an increase in mRNA expression of eNOS after GC exposure. However, SNP exposure tests the ability of vascular smooth muscle to respond to available NO rather than assessing NO bioavailability. Previous studies have shown that NO bioavailability and eNOS expression is reduced by GC exposure (35, 41). The reasons for the discrepancy between our findings and previously published data are unclear, but increased eNOS expression in the current study may represent a compensatory response to the culture conditions used. Unlike CPAs from normal pregnancies, vessels from FGR pregnancies maintain relaxation in response to high concentrations of SNP (10), and a similar response was also observed in cultured CPAs in the current study. This effect can also be reproduced by hypoxia (42). The comparable effect on vasodilatation in cultured vessels with the visible loss of the endothelium and in experimentally induced hypoxia is consistent with proposed endothelial cell dysfunction in FGR pregnancies (43).

The distribution of the 11β-HSD iso-enzymes within CPAs has not been examined previously. Although both isoforms are detectable in VSMCs and endothelial cells in vitro (44), 11β-HSD1 has been shown to be specifically expressed by endothelial cells in vitro, with 11β-HSD1 localized to VSMCs (14). Here we demonstrated 11β-HSD2 immunoreactivity in CPA endothelial cells, albeit at a low level, whereas 11β-HSD1 was undetectable. This latter finding contrasts with a previous study describing 11β-HSD1 immunoreactivity within the umbilical cord and villous tree vascular endothelium (45). This may be attributed to differences in antibody efficacy; however, the detection of strong and selective immunostaining in decidual cells from term fetal membranes suggests a high antibody specificity in the current study. CPA expression of 11β-HSD2 suggests a further level of protection for the fetoplacental vasculature against maternal cortisol.

In both acute and chronic experiments, hydrocortisone was less effective than dexamethasone. This may be due to metabolism by 11β-HSD2; however, 11β-HSD2 inhibition with carbenoxolone failed to increase the magnitude of the vasoconstrictor response to hydrocortisone. This is

Figure 6. Effect of hydrocortisone (HC) and dexamethasone (DEX) on mRNA expression of regulators of vascular tone in CPAs. Data are shown as fold change from control (CON); the line represents the median value. A–C, TBX-R and PROK1 were significantly up-regulated by GCs, whereas AGTR1 was unaffected. D–F, Bradykinin receptor and COX-2 were significantly down-regulated by glucocorticoids, whereas eNOS was significantly up-regulated by HC and DEX. * P < .05, ** P < .01, *** P < .001, compared with control CPA (Wilcoxon signed rank test).
unlikely to be due to a dosage effect because lower carbenoxolone concentrations have previously been demonstrated to reduce 11β-HSD2 activity (46, 47). The differences in the magnitude of response are therefore more likely to be due to the relatively higher potency of dexamethasone than hydrocortisone (48). Interestingly, carbenoxolone appeared to abolish the hydrocortisone-induced increase in U46619 vasoconstriction; this is likely to be an 11β-HSD2-independent effect of carbenoxolone on vascular function, as a gap junction inhibitor (49, 50), or by increasing apoptosis (20).

In summary, this study has demonstrated contrasting acute and chronic actions of GCs on CPAs, consistent with observations from other vascular beds. Blunted constriction to U46619 after acute GC exposure may explain the transient improvement in fetoplacental blood flow after antenatal GC treatment, whereas the enhanced vasoconstriction after longer-term exposure mimics the altered reactivity of CPA from pregnancies complicated by FGR. The exact mechanism behind this potentiation in constriction to thromboxane has not been fully elucidated; however, altered expression of vasoactive factors suggests dysregulation of autocrine/paracrine mediators of CPA tone in favor of vasoconstriction. Taken together, these findings support the hypothesis that exposure to elevated GCs may contribute to the pathogenesis of raised vascular resistance in the fetoplacental circulation in FGR.

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Address all correspondence and requests for reprints to: Dr Rebecca Lee Jones, Maternal and Fetal Health Research Centre, University of Manchester, St Mary’s Hospital, Research Fifth Floor, Oxford Road, Manchester M13 9WL, United Kingdom. E-mail: rebecca.lee.jones@manchester.ac.uk.

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