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Effect of oleic acid supplementation on prostaglandin production in maternal endometrial and fetal allantochorion cells isolated from late gestation ewes

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ABSTRACT

Introduction: Elevated circulating non-esterified fatty acids including oleic acid (OA) are associated with many pregnancy related complications. Prostaglandins (PGs) play crucial roles during parturition. We investigated the effect of OA supplementation on PG production using an *in vitro* model of ovine placenta.

Methods: Maternal endometrium (ME) and fetal allantochorion (FC) were collected in late pregnancy (day 135). Confluent cells were cultured in serum-free medium supplemented with 0, 20 or 100 μM OA and challenged with control medium, oxytocin (OT, 250 nM), lipopolysaccharide (LPS, 0.1 μg/ml) or dexamethasone (DEX, 5 μM). Spent medium was harvested at 2 and 24 h after challenge for quantifying PGs.

Results: In ME cells OA increased PGE_2 production moderately but attenuated $PGF_{2\alpha}$ production leading to a doubling of the PGE_2:$PGF_{2\alpha}$ ratio (E:F) ($P < 0.01$). Without OA, both OT and LPS stimulated PG production for about 3-fold ($P < 0.01$) without changing the E:F ratio. In the ME cells challenged with OT, OA decreased both PGE_2 and $PGF_{2\alpha}$ production by up to 70% ($P < 0.01$) whereas in LPS treated cells OA increased the E:F ratio. In FC cells PGE_2 production at 2 h was stimulated by 100 μM OA ($P < 0.05$). In these cells LPS caused a 3-fold increase in PGE_2 ($P < 0.01$), an effect which was completely inhibited by DEX.

Discussion: OA supplementation favours basal PGE_2 production in both ME and FC. In ME OA increased E:F ratios and antagonized the stimulatory effect of OT on PG production. This suggests that raised circulating OA may affect both the initiation and progression of parturition.

1. Introduction

Maternal obesity now affects around 20% of pregnancies in many westernised countries [1]. Obesity increases the risk of many pregnancy related complications including pre-eclampsia [2], pre-term birth [3], prolonged labour [4] and emergency caesarean section [5]. Obesity is associated with aberrant lipid metabolism and higher circulating concentrations of free fatty acids [6,7]. Women with non-esterified fatty acids (NEFA) levels in the highest tertile were at about twice the risk of spontaneous pre-term birth than those in the lowest tertile [3]. Amongst these, oleic acid (OA, 18:1n-9) accounts for about 30% of the NEFAs circulating during pregnancy in women and up to 45% in sheep [8]: its concentration increases in women with gestational diabetes mellitus [9] and OA was the NEFA with the greatest increase (67%) associated with pre-eclampsia [6]. OA also constituted around 10% of the fatty acids present in amniotic fluid [10] and 30% of total membrane fatty acids in immortalised pregnant human myometrial cells [11]. Circulating NEFA concentrations including OA also rise during negative energy balance when body lipids are mobilised to supply extra energy [7]. In addition, OA is a principal effective ingredient within foodstuffs such as olive oil and peanuts which are believed to have positive effects on human health, such as protection from cardiovascular disease [12,13]. Both *in vivo* and *in vitro* studies have shown that OA can reduce the negative metabolic effects of elevated dietary

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saturated fatty acids [14].

In mammals, various eicosanoids including prostaglandins (PGs) and leukotrienes are produced by the metabolism of n-6 polyunsaturated fatty acids (PUFAs). The key enzymes in this metabolic pathway, Δ6, Δ5 desaturase and prostaglandin-endoperoxide synthase (PTGS), are rate limiting to a large number of substrates, including 18 and 22 carbon fatty acids [15]. Therefore, OA supplementation may alter PUFA metabolism and PG synthetic pathways through enzyme competition. Unsaturated fatty acids (UFAs) are important signalling molecules controlling many cellular processes [16] so OA may compete with PUFAs for incorporation into phospholipid membranes and reduce availability of the PG precursors. Such evidence was found in a study showing that UFAs, including OA, inhibited induced activation of NFkB and *PTGS2* expression mediated through toll-like 4 receptors [16,17].

PGs, especially the 2-series, are key signalling molecules in reproductive processes including ovulation, luteolysis, menstruation and parturition [18,19]. Parturition in the ewe is initiated by activation of the fetal hypothalamic-pituitary-adrenal axis, followed by a concurrent increase in plasma cortisol and oestradiol, that up-regulates *PTGS2* expression and down-regulates PG dehydrogenase (*PGDH*), resulting in increased PG synthesis [20]. In the late pregnant ewe, PGE_2 is produced primarily by fetal component of the placentome, and concentrations rise over the last 3 weeks of gestation to aid fetal maturation [21]. At term, PGE_2 causes cervical dilatation whereas $PGF_{2\alpha}$ is released through oxytocin (OT) binding to its receptors (OTR) in the intercotyledonary endometrium and stimulates uterine contractions and delivery [20,22,23]. Synthetic glucocorticoids, such as dexamethesone (DEX) mimic the endogenous cortisol rise and induce parturition and pre-term birth [20]. Intrauterine infection is another major risk factor for preterm labour [24]. The bacterial endotoxin lipopolysaccharide (LPS) increases the activities of phospholipase (PL)A2 and PTGS2, and inhibits PGDH [25,26]. LPS induces NFkB activation, a key signalling pathway in infection-induced preterm labour [27].

The aim of the present study was, therefore, to test the hypothesis that supplementing maternal endometrial (ME) and fetal allantochorion (FC) cells isolated from late gestation ewes with different concentrations of OA would: (i) influence PG production and (ii) alter their response to the agonists OT, LPS and DEX.

2. Materials and methods

All reagents were from Sigma (Poole, Dorset, UK) or BDH Merck Ltd (Leics, UK) unless otherwise stated. All culture media used included 50,000 units/L penicillin and 50 mg/L streptomycin. All animal experiments were carried out under the Animal (Scientific Procedures, UK) Act 1986.

2.1. Animals, cell isolation and culture

Tissues for the study were obtained post mortem from 9 ewes on Day 135 of gestation (term is approximately 145 days). ME and FC cells were separated and cultured following the methods described previously [28]. The FC cells were taken from the intercotyledonary regions of the placenta to avoid possible contamination with maternal tissue.

2.2. OA supplementation and cell challenge

OA (cis-9-Octadecenoic acid, Sigma) was dissolved in 100% ethanol to 100 mM. It was diluted further to 10 mM in the dilution medium [serum free DMEM/F12 medium containing 0.1125% bovine serum albumin (BSA) as a carrier]. OT (Sigma) was dissolved in 0.01 M acetic acid at 5 mM. Further dilution to 500 μM was made in the dilution medium. LPS from *Escherichia coli* 026.B6 (Sigma) was dissolved in distilled water at 500 μg/ml then diluted to 50 μg/ml in dilution medium. DEX (Sigma) was dissolved in 100% ethanol to 20 mM, then diluted to 2 mM in the dilution medium. The solutions were sterilised by passing through a 0.20 μm filter (Nalge Nunc International, Rochester, NY14602-0365, USA).

The isolated endometrial cells were cultured in DMEM/F12 medium containing 10% fetal calf serum (FCS) and culture medium was changed every 48 h for 7–8 days to allow the cells to grow to confluence as checked using microscopy. Prior to the OA supplementation, the FCS present in the confluent cells was removed via incubation with 2 ml serum-free test medium (DMEM/F12 medium containing 0.1125% BSA, 50,000 units/L penicillin and streptomycin, and 1 ml/L ITS (0.5 mg/ml insulin, 0.5 mg/ml transferin and 0.5 μg/ml selenium; Sigma) for 3 h. The cells were then cultured in 2 ml test medium containing 0 (CONT), 20 or 100 μM OA for 45 h to allow sufficient time for OA incorporation into membrane phospholipids before challenge. After this, the cells were incubated for a further 2 h or 24 h with either: (i) control medium (CONT); (ii) 250 nM OT (endometrial cells only), (iii) 0.1 μg/ml LPS, (iv) 5 μM DEX or (v) 0.1 μg/ml LPS + 5 μM DEX in 1 ml of the above OA supplemented medium. Spent medium was collected and stored at −20 °C until analysis. There were four replicate wells per treatment for each ewe and all treatments were repeated in cells isolated from at least 3 separate ewes. The doses of OA and challenge were selected to fall within the physiological and effective range following preliminary work using a wider dose response curve. The maximum ethanol concentration in the incubation medium was less than 0.05%.

2.3. PG radioimmunoassay (RIA)

PGE_2 and $PGF_{2\alpha}$ in the spent medium were quantified using RIA methods as described previously [29]. The samples were diluted (×10–200) in the RIA buffer. This allowed the measured PG concentrations to fall within the analytical range and minimized the effect of medium on the RIA to a negligible level, making extraction unnecessary. The PG antisera were a kind gift from Dr N L Poyser (University of Edinburgh, Edinburgh, UK). Their cross-reactivities were as follows: $PGF_{2\alpha}$ antiserum, 34% with $PGF_{1\alpha}$ and 25% with $PGF_{3\alpha}$; PGE_2 antiserum, 23% with PGE_1 and 15% with PGE_3. The limit of detection was 2 pg/tube for PGE_2 and 1 pg/tube for $PGF_{2\alpha}$. The intra-assay and inter-assay coefficients of variation were 3.5% and 6.3% for PGE_2 ($n = 6$), and 4.1% and 9.6% for $PGF_{2\alpha}$, respectively ($n = 6$).

2.4. Statistical analysis

The data were expressed as mean ± standard error (S.E.) unless otherwise stated. Data analysis was carried out using an SPSS V22 software package (SPSS Inc, Chicago, IL, USA). The data were checked for distribution using a Levene's test of equality of error variance. Where necessary, logarithmic transformation was carried out to achieve homogeneity. Analysis of variance (ANOVA) with repeated measurement was carried out via a linear mixed effect model, which took the treatments, time, challenges and their interactions as the fixed effects and ewe as the random effect. Where a significant difference ($P < 0.05$) was achieved, Fisher's LSD for multiple comparisons were performed to examine the differences between the fixed effects.