REPRODUCTION
REVIEW

The role of endocannabinoids in pregnancy

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Abstract

Endocannabinoids are a family of lipid signalling molecules. As with prostaglandins (PGs), endocannabinoids are derived from polyunsaturated fatty acids and affect cell function via receptor-mediated mechanisms. They also bind to PG receptors, although at a lower affinity. The endocannabinoid network is regulated in pregnancy from embryo development to labour onset. Even small changes in endocannabinoid exposure can retard embryo development and affect implantation success. There is now compelling evidence that aberrant expression of factors involved in the endocannabinoid pathway in the placenta and circulating lymphocytes results in spontaneous miscarriage and poor pregnancy outcomes. It is likely that competition between endocannabinoids, PGs and other similar lipids ultimately determines how phospholipid/fatty acid substrates are metabolised and, thus, the balance between the uterotonic and tocolytic activities. We, therefore, hypothesise that endocannabinoid profiles may be used as a biomarker to predict and/or identify spontaneous labour onset.

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Introduction

The endocannabinoid system

Endocannabinoids are endogenous ligands derived from membrane phospholipids that bind to a family of G protein-coupled receptors, termed cannabinoid receptors. Five endocannabinoids have been identified to date in human tissues: *N*-arachidonoylethanolamide (anandamide; Devane *et al*. 1992), 2-arachidonoylglycerol (2-AG; Sugiura *et al*. 1995), homo-γ-linolenoylethanolamide (Hanus *et al*. 1993), docosatetraenoylethanolamide (Hanus *et al*. 1993), noladin ether (Hanus *et al*. 2001) and virodhamine (Porter *et al*. 2002). The two best-characterised endocannabinoids are anandamide and 2-AG, and of the five family members, only these molecules have been implicated in the processes of pregnancy and delivery. This review, therefore, focuses on anandamide and 2-AG. These endocannabinoids are not stored within cells, but are synthesised and released in response to increased substrate availability and synthase activity (Sugiura *et al*. 2002). Anandamide is thought to be released by a two-step enzymatic reaction in which arachidonic acid is transferred to a phospholipid precursor, phosphatidylethanolamine, by *N*-acyltransferase to produce *N*-arachidonoyl-phosphatidylethanolamine (NAPE), which is then cleaved by phospholipase D (PLD) (Fig. 1; Di Marzo *et al*. 1997). 2-AG, however, is synthesised from diacylglycerol (produced from phosphoinositides by phospholipase C) through the action of diacylglycerol lipase (DAGL; Bisogno *et al*. 2005). Phospholipase C-independent routes of 2-AG production involving retrograde transmission in the brain have also been reported (Di Marzo *et al*. 1994). Endocannabinoid degradation is mediated by integral membrane proteins, monoacylglyceride lipase (MAGL) and fatty acid amide hydrolase (FAAH), and results in the generation of arachidonic acid and subsequently prostaglandins (PG) (Fig. 1; Pacher *et al*. 2006). Other studies have demonstrated that endocannabinoids undergo oxidative metabolism by a number of fatty oxygenases: cyclooxygenases, lipoxygenases and cytochrome P450s, to produce PG glycerol esters (for 2-AG) and ethanolamides (for anandamide) (Bornheim *et al*. 1993, Edgemond *et al*. 1998, Kozak *et al*. 2000). The effects of endocannabinoids are dependent upon their half-life in the extracellular matrix, which is a product of the rate of synthesis and cellular uptake and the rate of degradation.

Two cannabinoid receptors, CB1 and CB2, have been identified and cloned (Matsuda *et al*. 1990, Munro *et al*. 1993). These receptors primarily couple to $G_{i/o}$ proteins and activate multiple signalling pathways via both the α and βγ subunits of G proteins: $G_{i\alpha}$ classically leads to adenylate cyclase inhibition, while $G_{i\beta\gamma}$ modulates calcium and potassium channels (Pertwee 2006). Other important actions include the activation of MAPKs and class IA and IB PI3K. CB receptors are desensitised (but not internalised) by the G protein-coupled receptor

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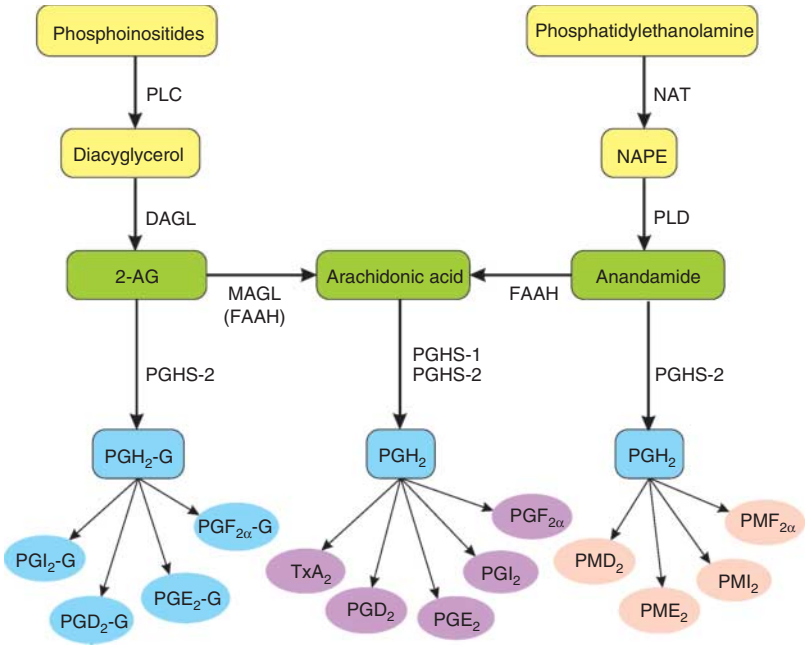

Figure 1 Metabolism of endocannabinoids. Anandamide and 2-AG are synthesised by a two-step enzymatic process from phospholipids, phosphoinositides and phosphatidylethanolamine. The endocannabinoids are converted to arachidonic acid by FAAH and MAGL respectively. Arachidonic acid is subsequently metabolised by PGHS-1 and PGHS-2 to produce prostaglandins (PGs). Alternatively, anandamide and 2-AG may be oxygenated by PGHS-2 and lipoxygenases to generate PG ethanolamides (prostamides (PMs)) and glycerol esters (-G). PLC, phospholipase C; DAGL, diacylglycerol lipase; NAT, *N*-acyltransferase; NAPE, *N*-arachidonoyl-phosphatidylethanolamine; PLD, phospholipase D; EA, ethanolamine.

kinases and β-arrestin-2 (Jin *et al.* 1999). The CB1 receptor is abundantly expressed in the CNS and peripheral tissues, whereas the CB2 receptor is predominantly detected in immune cells (Matsuda *et al.* 1990, Munro *et al.* 1993).

Local endocannabinoid signalling network in the placenta

Enzymes and proteins involved in the production and signalling of endocannabinoids are expressed in the term placenta, particularly in the villous trophoblast tissues (Table 1). This suggests that endocannabinoids can be produced locally in the placenta; however, this remains to be unequivocally established. Immunohistochemical studies have characterised changes in the expression of endocannabinoid-producing and -metabolising enzymes as well as cannabinoid receptors in first-trimester gestational tissue. The expression of human FAAH and CB1 proteins increases at weeks 9–10 of gestation before returning to basal levels (Habayeb *et al.* 2008, Taylor *et al.* 2011). The implication of these changes is that the expression of the CB1 receptor and FAAH is dramatically elevated (week 9) prior to the commencement of blood flow to the intervillous space (gestation weeks 10–12). This observation coupled with the low expression of NAPE-PLD suggests that local endocannabinoid production is limited and any available anandamide and 2-AG (presumably from the maternal circulation) are readily converted to other products (Fig. 1). The biological significance of the increased expression of the CB1 receptor remains to be elucidated.

More members of the endocannabinoid signalling network are expressed in the term placenta than in the first-trimester placenta (Table 1). This may be an indication that the developed placenta requires endocannabinoid regulation for the maintenance of pregnancy and preparation for labour.

Endocannabinoids, embryo development and implantation

Cannabinoid receptor expression during embryo development

The expression of the CB1 receptor is first detected at the two-cell to the blastocyst stage in the mouse embryo, and in later stages it is largely confined to the trophectoderm (Paria *et al.* 1995, Yang *et al.* 1996, Wang *et al.* 2003). In contrast, the CB2 receptor is present from the 1-cell to the blastocyst stage (Yang *et al.* 1996). Available data support a critical role of endocannabinoids in embryo development; for example, treatment of embryos with anandamide blocks blastocyst development, predominantly at the 8-cell/morula stage with some embryos arrested at the 2-cell stage (Paria *et al.* 1998). In the same study, 2-AG treatment arrested embryos at the 2-cell stage. The effects of both anandamide and 2-AG are reversible by pre-treatment with a CB1 receptor antagonist, but not with a CB2 receptor antagonist. Furthermore, the effect of endocannabinoids is primarily mediated by the CB1 receptor. These data are consistent with the hypothesis that the regulation of endocannabinoid synthesis is requisite for successful embryo development.

Table 1 Presence of expression/activity of endocannabinoid network proteins in the placental tissue.

Product	Protein	Decidua	Trophoblast	Amnion	Chorion	References
First trimester						
Anandamide	NAPE-PLD		+			Trabucco *et al.* (2009) and Taylor *et al.* (2011)
2-AG	PLC					
	DAGL					
Arachidonic acid	MAGL	+	+	+	+	Okazaki *et al.* (1981*a*)
	FAAH	+	+		+	Helliwell *et al.* (2004), Chamley *et al.* (2008), Trabucco *et al.* (2009), and Taylor *et al.* (2011)
Cannabinoid receptors	CB1	+	+			Habayeb *et al.* (2008), Trabucco *et al.* (2009), and Taylor *et al.* (2011)
	CB2	+	+			Habayeb *et al.* (2008) and Taylor *et al.* (2011)
Term						
Anandamide	NAPE-PLD	+	+			Taylor *et al.* (2011)
2-AG	PLC	+		+	+	Okazaki *et al.* (1981*a*)
	DAGL	+		+	+	Okazaki *et al.* (1981*b*)
Arachidonic acid	MAGL	+		+	+	Okazaki *et al.* (1981*a*, 1981*b*)
	FAAH	+	+	+		Park *et al.* (2003)
Cannabinoid receptors	CB1	+	+	+		Park *et al.* (2003) and Acone *et al.* (2009)
	CB2					

NB, + indicates detectable expression of the protein.

Paradoxically, *Cnr1*$^{-/-}$ and *Cnr2*$^{-/-}$ mouse embryos demonstrate impeded embryo development; by day 3 of pregnancy when wild-type mouse embryos develop into the 8-cell stage, these knockout embryos can mostly be found at the 4-cell stage (Paria *et al.* 2001, Xie *et al.* 2012). In agreement with earlier studies, Paria *et al.* (2001) also showed that treatment of 2-cell embryos with anandamide for 72 h significantly reduced the number of embryos that develop to the blastocyst stage in wild-type mice, and this has also been observed in the *Cnr2*$^{-/-}$ mouse embryos but not in the *Cnr1*$^{-/-}$ mouse embryos. Overall, CB receptors are required for embryo development, and the inhibitory effect of endocannabinoids on this process is likely to be mediated in part by receptor-independent mechanisms.

Endocannabinoid regulation of blastocyst development and implantation

Endocannabinoids are also instrumental in the embryo implantation process. The binding of anandamide to the blastocyst decreases prior to implantation (Paria *et al.* 2001), and the expression of the CB1 receptor is higher in the dormant blastocyst than in the activated blastocyst (Wang *et al.* 2003). Endocannabinoid signalling is regulated in the mouse uterus, where anandamide concentration increases almost threefold as the endometrium changes from a blastocyst-receptive to a non-receptive state (Paria *et al.* 2001). Once the blastocyst develops *in vitro*, anandamide and 2-AG exposure for 48 h promotes trophoblast differentiation and outgrowth, which occur through the CB1 receptor (Wang *et al.* 1999, Liu *et al.* 2002, Sun *et al.* 2010). After 96 h, however, the vehicle-treated blastocysts displayed a phenotype similar to that of the anandamide-treated cells. Similarly, a low anandamide concentration (14 nM) accelerated the attachment of blastocysts to a uterine epithelial cell monolayer, whereas a high anandamide concentration (56 nM) impeded the attachment (Liu *et al.* 2002). High anandamide concentrations also inhibited trophoblast differentiation (Wang *et al.* 1999). Liu *et al.* showed that these effects were mediated by the CB1 receptor, but not by the CB2 receptor. Recently, it has been reported that *Cnr1*$^{-/-}$ and *Faah*$^{-/-}$ mouse trophoblast stem cells demonstrate retarded cell migration, attachment and spreading (Xie *et al.* 2012) as well as reduced trophoblast stem cell proliferation (Sun *et al.* 2010).

Anandamide concentrations increase from days 1 to 4 following implantation in the mouse uterus, whereas 2-AG concentrations remain unchanged at levels that are markedly higher than that of anandamide (Wang *et al.* 2007). Additionally, anandamide and 2-AG are more abundant in the inter-implantation regions than in the implantation sites, which would serve to inhibit blastocyst attachment and trophoblast development as described earlier. Concomitantly, DAGLα (DAGLA) – which promotes 2-AG production – is highly expressed in the luminal epithelium of the inter-implantation sites and down-regulated in the myometrium after implantation. NAPE-PLD is expressed in the luminal epithelium; however, its expression is reduced at the time of implantation. It is more strongly expressed at the inter-implantation site, thus corresponding to the high anandamide concentration at this location (Guo *et al.* 2005, Wang *et al.* 2007). Both FAAH and MAGL are primarily localised in the luminal and glandular epithelia at the site of implantation before and after blastocyst attachment. This ensures low endocannabinoid signalling at the time of implantation for successful blastocyst attachment. In summary, implantation success requires low anandamide binding and signalling in the blastocyst, trophoblast and endometrium.

Endocannabinoids in ectopic pregnancies

Surprisingly, the opposite is true for ectopic pregnancies in the fallopian tube. FAAH, NAPE-PLD, and CB1 and CB2 receptors can be strongly detected in the luminal border of the fallopian tube epithelium (Gebeh *et al*. 2012). FAAH activity in peripheral blood cell membranes is significantly lower in women with ectopic pregnancies than in those with normal pregnancies (Gebeh *et al*. 2013). Similarly, the expression of *FAAH* mRNA is also significantly lower in the fallopian tubes of women with ectopic pregnancies than in those of women in the luteal or follicular phase of their menstrual cycle (Gebeh *et al*. 2012). Furthermore, plasma anandamide levels are significantly higher in women with ectopic pregnancies than in those with normal pregnancies (Gebeh *et al*. 2013). Also, oviductal transport of embryos is impaired in *Faah*$^{-/-}$ and *Cnr1*$^{-/-}$ mice (Wang *et al*. 2004, 2006). The absence of expression of the CB1 receptor does not affect embryo development; however, embryos of the *Faah*$^{-/-}$ mice show retarded development.

Presently, the contradictory findings that high plasma anandamide concentrations are associated with implantation in the fallopian tube (ectopic pregnancy) and failed implantation in the uterine wall (no pregnancy) cannot be resolved. The results suggest that the effect of abnormal anandamide exposure depends on its timing and location.

Endocannabinoids and labour

Changes in cannabinoid receptor expression in early-pregnancy miscarriages

Theoretically, increased endocannabinoid availability may up-regulate the activation and function of CB receptors. There are, however, discrepancies in the literature regarding the expression of cannabinoid receptors in miscarriages during the first trimester. It has been shown that the expression of placental CB1 receptor protein is elevated 2.5-fold in spontaneous miscarriage in the first trimester compared with levels observed in voluntary termination (Trabucco *et al*. 2009). In addition, Trabucco *et al*. found that the immunostaining of the CB1 receptor is poorly detected in placental villi from elective surgical terminations, which supports earlier observations from our laboratory that the expression of CB1 receptor mRNA and protein is not readily detected in the first-trimester placentae (weeks 9–13 of gestation; Helliwell *et al*. 2004). However, for gestational age-matched placentae of weeks 7–8, the expression of CB1 receptor protein in the trophoblast, mesenchymal core and decidua was approximately twofold lower in spontaneous miscarriage than in elective surgical termination (Taylor *et al*. 2011). The difference in results could be due to the tissue source as Trabucco *et al*. did not indicate which part of the placenta was analysed.

The expression of the CB2 receptor is relatively unchanged throughout the first trimester (Habayeb *et al*. 2008), and its expression in the second and third trimesters remains to be determined. The expression of the CB2 receptor in trophoblast cells and decidua is greatly up-regulated in placentae from spontaneous abortion compared with those from surgical terminations (Taylor *et al*. 2011). The significance of this is unknown, given that the effects of anandamide appear to be primarily dependent on the function of the CB1 receptor.

Alterations in endocannabinoid signalling promote miscarriage in early pregnancy

The expression of the anandamide-generating enzyme NAPE-PLD may be an indicator of anandamide concentrations and pregnancy outcomes; however, similar to those for the CB receptor, opposing results have been reported. The expression of NAPE-PLD was not significantly altered in the trophoblast, mesenchymal core or decidua of placentae from spontaneous miscarriage compared with that in placentae from elective surgical termination (Taylor *et al*. 2011). Conversely, the expression of *NAPE-PLD* mRNA is at least twofold higher in the first-trimester (weeks 9–12) placentae from elective surgical termination than in those from spontaneous miscarriage (Trabucco *et al*. 2009). This suggests that anandamide concentrations in the placenta are lower prior to spontaneous miscarriage. In direct contrast with these observations, several studies have found that plasma anandamide concentrations measured in the first trimester of women at risk of miscarriage are up to threefold higher in those who subsequently miscarried than in those who progressed to term (Habayeb *et al*. 2004, 2008, Taylor *et al*. 2011). A recent study, however, has failed to demonstrate a difference between plasma anandamide concentrations in women with normal pregnancies and those in women who subsequently miscarried (Tong *et al*. 2012). Collectively, the expression of NAPE-PLD and the content of anandamide in the placenta and their association with plasma anandamide concentrations require further characterisation.

FAAH is responsible for the conversion of anandamide to arachidonic acid, which then provides a source for labour-promoting PG production. As expected, anandamide content is higher in the uterus and placenta of *Faah*$^{-/-}$ mice than in wild-type mice (Sun *et al*. 2010). The expression and activity of *FAAH* mRNA and protein in peripheral lymphocytes have been found to be significantly lower in women who subsequently miscarried spontaneously or failed to maintain IVF pregnancy in the first trimester than in gestational age-matched women undergoing voluntary pregnancy termination or those whose pregnancies continued to term (Maccarrone *et al*. 2000, 2001, 2002). In the murine model, *Faah*$^{-/-}$ mice deliver prematurely

(Wang *et al*. 2006). Similarly, no immunostaining of FAAH could be detected in the trophoblast cells of first-trimester placentae obtained from women who miscarried, whereas the expression of FAAH could be observed in the trophoblast cells of placentae obtained from women who underwent voluntary termination (Trabucco *et al*. 2009). The absence of FAAH drives prostamide (PM), not PG, production (Fig. 1). The concentrations of PMs $F_{2\alpha}$, E_2 and D_2 are at least threefold higher in the tissues of anandamide-treated *Faah*$^{-/-}$ mice than in those of their wild-type controls (Weber *et al*. 2004). These findings provide strong evidence that i) interactions of FAAH and anandamide are essential for the normal progression of pregnancy and ii) a decreased activity of FAAH results in increased PM concentrations, which then have a negative impact on pregnancy. Other studies have shown that while there is no difference in the expression of FAAH protein in trophoblast cells of women with normal pregnancy and recurrent miscarriage, FAAH is more abundant in the decidual stromal cells of women with recurrent miscarriage than in the normal-pregnancy placentae (Chamley *et al*. 2008). These results suggest that the expression of FAAH is regulated in a tissue-specific manner and low expression and activity of FAAH are associated with early delivery.

Anandamide represses myometrial contractions

Anandamide represses oxytocin-induced contractions in human myometrial tissue (Dennedy *et al*. 2004). Inhibition is dose dependent and mediated by the CB1 receptor, but not by the CB2 receptor. In addition, daily i.p. injections of anandamide into rats during late pregnancy increased the length of gestation and the number of stillbirths per litter and decreased the levels of serum PGs $F_{1\alpha}$ and $F_{2\alpha}$ (Wenger *et al*. 1997). In support of these findings, *Cnr1*$^{-/-}$ mice demonstrated earlier labour onset compared with wild-type and *Cnr2*$^{-/-}$ mice (Wang *et al*. 2008). The same study also showed that treatment of wild-type mice with CB1 receptor antagonists from days 14 to 18 of pregnancy also resulted in premature labour. The absence of CB1 receptor signalling altered serum progesterone, oestrogen, corticosterone and corticotrophin-releasing hormone concentrations prior to parturition (Wang *et al*. 2008). Interestingly, a marked increase in human plasma anandamide concentrations has been reported as pregnancy progressed from the third trimester to term (but not during labour Habayeb *et al*. 2004). Because this was a cross-sectional study, it is difficult to determine whether or not this elevation plays a significant role in the timing of labour onset.

Once labour onset has commenced, the role of anandamide is unclear. When plasma anandamide concentrations in women undergoing labour induction by medical intervention were measured, no significant associations were found for pre-induction plasma anandamide concentrations and the length of induction to delivery time (Nallendran *et al*. 2010). This may be due to the variety of methods of labour induction that were used. In contrast, Habayeb *et al*. (2004) reported a moderate but positive correlation between plasma anandamide concentrations and duration of contractions/cervical dilatation at the time of sampling. Interestingly, although anandamide impedes labour induction, its concentration in plasma is elevated during active labour. Plasma anandamide concentrations were significantly elevated after induction compared with before induction; again, this may be confounded by the various method(s) used for inducing labour (Nallendran *et al*. 2010). The results are supported by other studies that reported a significant 1.7- to 3-fold increase in plasma anandamide concentrations of women at term in active labour (without prior intervention) than in women at term but not in labour (Habayeb *et al*. 2004, Lam *et al*. 2008). The expression of the CB1 receptor is markedly down-regulated in the villi of labouring placentae than in non-labouring placentae at term (Acone *et al*. 2009). This suggests that the increased pool of anandamide during labour is unlikely to be activating the CB1 receptor. Overall, anandamide has an inhibitory effect on myometrial contractions and extends gestation, but does not appear to have an effect once labour commences.

Endocannabinoids may promote labour via PG production

PGs are lipid mediators that play a critical role in the mechanisms of miscarriage (Sugino *et al*. 2000) and parturition at both term and preterm (Challis *et al*. 2000). Our laboratory demonstrated that endocannabinoids and a synthetic cannabinoid (CP55, 940) stimulate fetal membrane production of PGE_2 in a CB1 receptor-dependent manner (Mitchell *et al*. 2008). Similar results were also obtained using rat uterus, although in this tissue anandamide increased the levels of PGE_2 and $F_{2\alpha}$ in a CB2 receptor-dependent manner (Sordelli *et al*. 2012). PGs E and F have been implicated in the regulation of uterine contractions, cervical ripening and membrane rupture. PGI and thromboxanes contribute not only to these processes, but also to the vasculopathy of preeclampsia (Walsh & Wang 1998, Challis *et al*. 2000). Therefore, endocannabinoids have labour-promoting effects via PG production.

Paradoxical effects of anandamide on labour

The seemingly paradoxical effects of anandamide on labour onset may be cell- or species-specific; however, they may also be explained by the actions of PMs. Anandamide can be converted into two different groups of fatty acids: it is converted to arachidonic acid by FAAH and subsequently by PGHS-2 to an intermediate

endoperoxide, PGH_2, which can be further converted to PGs (Fig. 1). Anandamide can also be directly converted to PGH_2 by PGHS-2 and subsequently metabolised to form PMs; only anandamide-derived PGH_2-ethanolamine (PMH_2) is metabolised to PMs. Synthetic PG-amide (Bimatoprost, which activates PM-sensitive receptors) weakly elicits contractions in both pregnant and non-pregnant human isolated myometrial tissues, whereas the tissues are responsive to 17-phenylprostaglandin $F_{2\alpha}$ (Chen *et al.* 2005). The weak contractility induced by PMs could result from their low affinity for PG receptors (Ross *et al.* 2002). The affinity of PME_2 for PGE_2 receptors is at least 500-fold lower than that of PGE_2. If anandamide is being hydrolysed to PMs, then it may be that the increased PM concentrations may impede myometrial contractility and promote labour quiescence (Fig. 2). The paradox of the activity of anandamide may, therefore, be resolved by it being dependent upon its route of metabolism and the concurrent activity of FAAH; that is, the conditions that result in anandamide-inducing PG production are not the same as those that would have an overall relaxant effect on regular myometrial contractions (Fig. 2). If FAAH activity is high, increased amounts of anandamide would be metabolised via the central pathway (shown in Fig. 1), leading to elevated PG production. Alternatively, if FAAH activity is low, less amounts of anandamide would be converted to arachidonic acid, limiting the rate of PG production, but leaving anandamide available for PM production via the pathway shown on the right of Fig. 1.

A new model of preterm spontaneous onset of labour

Based on the studies described above and recent data from our laboratory, we propose a new model for the spontaneous onset of labour, where the endocannabinoid pathway is coupled to PG synthesis in the placenta (Fig. 2). According to the new model, increased plasma anandamide concentrations with normal FAAH activity predispose to labour onset. We have demonstrated that synthetic endocannabinoids stimulate the CB1 receptor and induce the production of labour-promoting PGs. In contrast, if FAAH activity is low or absent, anandamide is not readily converted to arachidonic acid and PGs. Instead, anandamide may be metabolised by PGHS-2 to produce PMs or may bind to the CB1 receptor, leading to PG production (Fig. 2). Each scenario results in distinctly opposing actions. Hence, the establishment of a normal range of PG:PM ratio for gestational age, and accurate measurement of the ratio in clinical samples, would be a useful tool in assessing the risk of premature labour.

Challenges in PG quantification

The measurement of PG concentrations in fluids and tissues is traditionally performed using RIAs or ELISAs.

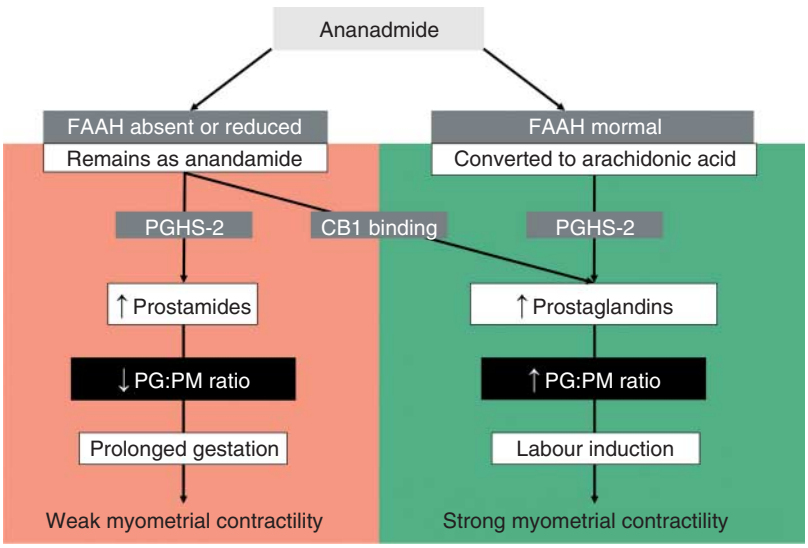

Figure 2 A new model for the spontaneous onset of preterm labour that implicates the metabolism of anandamide and its effect on the prostaglandin:prostamide (PG:PM) ratio as a biological marker for the risk of initiation of uterine contractions. The presence and absence of fatty acid amide hydrolase (FAAH) and PGH synthase (PGHS) are variables in the new model, leading to three possible outcomes from the introduction or production of anandamide: FAAH absent or reduced – anandamide is unable to be converted to arachidonic acid, resulting in increased anandamide concentrations – i) anandamide may be metabolised by PGHS-2, which increases PM production and therefore delays labour induction and prolongs labour, ii) anandamide may bind to the CB1 receptor, which has been shown to stimulate PG production by our laboratory, and this may lead to an increased PG:PM ratio and drive labour induction and iii) lastly, normal or increased amounts of FAAH convert anandamide to arachidonic acid, increasing the availability of PG substrate and, in doing so, increasing the PG:PM ratio during labour induction.

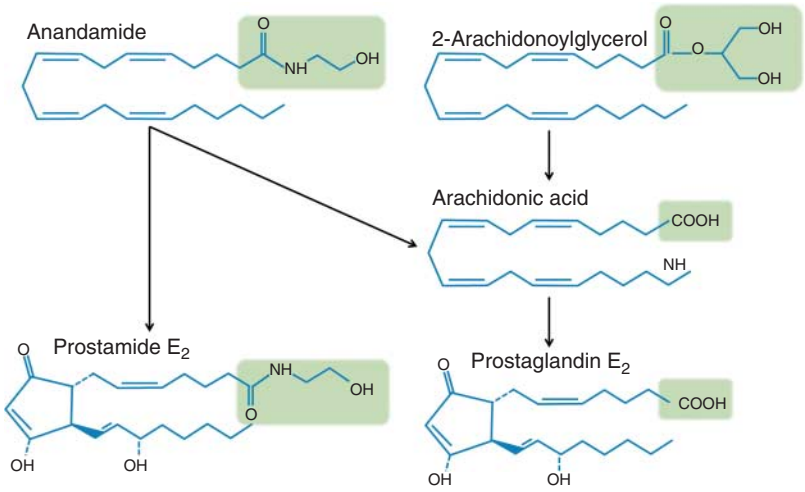

Figure 3 Structural similarities between arachidonic acid and the endocannabinoids, anandamide and 2-arachidonoylglycerol (2-AG), and prostaglandin (PG) E_2 and prostamide (PM) E_2. Note that PME_2 is closer in structure to the antigen than PGE_2 itself due to its larger moiety at the carboxyl end. Adapted from **McKirdy NC, Rice GE & Mitchell MD** 2013 Eicosanoids as diagnostics for preterm labor. *Reproductive Biology Insights* **6** 1–10.

The sensitivity of these assays is dependent upon the sensitivity and specificity of antibodies raised against PG metabolites. PGs and endogenous cannabinoid metabolites share the same lipid backbone with differing polar head groups at exactly the same position (Fig. 3; reviewed in McKirdy *et al.* (2013)). Small lipid molecules display low immunogenicity – to elicit an immune response, they must be conjugated to a carrier protein (commonly BSA). We report a major cross-reactivity of commercial antisera raised against PG PGE_2 with its endocannabinoid counterpart, PM PME_2 (Glass *et al.* 2005). Similarly, this has been observed for $PGF_{2\alpha}$ and $PMF_{2\alpha}$. Additionally, these antibodies demonstrate a higher affinity for the endocannabinoid metabolites than their supposed PG target.

This has important implications for the current literature and may change our view of the role that PGs play in human pregnancy and parturition. To illustrate, a hypothetical dataset representing the quantification of eicosanoids by immunoassay and mass spectrometry is presented in Fig. 4. The RIA data demonstrate a general increase in PG concentrations with labour (the two left columns) sufficient for statistical significance; however, the wide distribution of data points prevents their clinical utility in diagnostic testing. The immunoassay-based measurement of PGE_2 is a conflation of antibody reactivity with both PGE_2 (middle two columns) and PME_2 (the two right columns). The contribution of PGE_2 and PME_2 to the observed immunoreactivity can be resolved using mass spectrometry approaches. Such deconvolution of the immunoassay data, thus, may provide a different insight into labour-associated changes in the concentrations of these lipid-derived mediators. The capacity to establish labour-associated changes in individual lipid metabolites also affords an opportunity to develop multivariate index assays that may improve diagnostic efficiency to identify labour onset at both term and preterm.

Summary

Endocannabinoid signalling is regulated during embryo development, blastocyst implantation, placentation and labour. There is direct evidence that alterations in endocannabinoids and the enzymes that regulate their production and metabolism lead to retarded embryo development, poor blastocyst attachment and miscarriage. It is becoming increasingly evident that elevated anandamide concentrations promote spontaneous contractions, and this is likely to be mediated by PG production. We propose that differences in the profile of endocannabinoids present in biofluids may be of utility in the prediction and/or diagnosis of spontaneous labour onset at both term and preterm.

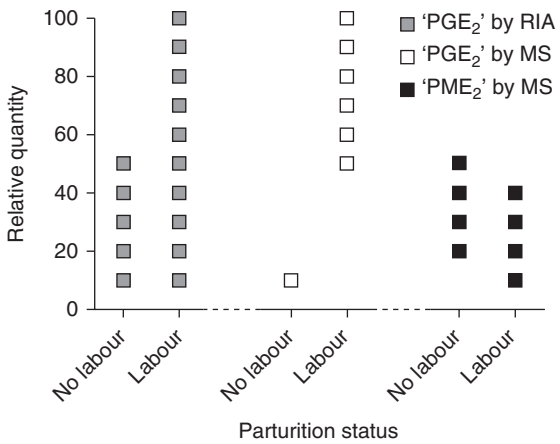

Figure 4 A hypothetical dataset demonstrating the putative confounding effects of antibody cross-reactivity on the quantification of immunoreactive PGE_2. Immunoassay-based measurement of PGE_2 (grey squares) is a conflation of antibody reactivity with both PGE_2 (open squares) and PME_2 (black squares). PGE_2 and PME_2 contributions can be resolved using mass spectrometry approaches. Such deconvolution of the immunoassay data, thus, may provide a different insight into labour-associated changes in the concentrations of these lipid-derived mediators.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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