

# Cannabinoids stimulate prostaglandin production by human gestational tissues through a tissue- and CB1-receptor-specific mechanism

Murray D. Mitchell, Timothy A. Sato, Anderson Wang, Jeffrey A. Keelan, Anna P. Ponnampalam, and Michelle Glass

Liggins Institute and Department of Pharmacology and Clinical Pharmacology, University of Auckland, Auckland, New Zealand

**Mitchell MD, Sato TA, Wang A, Keelan JA, Ponnampalam AP, Glass M.** Cannabinoids stimulate prostaglandin production by human gestational tissues through a tissue- and CB1-receptor-specific mechanism. *Am J Physiol Endocrinol Metab* 294: E352–E356, 2008. First published November 27, 2007; doi:10.1152/ajpendo.00495.2007.—Endocannabinoids have been implicated in the mechanisms of implantation, maintenance of pregnancy, and parturition in women. Intrauterine prostaglandin production and actions are also critical in each of these mechanisms. Hence, we have evaluated the effects of cannabinoids on prostaglandin biosynthesis by human gestational membranes. Explants of term amnion and choriodecidua were established and treated with the endogenous endocannabinoids 2-arachidonoyl glycerol and anandamide, as well as the synthetic cannabinoid CP55,940, to determine their ability to modulate PGE<sub>2</sub> production. The explants were also treated with CP55,940 in the presence of either SR141716A (a potent and selective antagonist of the cannabinoid receptor CB1) or NS398 [a cyclooxygenase (COX)-2 inhibitor] to determine whether any observed stimulation of PGE<sub>2</sub> production was mediated through the CB1-receptor and/or COX-2 activity. All three cannabinoids caused a significant increase in PGE<sub>2</sub> production in the amnion but not in the choriodecidua. However, separated fetal (chorion) explants responded to cannabinoid treatment in a similar manner to amnion, whereas maternal (decidual) explants did not. The enhanced PGE<sub>2</sub> production caused by CP55,940 was abrogated by cotreatment with either SR141716A or NS398, illustrating that the cannabinoid action on prostaglandin production in fetal membranes is mediated by CB1 agonism and COX-2. Data from Western blotting show that cannabinoid treatment results in the upregulation of COX-2 expression. This study demonstrates a potential role for endocannabinoids in the modulation of prostaglandin production in late human pregnancy, with potentially important implications for the timing and progression of term and preterm labor and membrane rupture.

endocannabinoids; prostaglandin; placenta; amnion; chorion; decidua

SEVERAL STUDIES (5, 21) have indicated that the incidence of cannabis use during pregnancy is ~5–15%. These studies were mainly conducted in intercity hospitals, and in some cases their findings may be confounded by the use of other illicit substances as well as smoking. Cannabis use during pregnancy has been associated with many adverse outcomes such as low fetal birth weight, preterm delivery, and miscarriage (25).

The active ingredient in cannabis exerts its actions through brain-type (CB1) and spleen-type (CB2) cannabinoid receptors. Two key naturally occurring endogenous cannabinoid ligands, 2-arachidonoyl-glycerol (2-AG) and anandamide (AEA), have been identified (25), and recent years have seen the identification of a number of enzymes involved in their

synthesis and degradation (1). AEA is degraded by the enzyme fatty acid amide hydrolase (FAAH), which metabolizes it into arachidonic acid and ethanolamide. CB1, CB2, and FAAH have all been identified and their expression characterized in gestational tissues (8, 17).

Disruptions in the balance of AEA levels and FAAH activity have been implicated in poor implantation in the mouse (15). Low FAAH activity and high levels of AEA are apparent in the blood of women who suffer from spontaneous miscarriages (14). Furthermore, increased circulating AEA levels lead to poor implantation in patients undergoing in vitro fertilization as well as embryonic transfer procedures (13).

It is well established that prostaglandins play a critical part in multiple aspects of pregnancy, including delivery at term and preterm (1<sup>st</sup> and 2<sup>nd</sup> trimester), and that the fetal membranes play a major role in intrauterine prostaglandin production. Since endocannabinoids are both a source of AA that can then be a substrate for the production of prostaglandins and are also CB ligands with potential effects on placental tissues, the aim of the present study was to determine the effects of endocannabinoids on the production of prostaglandins by extraplacental membranes: amnion, chorion, and decidua.

## MATERIALS AND METHODS

**Patients and consent.** Placentas were obtained from women undergoing a Caesarean section at term before the onset of labor because of prior Caesarean section or cephalo-pelvic disproportion. All women gave informed consent for the use of their placentas as approved by the local human ethics committee.

**Materials.** DME-199 and Ham's/F-12 culture media were obtained from Irvine Scientific (Santa Ana, CA). FCS and streptavidin-alkaline phosphatase were purchased from Invitrogen (Auckland, New Zealand). Bovine  $\gamma$ -globulin and LPS (serotype 055:B5) were purchased from Sigma Chemical (St. Louis, MO). 2-AG and AEA were purchased from Cayman Chemicals (Ann Arbor, MI), and the synthetic THC analog CP55,940 was from Tocris Cookson (Bristol, UK). SR141716A was provided by Research Biochemicals as part of the chemical synthesis program of the National Institute of Mental Health (contract NO1-MH-30003). Tritiated PGE<sub>2</sub> was purchased from Amersham-Pharmacia Biotech (Aylesbury, UK). NS398 was purchased from Cayman Chemicals.

**Explant culture.** After the amnion was manually removed, chorio-decidual membranes were washed carefully in media to remove residual red blood cells without causing damage to the integrity of the membrane. Tissue explants (6-mm disks) were excised with a cork borer as described previously (22). In some experiments, the decidua and chorion were further separated. The decidua was removed using glass slides until the semitransparent chorion remained. Explants were

Address for reprint requests and other correspondence: M. D. Mitchell, Liggins Institute, Univ. of Auckland, Private Bag 92019, Auckland, New Zealand (e-mail: m.mitchell@auckland.ac.nz).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

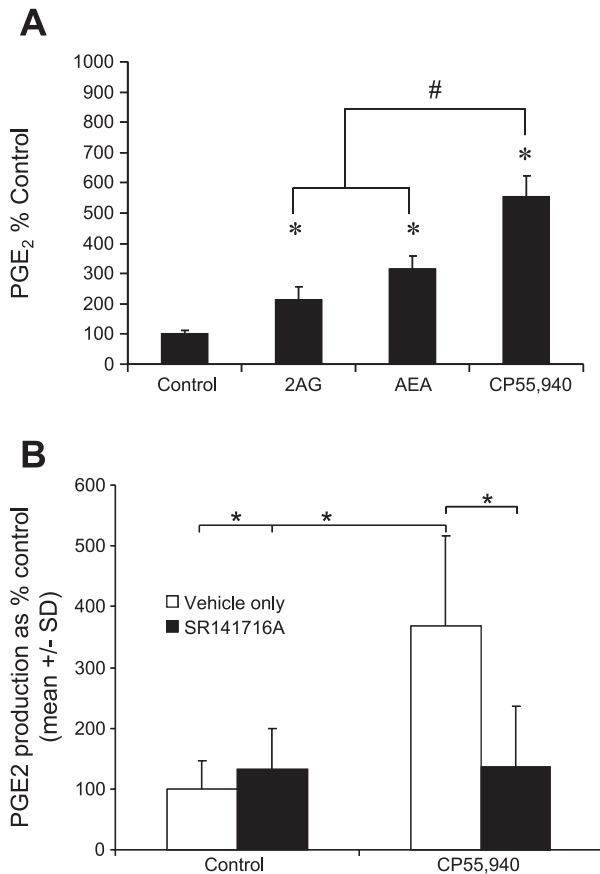


Fig. 1. Effects of endocannabinoids and CP55,940 on PGE<sub>2</sub> production by human amnion explants. *A*: explants were treated with 30  $\mu$ M of the endocannabinoids or 1  $\mu$ M CP55,940, and the media were harvested after 24 h for determination of PGE<sub>2</sub> production by RIA. *B*: explants were treated with 1  $\mu$ M of CP55,940 with or without 0.1  $\mu$ M SR141716A for 24 h and media were removed for PGE<sub>2</sub> analysis. Results, initially derived as pg/mg wet tissue wt, are expressed as percentage of control for each time point (means  $\pm$  SE;  $n = 3$  placentas). \* $P < 0.05$ . #Significant increase in PGE<sub>2</sub> production induced by CP55,940 compared with that induced by 2-arachidonoyl glycerol (2-AG) and anandamide (AEA).

pooled and randomly distributed into 12-well plates (3 explants per well; 3 wells per treatment) containing medium supplemented with 10% FCS and antibiotics. The explants were allowed to equilibrate overnight at 37°C in a humidified atmosphere of 5% CO<sub>2</sub>-95% air. The following day, media were replaced with serum-free media containing 0.1% bovine  $\gamma$ -globulin and antibiotics. Explants were then treated with the various test substances or the appropriate vehicle control. After an additional 24 h, media were collected and prostaglandin production rates were derived and normalized to the wet weight of the explants in the individual wells.

**Immunoassay.** PGE<sub>2</sub> production was determined using RIA as described previously (10) using an antiserum generated in our laboratory. The lower limit of sensitivity for the assay is 0.76 pg/ml, and all samples tested were within the range of the standard curve (0–5,000 pg/ml).

**Data presentation and statistics.** Prostaglandin production rates, calculated as picograms per milligrams of wet weight per 24 h, are represented as a percentage of control for each experiment (mean  $\pm$  SE). Results are pooled data from multiple experiments performed in triplicate. Statistical significance was determined by ANOVA followed by Dunnett's test. A  $P$  value  $< 0.05$  was considered to be significant.

## RESULTS

**Effects of cannabinoids on PGE<sub>2</sub> production by human amnion explants.** Results presented in Fig. 1*A* demonstrate the effects of endocannabinoids (AEA and 2-AG) and a synthetic cannabinoid ligand (CP55,940) on PGE<sub>2</sub> production by human amnion explants. Both the endogenous endocannabinoids (30  $\mu$ M) as well as the synthetic cannabinoid (1  $\mu$ M) stimulated a statistically significant increase in PGE<sub>2</sub> production. CP55,940 exerted a significantly greater effect than either AEA or 2-AG.

To determine whether the cannabinoid-mediated stimulation of PGE<sub>2</sub> production is mediated through the CB1 receptor, we treated explants with CP55,940 alone (1  $\mu$ M) and in the presence of SR141716A (0.1  $\mu$ M), a potent and selective antagonist of the CB1 receptor (19). CP55,940 induced a severalfold increase in PGE<sub>2</sub> production that was abolished by SR141716A (Fig. 1*B*). By itself, SR141716A ( $10^{-10}$ – $10^{-6}$  M) had no effect on amnion PGE<sub>2</sub> production.

**Effects of endocannabinoids on choriodecidual PGE<sub>2</sub> production.** Neither 2-AG, AEA, nor CP55,940 had a statistically significant effect on PGE<sub>2</sub> production by human choriodecidual explants (Fig. 2*A*). However, when the membranes were separated into isolated chorion and decidual explants (Fig. 2*B*), 2-AG, AEA, and CP55,940 all exerted significant stimulatory

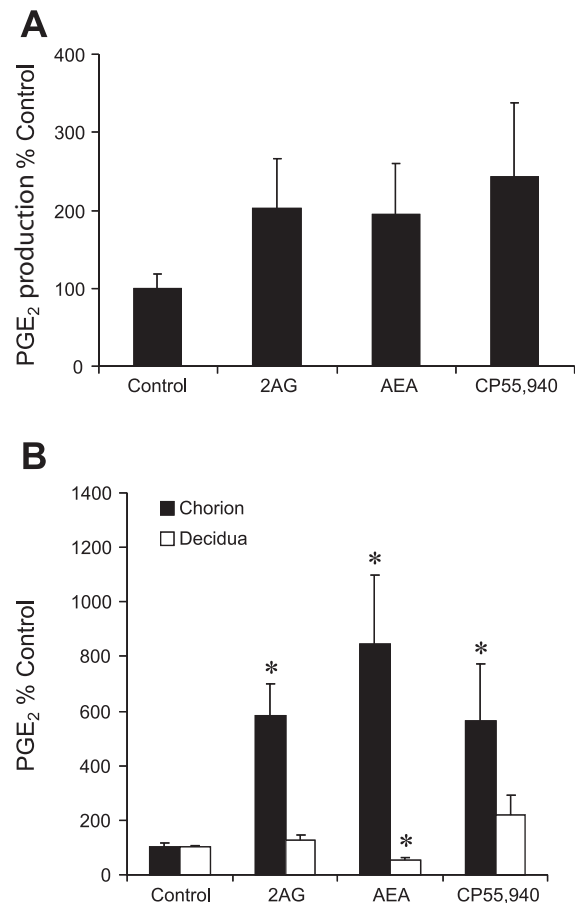


Fig. 2. Effects of cannabinoids on choriodecidual PGE<sub>2</sub> production. *A*: choriodecidual explants were treated with the cannabinoids, and the media were harvested after 24 h. *B*: chorion and decidual explants were separately treated with cannabinoids for 24 h. Results, initially derived as pg/mg wet tissue wt, are expressed as percentage of control for each time point (means  $\pm$  SE;  $n = 3$  placentas). \* $P < 0.05$  compared with control.

effects on PGE<sub>2</sub> production by chorionic (fetal) tissue but not by decidual (maternal) explants. Indeed, AEA significantly reduced PGE<sub>2</sub> production by decidual tissue (Fig. 2B).

**Effect of the cyclooxygenase-2 inhibitor NS398 on cannabinoid-induced PGE<sub>2</sub> production.** To determine whether cannabinoid-induced PGE<sub>2</sub> production occurs through the cyclooxygenase (COX)-2 pathway, we treated explants with CP55,940 alone and in the presence of NS398 (20 μM), a COX-2 inhibitor. We observed that the enhanced PGE<sub>2</sub> production caused by CP55,940 was abrogated by cotreatment with NS398 in both amnion (Fig. 3A) and chorion (Fig. 3B) explants. NS398 treatment alone did not have any significant effect on the basal level of PGE<sub>2</sub> production in the explants.

**Effect of CP55,940 on the protein expressions of COX-2, COX-1, and cytosolic PGE synthase.** The effect of CP55,940 on the protein levels of COX-2, COX-1, and cytosolic PGE synthase was determined by Western blotting. COX-2 expression was induced by CP55,940 treatment (Fig. 4). However, no

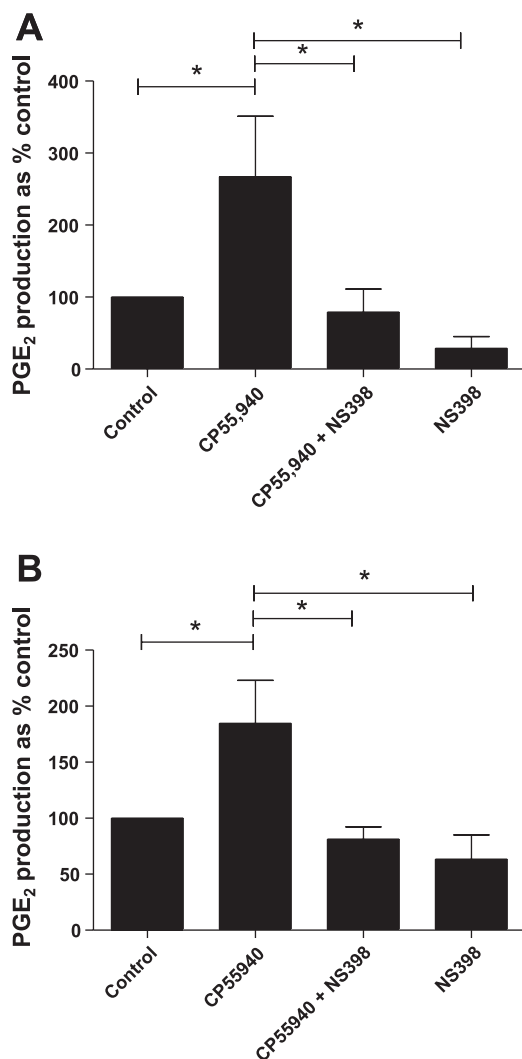


Fig. 3. Effect of cyclooxygenase (COX)-2 inhibitor NS398 on cannabinoid-induced PGE<sub>2</sub> production in amnion (A) and chorion (B) explants. Explants were treated with 1 μM CP55,940 with or without NS398 (20 μM) for 24 h, and the media were removed for PGE<sub>2</sub> analysis. Results, initially derived as pg/mg wet tissue wt, are expressed as percentage of control for each time point (means ± SE; n = 3 placentas). \*P < 0.05.

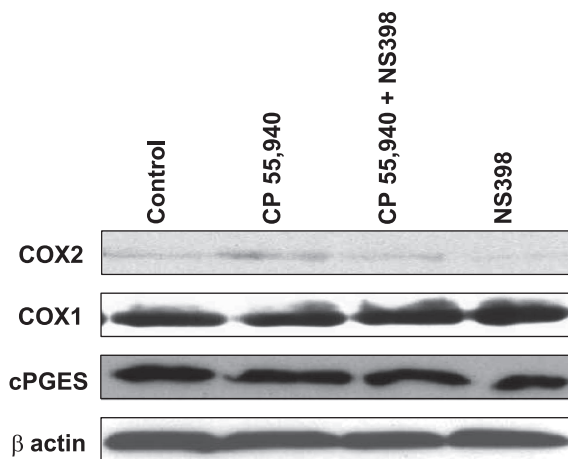


Fig. 4. Effect of cannabinoid treatment on the expression of COX-1, COX-2, and cytosolic PGE synthase (cPGES) proteins. Representative SDS page micrographs show that COX-2 protein expression was induced with CP55,940 treatment. The treatment had no effects on COX-1 and cytosolic PGES protein levels. β-actin was used as a loading control.

differences in the levels of COX-1 and cytosolic PGE synthase protein expression were observed between treatment groups.

#### DISCUSSION

We have demonstrated for the first time that endocannabinoids acting via CB1 receptors stimulate increased fetal membrane production of PGE<sub>2</sub>. This is a critical event in the mechanisms of labor at term and preterm. The tissue specificity for fetal tissues and not the adjacent maternal decidua is intriguing. Our data also imply that the upregulation of PGE<sub>2</sub> synthesis by cannabinoids is caused through the induction of COX-2. The most exciting finding, however, is that the CB1 receptor may present a new therapeutic target for the treatment of preterm labor.

The events that initiate the onset of labor involve increased local production of prostaglandins (2). The amnion is considered the most important tissue in this regard, being the most abundant source of PGE<sub>2</sub>, whereas the chorion as the major site of expression of the prostaglandin-metabolizing enzyme 15-hydroxyprostaglandin dehydrogenase (PGDH) is significant with respect to metabolic inactivation of prostaglandins. Results presented in this study strongly indicate that endogenous endocannabinoids may contribute to the production of prostaglandins by human fetal membranes. Interestingly, this effect does not appear to be manifested in the adjacent maternal (decidual) membrane, suggesting that the effects observed represent a fetal, but not maternal, response. The decidua has been shown to be responsive to CB1 agonism, although the effects observed on decidual formation were inhibitory (11). It has been shown that the plasma levels of AEA in women in labor at term are increased dramatically compared with those not in labor ( $2.5 \pm 0.22$  vs.  $0.68 \pm 0.09$  nM; Ref. 7). While it is not known whether concentrations within the amniotic cavity and fetal membranes are also increased, it is tempting to speculate that elevated endocannabinoid concentrations at or during labor may be involved in driving increased prostaglandin production by the fetal membranes at this time.

The concentration of the endocannabinoids used in this study were high but in line with those used by others (3, 20).



Concentration-dependency experiments indicated that 10–30  $\mu\text{M}$  was the optimal concentration to observe increased  $\text{PGE}_2$  production by the amnion explants (data not shown). The physiological concentration of the endocannabinoids present in human gestational tissue remains unknown. The concentrations required to produce a robust response may be influenced by the ability of endocannabinoids to penetrate the tissue, as these studies were performed in intact explants. The permeability issue in conjunction with the endocannabinoids propensity to stick to plastic raises the possibility that the effects that we observed may be a result of much lower endocannabinoid concentrations than were originally added to the culture media.

We have previously shown that the CB1 and CB2 receptors are present in both early and late human gestational tissues (8, 17). The CB1 receptor is present in all layers of the fetal membranes, with particularly strong expression in the amniotic epithelium and reticular cells, and cells of the decidua. Data from the present study imply that the effect of endogenous cannabinoids on prostaglandin production in placenta is mediated predominantly through CB1 and involves COX-2 induction. This is in agreement with the study by Denny et al. (4), which clearly demonstrated a CB1-mediated effect of endocannabinoids on human pregnant myometrium. The ability of the synthetic cannabinoid CP55,940 to stimulate  $\text{PGE}_2$  production via COX-2 induction in a CB1-receptor-dependent manner has been shown previously in guinea pigs (24). COX-2 induction by endocannabinoids has also been demonstrated (9, 18). In addition to breakdown into arachidonic acid, AEA and 2-AG can also be metabolized by COX into prostaglandin ethanolamides and prostaglandin glycerols, respectively (12, 27). We have previously demonstrated that  $\text{PGE}_2$ -ethanolamide cross-reacts significantly in our  $\text{PGE}_2$  RIA, raising the possibility that the increased  $\text{PGE}_2$  production measured may reflect endocannabinoid conversion to prostamides (6). However, since CP55,940 is structurally unrelated to arachidonate, prostamide cross-reactivity cannot explain the stimulatory ability of this ligand.

In addition to the upregulation of biosynthesis via increased COX expression and activity, prostaglandin concentrations can also be regulated by metabolism. A reduction of chorion PGDH activity and expression has been demonstrated in association with preterm birth, particularly in pregnancies with intrauterine infection (23). While the effects of cannabinoids on chorionic  $\text{PGE}_2$  production could theoretically be due to inhibition of PGDH activity, in the amnion levels of PGDH are low or undetectable, so this mechanism cannot contribute to the effects observed.

In conclusion, the results presented in this study demonstrate a potential role for endocannabinoids in the modulation of prostaglandin production in late human pregnancy, with potentially important implications for the timing and progression of term and preterm labor and rupture of membranes. It has been observed that  $\sim 40\%$  of  $\text{CB1}^{-/-}$  mice show pregnancy loss (16, 26). If these results are also relevant to the placenta, endocannabinoid-mediated effects on prostaglandin production may also have importance in the implantation and maintenance of placental capillary tone. Further studies will be necessary to ascertain the precise role of endocannabinoids in regulating prostaglandin pathways during human pregnancy.

## ACKNOWLEDGMENTS

We thank the theatre staff at National Women's Hospital for the collection of placentas, with special thanks to O. Tupusi for the organization of patient consent; C. Osephook for technical assistance; and E. Robinson (Department of Community Health, University of Auckland, New Zealand) for advice on the statistical analyses of the data.

Present address of J. A. Keelan: School of Women's and Infant's Health, University of Western Australia, King Edward Memorial Hospital, Perth, Australia.

## GRANTS

This work was funded by a Programme Grant to M. D. Mitchell from the Health Research Council of New Zealand. A. P. Ponnampalam is supported by a fellowship from Invitrogen. M. D. Mitchell is supported by NRCGD.

## REFERENCES

- Bari M, Battista N, Fezza F, Gasperi V, Maccarrone M. New insights into endocannabinoid degradation and its therapeutic potential. *Mini Rev Med Chem* 6: 257–268, 2006.
- Challis JR, Sloboda DM, Alfaidy N, Lye SJ, Gibb W, Patel FA, Whittle WL, Newnham JP. Prostaglandins and mechanisms of preterm birth. *Reproduction* 124: 1–17, 2002.
- Chang YH, Lee ST, Lin WW. Effects of cannabinoids on LPS-stimulated inflammatory mediator release from macrophages: involvement of eicosanoids. *J Cell Biochem* 81: 715–723, 2001.
- Denny MC, Friel AM, Houlihan DD, Broderick VM, Smith T, Morrison JJ. Cannabinoids and the human uterus during pregnancy. *Am J Obstet Gynecol* 190: 2–9, 2004.
- Fergusson DM, Horwood LJ, Northstone K. Maternal use of cannabis and pregnancy outcome. *BJOG* 109: 21–27, 2002.
- Glass M, Hong J, Sato TA, Mitchell MD. Misidentification of prostamides as prostaglandins. *J Lipid Res* 46: 1364–1368, 2005.
- Habayeb OM, Taylor AH, Evans MD, Cooke MS, Taylor DJ, Bell SC, Konje JC. Plasma levels of the endocannabinoid anandamide in women—a potential role in pregnancy maintenance and labor? *J Clin Endocrinol Metab* 89: 5482–5487, 2004.
- Helliwell RJ, Chamley LW, Blake-Palmer K, Mitchell MD, Wu J, Kearn CS, Glass M. Characterization of the endocannabinoid system in early human pregnancy. *J Clin Endocrinol Metab* 89: 5168–5174, 2004.
- Hinz B, Ramer R, Eichele K, Weinzierl U, Brune K. Up-regulation of cyclooxygenase-2 expression is involved in R(+)-methanandamide-induced apoptotic death of human neuroglioma cells. *Mol Pharmacol* 66: 1643–1651, 2004.
- Keelan JA, Sato TA, Hansen WR, Gilmour JS, Gupta DK, Helsby NA, Mitchell MD. Interleukin-4 differentially regulates prostaglandin production in amnion-derived WISH cells stimulated with pro-inflammatory cytokines and epidermal growth factor. *Prostaglandins Leukot Essent Fatty Acids* 60: 255–262, 1999.
- Kessler CA, Moghadam KK, Schroeder JK, Buckley AR, Brar AK, Handwerger S. Cannabinoid receptor I activation markedly inhibits human decidualization. *Mol Cell Endocrinol* 229: 65–74, 2005.
- Kozak KR, Rowlinson SW, Marnett LJ. Oxygenation of the endocannabinoid, 2-arachidonylglycerol, to glyceryl prostaglandins by cyclooxygenase-2. *J Biol Chem* 275: 33744–33749, 2000.
- Maccarrone M, Bisogno T, Valensise H, Lazzarin N, Fezza F, Manna C, Di Marzo V, Finazzi-Agro A. Low fatty acid amide hydrolase and high anandamide levels are associated with failure to achieve an ongoing pregnancy after IVF and embryo transfer. *Mol Hum Reprod* 8: 188–195, 2002.
- Maccarrone M, Valensise H, Bari M, Lazzarin N, Romanini C, Finazzi-Agro A. Relation between decreased anandamide hydrolase concentrations in human lymphocytes and miscarriage. *Lancet* 355: 1326–1329, 2000.
- Paria BC, Dey SK. Ligand-receptor signaling with endocannabinoids in preimplantation embryo development and implantation. *Chem Phys Lipids* 108: 211–220, 2000.
- Paria BC, Song H, Wang X, Schmid PC, Krebsbach RJ, Schmid HH, Bonner TI, Zimmer A, Dey SK. Dysregulated cannabinoid signaling disrupts uterine receptivity for embryo implantation. *J Biol Chem* 276: 20523–20528, 2001.
- Park B, Gibbons HM, Mitchell MD, Glass M. Identification of the CB1 cannabinoid receptor and fatty acid amide hydrolase (FAAH) in the human placenta. *Placenta* 24: 990–995, 2003.

18. **Ramer R, Brune K, Pahl A, Hinz B.** R(+)-methanandamide induces cyclooxygenase-2 expression in human neuroglioma cells via a non-cannabinoid receptor-mediated mechanism. *Biochem Biophys Res Commun* 286: 1144–1152, 2001.
19. **Rinaldi-Carmona M, Barth F, Heaulme M, Shire D, Calandra B, Congy C, Martinez S, Maruani J, Neliat G, Caput D, et al.** SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett* 350: 240–244, 1994.
20. **Rockwell CE, Snider NT, Thompson JT, Vanden Heuvel JP, Kaminski NE.** Interleukin-2 suppression by 2-arachidonyl glycerol is mediated through peroxisome proliferator-activated receptor gamma independently of cannabinoid receptors 1 and 2. *Mol Pharmacol* 70: 101–111, 2006.
21. **Sherwood RA, Keating J, Kavvadia V, Greenough A, Peters TJ.** Substance misuse in early pregnancy and relationship to fetal outcome. *Eur J Pediatr* 158: 488–492, 1999.
22. **Simpson KL, Keelan JA, Mitchell MD.** Labour-associated changes in the regulation of production of immunomodulators in human amnion by glucocorticoids, bacterial lipopolysaccharide and pro-inflammatory cytokines. *J Reprod Fertil* 116: 321–327, 1999.
23. **Van Meir CA, Sangha RK, Walton JC, Matthews SG, Keirse MJ, Challis JR.** Immunoreactive 15-hydroxyprostaglandin dehydrogenase (PGDH) is reduced in fetal membranes from patients at pre-term delivery in the presence of infection. *Placenta* 17: 291–297, 1996.
24. **Vannacci A, Giannini L, Passani MB, Di Felice A, Pierpaoli S, Zagli G, Fantappie O, Mazzanti R, Masini E, Mannaioni PF.** The endocannabinoid 2-arachidonylglycerol decreases the immunological activation of Guinea pig mast cells: involvement of nitric oxide and eicosanoids. *J Pharmacol Exp Ther* 311: 256–264, 2004.
25. **Wang H, Dey SK, Maccarrone M.** Jekyll and hyde: two faces of cannabinoid signaling in male and female fertility. *Endocr Rev* 27: 427–448, 2006.
26. **Wang H, Guo Y, Wang D, Kingsley PJ, Marnett LJ, Das SK, DuBois RN, Dey SK.** Aberrant cannabinoid signaling impairs oviductal transport of embryos. *Nat Med* 10: 1074–1080, 2004.
27. **Yu M, Ives D, Ramesha CS.** Synthesis of prostaglandin E2 ethanolamide from anandamide by cyclooxygenase-2. *J Biol Chem* 272: 21181–21186, 1997.

