Evidence for the presence of AH 13205-sensitive EP2-prostanoid receptors in the pregnant baboon but not in the pregnant sheep myometrium near term
Rafael Garcia Villar, L.R. Green, S.L. Jenkins, R.A. Wentworth, R.A. Coleman, P.W. Nathanielsz

To cite this version:
Rafael Garcia Villar, L.R. Green, S.L. Jenkins, R.A. Wentworth, R.A. Coleman, et al.. Evidence for the presence of AH 13205-sensitive EP2-prostanoid receptors in the pregnant baboon but not in the pregnant sheep myometrium near term. Journal of the Society for Gynecologic Investigation, 1995, 2 (1), pp.6-12. hal-02713643

HAL Id: hal-02713643
https://hal.inrae.fr/hal-02713643
Submitted on 1 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution - ShareAlike 4.0 International License
Evidence for the Presence of AH 13205-Sensitive EP$_2$-Prostanoid Receptors in the Pregnant Baboon But Not in the Pregnant Sheep Myometrium Near Term

Raphael Garcia-Villar, PhD, Lucy R. Green, BSc, Susan L. Jenkins, MS, Richard A. Wentworth, PhD, Robert A. Coleman, PhD, and Peter W. Nathanielsz, MD, PhD, ScD

OBJECTIVE: Our purposes were to assess the effects of prostaglandin (PG) E$_2$ and PGE$_{2a}$ on myometrial contractility in pregnant sheep and baboons in an in vitro superfusion study, and to characterize further the PGE-sensitive (EP) receptor subtype involved in the myometrial response to PGE$_2$ by using the selective prostanoïd EP$_2$ agonist AH 13205.

METHODS: Strip preparations of uterine muscle from 15 sheep (107–145 days' gestational age) and ten baboons (138–185 days' gestation) were studied. Cumulative concentration-response curves (CRC) were constructed to oxytocin (8.2 nmol/L to 0.42 nmol/L, PGE$_2$ (0.1 nmol/L to 1 nmol/L), and PGE$_{2a}$ (1 nmol/L to 100 nmol/L), and 50% effective concentration (EC50) values (mean and 95% confidence interval) were calculated. We also tested the hypothesis that PGE$_2$-induced myometrial relaxation in pregnant baboons could be mediated by EP$_2$-prostanoid receptors. Myometrial strips were stimulated by oxytocin (0.42 nmol/L), and CRCs to the EP$_2$-agonist AH 13205 (0.1 nmol/L to 10 nmol/L) were constructed.

RESULTS: Prostaglandin F$_{2a}$ stimulated myometrial activity in a concentration-related fashion in all preparations from both sheep and baboons. The EC50 in the sheep myometrium for PGE$_{2a}$ (52 nmol/L, 95% confidence interval [CI] 25–110) was significantly (P < .05) lower than that in baboon myometrium (183 nmol/L, 95% CI 93–353). Oxytocin stimulated myometrial activity in preparations of both sheep (EC50 = 0.29 nmol/L, 95% CI 0.11–0.71) and baboon (EC50 = 0.31 nmol/L, 95% CI 0.18–0.52). In contrast, responses to PGE$_2$ were species-related: PGE$_2$ caused concentration-related stimulation of myometrial activity in sheep tissue (EC50 = 3.2 nmol/L, 95% CI 2.0–5.0), but induced concentration-related inhibition of activity in baboon myometrium (50% inhibitory concentration [IC50] = 21 nmol/L, 95% CI 12.2–203). A concentration-related inhibitory response to AH 13205 (IC50 = 3.56 nmol/L, 95% CI 1.28–5.99) was obtained in the baboon. In contrast, AH 13205 failed to inhibit comparable myometrial strip preparations from pregnant sheep.

CONCLUSIONS: The present studies suggest that both sheep and baboon myometrium contain prostanoid receptors that mediate stimulation. In addition, baboon myometrium, like that from the human, contains AH 13205-sensitive EP receptors (EP$_2$ receptors), which mediate inhibition. The pregnant baboon may therefore represent a suitable animal model for investigations into the use of EP$_2$ agonists for the prevention of premature labor in humans. (J Soc Gynecol Invest 1995;2:6–12)

KEY WORDS: Receptors, prostaglandins, myometrium, sheep, baboon, pregnancy, PGE$_2$, EP$_2$ receptors.

Prostaglandins, especially prostaglandins (PGs), are involved in most aspects of uterine physiology. Prostaglandins have been implicated in the mechanism of parturition both in the human and in several animal species, including nonhuman primates and sheep. Parturition can be delayed by the administration of PG synthase inhibitors in both the rhesus monkey and the rhesus baboon.
key, and women. Moreover, several motility-related pathophysiologic conditions such as preterm or dysfunctional labor are associated with abnormal release of PGs. The PGs of both the E and F series (mainly PGE₂ and PGF₂α) appear to be among the major factors governing the contractility of the myometrium. Although PGF₂α is consistently excitatory on myometrial preparations from all species reported to date,8–11 the effects of PGE₂ are less consistent. Depending on the species and experimental conditions, PGE₂ has been shown to cause both excitatory and inhibitory effects on myometrial contractility.12–14 The stimulatory effect of PGE₂ may be mediated by specific PGF₂α-sensitive (EP) receptors, which may act through a variety of different post-receptor mechanisms.12–14 Although EP receptors are present in myometrium from the rat, hamster, and probably human, they appear to be absent in myometrium from the guinea pig and cat, and in these latter preparations, PGF₂α causes its excitatory effects through interaction with the thromboxane-sensitive (TP) or even the PGE-sensitive (EP) receptor.16

The cellular effects of PGE₂ are mediated predominantly by EP receptors, of which there exist several subtypes. Radioligand-binding studies have identified one or two (likely two affinity states) receptors for PGE₂ in uterine tissues (myometrium and/or endometrium) in several species, including the human.18–21 However, little information is available on the subcellular pathway and/or the functional response involved in receptor activation. Functional studies have identified at least three E-prostanoid (EP) receptor subtypes, namely EP₁, EP₂, and EP₃ receptors, in smooth-muscle tissues including myometrium.24–25 Thus, both EP₁ and EP₂ receptors mediate smooth-muscle cell contraction by increasing intracellular calcium levels through phospholipase C-mediated inositol phosphate catalysis and/or by a Gi protein-mediated lowering of intracellular cAMP. In contrast, it appears that EP₂ receptors cause relaxant effects in myometrial cells by a Gs protein-mediated increase in intracellular cAMP.26 The dual actions (stimulatory and/or inhibitory) of PGE on uterine motility found in some studies can, therefore, be explained in terms of interaction with either receptor subtype: the nature of the uterine contractile response to endogenous or exogenous PGE₂ depends on which EP receptors are present and functional in the plasma membrane of myometrial cells in a given physiologic situation. The hamster is the only animal species for which EP-inhibitory receptors have been identified firmly in myometrium. Experimental evidence based on differences with the EP₂ subtype in the relative rank order of potency for selective agonists19 has indicated that this inhibitory receptor is more likely to be of the recently identified EP₄ subtype. So far, only human myometrium appears to contain EP₂ receptors.13 The lack of an adequate animal model with which to complement and extend human studies precludes the development of EP₂ agonists as tocolytic agents for the prevention of premature labor in pregnant women.

The present study was performed on isolated, perfused myometrial strips obtained under halothane anesthesia from late-pregnant ewes and baboons. The objectives of the study were to evaluate the direct actions of PGE₂ and PGF₂α on myometrial contractility and to produce evidence for inhibitory EP receptors by using the selective EP₂ agonist AH 132058–11 on myometrial strips from both species, precontracted with oxytocin. AH 13205 is an EP₂-receptor agonist with little or no activity either at other types of EP receptor27 or at the other types of prostanoid receptor (DP, FP, IP, and TP receptors, defined as receptors with the highest affinity for PGD₂, PGF₂α, PGI₂, and TXA₂, respectively).29

MATERIALS AND METHODS

Processing of Myometrial Tissue

Pieces of full-thickness uterine wall were collected at the beginning of surgical procedures under halothane general anesthesia from pregnant animals of known gestational age. In sheep, these specimens were taken midway from the uterotubal junction and the cervix in the antimesometrial border (great curvature) of the fetus-bearing uterine horn. In baboons, the myometrial tissue was taken from the ventral surface of the uterus in the fundal region or body. The exact site depended upon the location of the placenta. We studied a total of 15 Columbia × Rambouillet cross-breed ewes (Ovis aries, 50–70 kg) between 107 and 145 days of gestation with singleton or multiple pregnancies (term 148 days), and ten baboons (Papio cynocephalus, 24–30 kg) between 158 and 185 days of gestation and bearing a single fetus (term 184 days). Tissues were placed rapidly at 4°C in Krebs buffer of the following composition (mmol/L): NaCl, 118.0; NaHCO₃, 25.0; KCl, 5.0; KH₂PO₄, 1.0; glucose, 11.0; and CaCl₂, 1.3. The cyclo-oxygenase blocker indomethacin (3 μmol/L) was added to the buffer to prevent post-collection synthesis of endogenous PGs, which could interfere with the contractility of the collected tissue. Studies were approved by the Cornell University Institutional Animal Care and Use Committee.

Tissue Preparation

The endometrium was removed gently with the aid of a glass slide and dissection forceps. Myometrial tissue was then cut with a scalpel blade. Individual strips (approximately 0.4 × 1 cm) were prepared in the direction of the longitudinal muscle fibers. A long cotton thread was sutured to the upper end of each muscle strip for attachment to a force transducer (UFI, Pioden Controls Ltd., Canterbury, UK), and the lower end was anchored to the bottom of the superfusion chamber. The distance between the two sutures, measured under 1 g of tension, was found to be in the range of 9–12 mm for all the strips.
used in the study. Care was taken not to allow the tissues to dry during preparation.

**Muscle Superfusion System**

The superfusion system used was similar to those described previously. In each study, eight strips of myometrium were placed in individual chambers. Oxygenated (95% O₂/5% CO₂) Krebs buffer (35–37°C), of the composition described above and containing indo- methacin (3 μmol/L), was superfused onto the myometrial strips using eight channels of a 16-channel individual-catheter peristaltic pump (CR 07618-60, Ismatec, Cole-Parmer, Chicago, IL) equipped with calibrated vinyl tubing (2.5 mm internal diameter). The system achieved a flow rate of 2.0 mL/minute. Care was taken that the nutrient buffer dripped down the cotton thread and spread over the whole strip in each chamber. A lower hole in the chamber was connected to a waste collector. Drugs were instilled into the flow of nutrient buffer using the remaining eight channels of the peristaltic pump, which were equipped with narrow-bore calibrated vinyl tubings (0.25 mm internal diameter) to produce a rate of flow for drug solutions 1/100 of that for the buffer. This dilution was taken into account when determining the final concentration of drug that actually came in contact with the superfused tissues.

**Contractility Data Acquisition**

After placement in the superfusion chamber, a resting tension of 1 g was applied to each myometrial strip, which was then allowed to equilibrate for 1–2 hours until a regular contractility pattern developed. Tension changes produced during the study ranged from 1–20 g, and were sampled at 32 Hz using a Data Acquisition System connected to an IBM class PC computer. The computer was programmed for real-time analysis, and printers were used to output strip-chart representations of muscle activity and integrated drug-induced effects. Baseline contractility activity for each channel was defined as the average tension (g) computed for the 10-minute period immediately before administration of a drug. Drugs were administered for 12 minutes. The differences between baseline myometrial activity and the levels achieved during the final 10 minutes of the 12-minute period of drug treatment were computed on-line. Drug concentrations used were from the range from that producing no effect to that achieving a maximal activity response, as determined in previous pilot studies.

**Experimental Schedule**

In the first study, myometrial strips were superfused with PGE₂ at concentrations ranging from 100 pmol/L to 1 μmol/L (six sheep and four baboons), and with PGF₂α from 1 nmol/L to 100 μmol/L (nine sheep and five baboons).

In the second study, to evaluate the effects of AH 13205, the muscle strips were precontracted. This was considered necessary because in tracheal smooth muscle, PGE₂ has been shown to be stimulatory on low-tone preparations but consistently inhibitory on precontracted strips. Thus, concentration-response curves (CRCs) to oxytocin were constructed cumulatively in myometrial strips from five sheep and five baboons using concentrations of 4.2 pmol/L to 0.42 μmol/L. Myometrial activity was driven by adding oxytocin up to the 50% effective concentration (EC50) into the nutrient Krebs buffer. For the study of AH 13205, the CRCs were constructed over the range of 0.1 nmol/L to 10 μmol/L of AH 13205 in muscle stimulated with 0.42 nmol/L oxytocin.

**Curve Fitting**

Contractility data from the different replicates (two to eight for each concentration in each animal) were averaged and normalized to percent of maximal effect. A nonlinear regression curve-fitting program (GraphPad V2.0, ISI software, Philadelphia, PA) was used for fitting the replicates for each drug-concentration point for all the animals of each experimental group to a sigmoid curve, using the logistic equation:

\[ E = E_{\text{max}} \times \frac{[D]_s}{EC50s + [D]}, \]

in which \( E \) = the effect of a given concentration of drug; \( E_{\text{max}} \) = the maximal achievable effect (top of the curve); \([D] = \) the concentration of drug; \( EC50 = \) the concentration of drug that achieves 50% of \( E_{\text{max}} \) (for investigations of inhibitory effects, the term EC50 was replaced by IC50, with a similar definition); and exponent \( s = \) the slope factor. From this overall CRC fit, drug potencies were calculated for each species as the EC50 or its negative logarithm (−log EC50 or pD₂).

**Statistics**

Because EC50s for a given drug are not normally distributed, we used the corresponding mean pD₂ (± standard error of the mean [SEM]) values to calculate the 95% confidence interval (95% CI, calculated as ±t × SEM, using t values for the corresponding degrees of freedom) as an indication of the dispersion of the overall mean EC50 for each drug and each species. Intra-species differences (sheep versus baboon) in drug potencies, or effects of treatments (saline versus drug), were assessed where relevant using the Mann-Whitney test. Differences with \( P < .05 \) were considered significant.

**Drugs**

Prostaglandins \( E₂ \) (Dinoprostone, MW 352.5) and \( F₂α \) (Dinoprost tromethamine [THAM] salt, MW 475.6) were gifts from the Upjohn Company (Kalamazoo, MI). Stock solutions of PGE₂ (1 mmol/L) were prepared in absolute ethanol and stored at −20°C. Serial dilutions
were done in phosphate-buffered saline (NaCl, 120 mmol/L; KCl, 2.7 mmol/L; KH$_2$PO$_4$-Na$_2$HPO$_4$, 10.0 mmol/L; Sigma Chemicals, St. Louis, MO). Prostaglandin F$_{2\alpha}$ as a 5-mg/mL (11 mmol/L) THAM salt (LUTALYSE) was stored at room temperature. Oxytocin (Butler, Columbus, OH) was stored at 4°C as a saline solution (42 µmol/L). The EP$_2$ receptor subtype selective agonist AH 13205 (trans-2-(4-[1-hydroxyhexyl]phenyl)-5-oxycyclopentenehexanoic acid; gift from Glaxo, Ware, Herts, UK) was stored at −20°C as a 1-mmol/L solution in absolute ethanol. Indomethacin (Sigma Chemicals) was dissolved (5 mg/mL) in absolute ethanol before being added to Krebs buffer at a final concentration of 3 µmol/L. Fresh solutions were prepared each day, left in ice until use, and discarded after each experiment.

**RESULTS**

**Spontaneous Contractility Pattern**

After equilibration, all myometrial strips studied displayed a characteristic pattern of contractility. The pattern consisted of the cyclic occurrence of contractile episodes of slightly less than 1 minute in duration, followed by relatively longer periods of quiescence. In sheep myometrium, episodes of 51.6 ± 5.0 seconds and a mean amplitude of 5.37 ± 0.34 g occurred at intervals of 143.4 ± 10.6 seconds (mean ± SEM, n = 10). The mean values calculated for each sheep were obtained from at least 20 contractile episodes per animal. In baboon myometrium, episodes lasted 33.1 ± 8.3 seconds, had a mean amplitude of 1.9 ± 0.3 g, and occurred at intervals of 144.4 ± 53.7 seconds (mean ± SEM, n = 5). The mean values calculated for each baboon were obtained from at least ten contractile episodes per animal.

**Effects of PGE$_2$ and PGF$_{2\alpha}$ on Myometrial Contractility**

Table 1 summarizes the agonist potencies of PGE$_2$ and PGF$_{2\alpha}$ on myometrial strips from both species. In sheep myometrium, both PGE$_2$ and PGE$_2$ caused concentration-related increases in myometrial activity (Figure 1), with EC50 values of 52 and 3.2 nmol/L, respectively. In contrast, in baboon myometrium, PGE$_2$ again caused concentration-related stimulant activity (EC50 = 183 nmol/L), but PGE$_2$ caused concentration-related inhibition (IC50 = 21 nmol/L) (Figure 1).

**Effects of Oxytocin**

As expected, oxytocin (4.2 pmol/L to 0.42 µmol/L) caused concentration-related increases in myometrial activity in both species (Figure 2). In sheep myometrium, the EC50 for oxytocin was 0.29 nmol/L (95% CI 0.11–0.71), and in baboon myometrium, the EC50 value was 0.31 nmol/L (95% CI 0.18–0.52) (Table 2). These values were not significantly different from each other. Oxytocin (0.42 nmol/L) consistently increased the activity of myometrial strips from both baboons and sheep by two to three times the spontaneous activity level. This effect was maintained for at least 2 hours (data not shown). Consequently, this concentration of oxytocin was chosen to drive myometrial motility, and the activity level obtained was considered as 100% baseline activity for the following study.

**Effects of AH 13205**

Both AH 13205 superfused at increasing concentrations (0.1 nmol/L to 10 µmol/L) and saline vehicle failed to modify the level of oxytocin-driven activity in sheep myometrial preparations (Figure 3A). In baboon myometrium, however, the same range of concentrations of AH 13205 caused a concentration-related inhibition of oxytocin-driven myometrial activity (IC50 = 3.56 nmol/L, 95% CI 1.28–5.99), whereas saline vehicle again produced no effect (Figure 3B, Table 2).

**DISCUSSION**

The present study, using an in vitro smooth muscle superfusion system, confirms the already well-documented stimulant action of PGE$_2$ on both pregnant sheep and baboon myometrium in late gestation. Myometrial contractile responses to PGE$_2$ analogues have been reported both in vivo and in vitro, e.g., in humans, non-human primates, sheep, and rats. However, our data also show a species-dependent effect of PGE$_2$ on myometrial contractile activity. Prostaglandin E$_2$ caused a clear concentration-related stimulation of motility in sheep myometrial strips, but caused a concentration-related inhibition of motility in comparable strips from the baboon myometrium.

A species-specific, concentration-related effect of the selective prostaglandin EP$_2$ agonist, AH 13205, on oxytocin-induced contractile activity was demonstrated in myometrial muscle from the pregnant baboon. In contrast, there was no effect of AH 13205 on the motility of sheep myometrium.
comparable strips from pregnant sheep myometrium. Because AH 13205 has been shown to be essentially inactive at other potentially inhibitory prostanoid receptors, such as IP or DP receptors, our findings suggest that functional EP receptors are present on the plasma membrane of myometrial cells and mediate the inhibitory effect of AH 13205 in the baboon. In contrast, EP3 receptors are either absent or not functionally coupled in the myometrium of late-pregnant ewes.

The species-specific myometrial response to PGE2 is interesting. Prostaglandin E2 is thought to exert its cellular effects primarily by an action on prostanoïd EP receptors, of which there are at least three subtypes: EP1, EP2, and EP3. The interaction of endogenous and/or exogenous PGE2 with these receptor subtypes initiates different intracellular regulatory cascades in myometrial smooth-muscle cells. Both EP1 and EP3 receptor subtypes mediate excitatory responses by increasing intracel-

**Figure 2.** Concentration-response curves (mean ± SEM) to oxytocin for myometrial strips from five pregnant sheep at 122–144 days’ gestation (open squares) and five baboons at 158–185 days’ gestation (closed squares). Tension is represented as a percentage of maximum oxytocin response. Values from each animal were obtained from at least two replicates per data point and per animal. The plotted curves are lines of best fit obtained by nonlinear regression analysis.

| Oxytocin | Sheep (n = 5, 122–144 days’ gestation) | Baboon (n = 5, 158–185 days’ gestation) |
|----------|---------------------------------------|----------------------------------------|
| EC50 (μmol/L) | 0.29 | 0.31 |
| 95% CI | 0.11–0.71 | 0.18–0.52 |
| AH 13205 | No effect | 3.56 |
| IC50 (μmol/L) | No effect | 1.28–5.99 |

Abbreviations as in Table 1.
for the presence of functional EP2 receptors in the myometrium has been restricted to the human.\textsuperscript{13} Drawing a comprehensive picture of uterine prostanoid receptors would have important implications in clinical obstetrics. Indeed, PGE\textsubscript{2} has been used successfully to improve the rate of cervical ripening during term labor in humans,\textsuperscript{35} and it has been shown to soften the ovine cervix in vitro.\textsuperscript{36} However, the lack of consistency of PGE\textsubscript{2} effects on uterine contractility (inhibition or activation) remains a concern for the clinician. Synthetic analogues of PGE\textsubscript{2} would be most likely to be effective if their stimulatory effects (cervical ripening and myometrial stimulation) and their inhibitory effects (myometrial tocolysis) could be dissociated. This could be achieved if these effects were mediated by different receptor subtypes and if selective agonists and antagonists for each receptor subtype become available. Thus, EP\textsubscript{1} and/or EP\textsubscript{3} agonists could be used to improve management of problems due to inadequate myometrial activity during parturition, whereas EP\textsubscript{2} agonists could be intended for clinical use as tocolytics in the treatment of premature labor. In addition, EP\textsubscript{2} agonists such as AH 13205 might be useful when PGE\textsubscript{2} is used to induce cervical dilatation, if the effects of PGE\textsubscript{2} on the cervix were unaffected by AH 13205, while permitting AH 13205 to inhibit any unwanted uterotonic action of PGE\textsubscript{2}. So far, the lack of an appropriate animal model for human myometrial tissue has impeded progress in the development of EP\textsubscript{2}-receptor selective drugs.

In conclusion, the PGF\textsubscript{2}\alpha-induced stimulation of the myometrium of both pregnant baboons and sheep is in agreement with results obtained with a wide range of species, including the human. In view of its high potency, PGE\textsubscript{2}-induced stimulation of sheep myometrium can be explained most likely by an interaction with stimulatory EP\textsubscript{2} and/or EP\textsubscript{3} receptors. Because PGF\textsubscript{2}\beta alone caused contraction of baboon myometrium, it is tempting to speculate that FP receptors are involved. However, PGF\textsubscript{2}\alpha demonstrated a rather low absolute potency in this effect. Thus, further experiments with other more selective agonists and antagonists will be required to characterize definitively the receptors involved in the baboon myometrial contractile response to PGF\textsubscript{2}\beta. We hypothesize, however, that the stimulatory response of the myometrium to PGF\textsubscript{2}\alpha is mediated by TP or even EP\textsubscript{2}, and/or EP\textsubscript{3} receptors, as in other tissues and species.\textsuperscript{16} The data obtained in the second part of our study, featuring oxytocin-induced stimulation of myometrial strips from both pregnant baboons and sheep and subsequent challenge with the selective EP\textsubscript{1}-receptor agonist AH 13205, provide pharmacologic evidence that the prostanoid receptor(s) involved in the inhibitory effect of PGE\textsubscript{2} in baboon myometrium is of the EP\textsubscript{2} subtype.

Further in vitro experiments testing other selective EP agents in parallel functional and radioligand binding studies in baboon myometrium would provide more definitive evidence as to the nature of the different EP receptors actually present in the myometrium (stimulatory and/or inhibitory), and would determine any regional or gestational-age-related changes in both non-human and human myometrium. However, the present work clearly indicates that the baboon may represent a suitable animal model for investigating the potential of EP\textsubscript{2} agonists in the control of myometrial hyperactivity in humans. A major advantage of this model is the ability to study activity in vivo using chronic recording of myometrial electrical activity and changes in intrauterine pressure\textsuperscript{37} in the pregnant baboon. Thus, in vitro contractility of myometrial preparations can be equated precisely with the in vivo function of the same specimens.

REFERENCES

1. Flint APF, Hillier K. Prostaglandins and reproductive processes in female sheep and goats. In: Karin SMM, ed. Prostaglandins and reproduction. London: MTP Press, 1976: 271–90.
2. Novy MJ, Liggins GC. Role of prostaglandins, prostacyclin, and thromboxanes in the physiologic control of the uterus and in parturition. Semin Perinatol 1980;4:45–66.
3. Thorburn GD. The placenta, prostaglandins and parturition: A review. Reprod Fertil Dev 1991;3:277–94.
4. Novy MJ, Cook MJ, Manugh L. Indomethacin block of normal onset of parturition in primates. Am J Obstet Gynecol 1974;118:412–6.
5. Lewis R, Schulte JD. Influence of acetylsalicylic acid, an inhibitor of prostaglandin synthesis, on the duration of human gestation and labour. Lancet 1973;i:1139–61.

6. Romero R, Emamian M, Wan M, Quintero R, Hobbins JC, Mitchell MD. Prostaglandin concentrations in amniotic fluid of women with intra-amniotic infection and preterm labor. Am J Obstet Gynecol 1987;157:1461–7.

7. Norman RJ, Reddi K. Prostaglandins in dysfunctional labour: Evidence for altered production of prostaglandin F2α. Reprod Fertil Dev 1990;2:563–74.

8. Lauersen NH, Raghavan KS, Wilson KH, Fuchs F, Niemann WH. Effects of prostaglandin F2α, oxytocin, and ethanol on the uterus of the pregnant baboon. Am J Obstet Gynecol 1973;115:912–8.

9. Garcia-Villar R, Marner PG, Laurentie MP, Toutain PL. Relative oxytocic properties of prostaglandins compared with cloprostenol, prostaglandin F2α and oxytocin in the ovariectomized ewe. Am J Vet Res 1985;46:841–4.

10. Dyal R, Crankshaw DJ. The effects of some synthetic prostanooids on the contractility of the human lower uterine segment in vitro. Am J Obstet Gynecol 1986;158:281–5.

11. Crankshaw DJ, Gaspar V. Effects of prostanooids on the rat’s myometrium in vitro during pregnancy. Biol Reprod 1992;46:392–400.

12. Toppozzada M, Gaspar A, Said S. In vivo inhibition of the human non-pregnant uterus by prostaglandin E2. Prostaglandins 1974;4:401–10.

13. Senior J, Marshall K, Sangha R, Baxter GS, Clayton JK. In vitro characterization of prostanooid EP-receptors in the non-pregnant human myometrium. Br J Pharmacol 1991;102:747–53.

14. Yeardley HL, Coleman RA, Marshall K, Senior J. The effects of PGE2, sulprostone and AH 13205 on human uterine action in vitro. Br J Pharmacol 1992;105(Proc Suppl):241p.

15. Hertleendy F, Molnar M. Mode of action of prostaglandins in myometrial cells. In: Garfield RE, ed. Uterine contractility. Boston: Serono Symposium, 1990:221–36.

16. Coleman RA, Kennedy I, Humphrey PPA, Bunce KT, Lumley P. Prostanooid receptors and their receptors. In: Hansch C, Sannes PG, Taylor JB, ed. Comprehensive medical chemistry. Vol. 3. Oxford: Pergamon Press, 1990:643–714.

17. Goureau O, Tanfin Z, Marc S, Harbon S. Diverse prostanooid receptors activate distinct signal transduction pathways in rat myometrium. Am J Physiol 1992;263:C257–65.

18. Kennedy TG, Martel D, Psychoyos A. Endometrial prostaglandin E2 binding: Characterization in rats sensitized for the decidual cell reaction and changes during pseudopregnancy. Biol Reprod 1983;29:556–64.

19. Asboth G, Todd H, Toth M, Hertleendy F. PGE2 binding, synthesis, and distribution in human ovine. Am J Physiol 1985;248:E80–8.

20. Giannopoulos G, Jackson K, Kredentser J, Tulchinsky D. Prostaglandin E and F2α receptor in human myometrium during the menstrual cycle and in pregnancy and labor. Am J Obstet Gynecol 1985;153:904–10.

21. Kennedy TG, Keys JL, King GJ. Endometrial prostaglandin E2-binding sites in the pig: Characterization and changes during the estrous cycle and early pregnancy. Biol Reprod 1986;35:624–32.

22. Hodam JR, Snakes MC, Kuehl TJ, Jones MA, Harper MJK. Characterization of prostaglandin E2 binding to uterine membranes from baboon, rabbit, and tree shrew (Tupaia belangeri). J Mol Endocrinol 1989;3:33–42.

23. Lerner RW, Lopachuk GD, Olley PM. High affinity prostaglandin E receptors: attenuate adenylyl cyclase activity in isolated bovine myometrial membrane. Can J Physiol Pharmacol 1990;68:1574–80.

24. Kennedy I, Coleman RA, Humphrey PPA, Levy GP, Lumley P. Studies on the characterization of prostanooid receptors: A proposed classification. Prostaglandins 1982;24:667–89.

25. Coleman RA, Kennedy I, Sheldrick RLG. Evidence for the existence of three subtypes of PGE2 sensitive (EP) receptors in smooth muscle. Br J Pharmacol 1987;91(Proc Suppl):323.

26. Davies P, MacIntyre DE. Prostaglandins in inflammation. In: Gallin JJ, Goldstein JM, Snyderman R, ed. Inflammation: Basic principles and clinical correlates. 2nd ed. New York: Raven Press, 1992:123–38.

27. Smith WL. Prostanooid biosynthesis and mechanisms of action. Am J Physiol 1992;263:F181–91.

28. Coleman RA, Grix SP, Head SA, Louitr J, Mallet A, Sheldrick RLG. A novel inhibitory prostanooid receptor in piglet saphenous vein. Prostaglandins 1994;47:151–68.

29. Watson S, Girdlestone D. Receptor nomenclature. Trends Pharmacol Sci 1995(suppl):S1–S43.

30. Nials AT, Vardey CF, Denyer LH, et al. AH 13205, a selective prostanooid EP2-receptor agonist. Cardiac Drug Rev 1993;11:165–79.

31. Nials AT, Coleman RA, Hartley D, Sheldrick RLG. AH 13205—a novel, selective prostanooid EP2-agonist. Br J Pharmacol 1991;102:24P.

32. Coleman RA, Nials AT. Novel and versatile superfusion system. Its use in the evaluation of some spasmogamic agents using guinea-pig isolated tracheal smooth muscle. Br J Pharmacol Methods 1989;21:71–86.

33. Figueroa JP, Mahan S, Poore EB, Nathanber PW. Characteristics and analysis of uterine electromyoagographic activity in the pregnant sheep. Am J Obstet Gynecol 1985;151:524–31.

34. Coleman RA, Kennedy I. Contractile and relaxant actions of prostaglandins on guinea-pig isolated trachea. Br J Pharmacol 1980;68:533–9.

35. Rayburn WF. Prostaglandin E2 gel for cervical ripening and induction of labor: A critical analysis. Am J Obstet Gynecol 1989;160:529–34.

36. Ownway JR, Fitzpatrick RJ. Effect of intravaginal application of prostaglandin E2 gel on the mechanical properties of the ovine cervix uterus at term. Am J Obstet Gynecol 1990;163:657–60.

37. Morgan MA, Silavon SL, Wentworth RA, et al. Different patterns of myometrial activity and 24h rhythms in myometrial contractibility in the gravid baboon during the second half of pregnancy. Biol Reprod 1992;46:1158–64.
