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ANTIPROGESTAGENIC INHIBITION OF UTERINE PROSTAGLANDIN INACTIVATION: A PERMISSIVE MECHANISM FOR UTERINE STIMULATION

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Summary—The use of antiprogestins as abortifacients is more effective when antiprogestin priming is followed by the administration of a small dose of synthetic prostaglandin. This increased myometrial sensitivity towards PG has not been explained and experiments in the guinea-pig where no myometrial activity is observed after 48 h of antiprogestin administration together with measurements of PG metabolites in uterine vein blood have given rise to the suggestion that prostaglandin synthesis is inhibited by antiprogestins. We have treated groups of 50 day pregnant guinea-pigs with 10 mg RU486 or vehicle alone and examined the ability of homogenised uterine tissues (myometrium/decidua, cervix, chorion and amnion) to metabolize PGE when given a large excess of substrate and sufficient cofactors. In addition we have examined the ability of these homogenates to synthesis PG. Antiprogestin treatment *in vivo* resulted in a 9-fold reduction in metabolic activity in chorion ($P < 0.02$) and a 4-fold reduction in myometrium/decidua ($P < 0.02$). Reduction in activity seen in amnion and cervix was not significant. The maximum metabolism was seen in the chorion and minimal metabolism in the amnion. Maximum PG production was seen in the amnion and minimum in the chorion. These results show that the effect of antiprogestin in reducing prostaglandin catabolism would reduce the threshold above which PG production would cause contractions which would in turn stimulate PG production. Thus an explanation is provided of how low doses of exogenous PGs or transient synthesis of endogenous PG within an antiprogestin treated uterus can led to a self sustaining cycle of stimulation which will lead to abortion.

INTRODUCTION

Antiprogestins used in conjunction with synthetic prostaglandins have proved effective alternatives to surgical intervention for the termination of first trimester pregnancy [1–4]. However these drugs have undeveloped potential for use in preventing ovulation [5] and implantation [6], induction of labour [7] and in cervical ripening [8]. At present, little is known about the action of these compounds except that they are antagonists of the action of progesterone at the receptor level [9]. Although RU486 (mifepristone) was the first truly antiprogestagenic steroid, it displayed high relative binding affinities for both the progesterone and the glucocorticoid receptor [10]. Antiprogestins have subsequently been synthesised which possess reduced antiglucocorticoid activity [11] but the two antiprogestins currently under-going clinical trials (RU486 and ZK98,299 [onapristone]) are antiglucocorticoids.

The guinea-pig has been widely used as a model for studying antiprogestagenic activity in women since both guinea-pigs and women display a luteal-placental shift which occurs around days 35–40 in the guinea-pig and both start labour with high circulating progesterone levels. Such research has provided valuable information on the effects of a range of antiprogestins and has demonstrated that RU486 and ZK98,299 sensitize the uterus to exogenous prostaglandin (PG) but in the guinea-pig experiments virtually no myometrial activity is seen in such primed uteri until administration of PG [12]. In addition, the cervix softens and exhibits increased extensibility [13] and electron microscopy studies have shown that there is an influx and activation of white cells [14] which may play a critical part in the remodelling of these tissues. Although prostaglandin production has been shown to fall in response to progesterone and rise in response to antiprogestins in both cells [15] and tissue

explants [16] of human endometrium and in explants of rat myometrium [17], there is only indirect evidence that the action of antiprogestins *in vivo* is exerted through an action on PG metabolism. Evidence from *in vitro* incubations with decidual cells [18] and decidual explants [16] of human decidua show only a modest response to progesterone and antiprogestins. Indeed the quiescent myometrium after antiprogestin priming together with the observation that PGF metabolite levels, measured in the uterine vein of the normally cycling guinea-pig, fall after antiprogestin treatment have led to the suggestion that PG production in the uterus might be inhibited by antiprogestins [19, 20]. In this study we have investigated the metabolism of PGs to the inactive 13,14-dihydro-15-keto forms by uterus, cervix and membranes of the pregnant guinea-pig in order to explain the previous anomalous results and to extend our knowledge of how these compounds exert their effects.

MATERIALS AND METHODS

RU486 {17α-hydroxy-11β-(4-dimethylaminophenyl)-17β-(1-propynyl)estra-4,9-dien-3-one} was a gift from D. Philibert (Roussel–Uclaf, Paris). All other reagents cofactors etc were obtained from Sigma Ltd, Poole, Dorset. Protein assay was that described by Lowry *et al.* [21]. Guinea-pigs of timed gestation as judged by post-partum mating were injected subcutaneously with 10 mg RU486 in 0.2 ml ethyl oleate/corn oil (1:1) or with vehicle alone. Animals were killed 24 h later by cervical dislocation. A sample of peripheral blood was obtained by cardiac puncture and tissues were excised and washed in physiological saline and kept on ice. Mean placental weights and mean fetus weights were recorded. Tissue was homogenised in 50 mM Tris buffer pH 7.4 centrifuged at 12,000 *g* for 2 min (Biofuge) to remove macroparticular material and diluted by 10 and by 100 to give a protein concentration in the range of 4–12 mg/ml (for the 10-fold dilution).

Metabolism

1 ml of the 100-fold dilution was incubated for 15 min in a shaking water bath with 1.0 mM NAD, 1.0 mM NADPH and 300 ng/ml PGE_2. After 15 min the reaction was stopped by the addition of 1.0 ml methyloximating solution (10 mg/ml methoxyamine hydrochloride in 0.5 M acetate buffer pH 5.6 [22]) and the vials were left overnight at room temperature prior to assay.

Synthesis

1 ml of the × 10 dilution of supernatant from tissue homogenisation was incubated at 37°C for 15 min in a shaking waterbath to investigate synthetic potential of the tissue. The reaction was stopped after 15 min by the addition of methyloximating solution as above. Samples were also treated with methyl oximating solution as above without incubation at 37°C.

Assay

Prostaglandins were assayed as reported previously [15, 22, 23]. All assays were performed using PG linked to Pro-Gly-Tyr peptide [23] and separation was achieved using donkey anti-rabbit γ-globulin attached to magnetic particles, incubating the particle suspension with label/antibody mixture for 30 min and then precipitating second antibody with a magnet (Amersham).

Statistical evaluation

Significance of difference was assessed using analysis of variance on log transformed data using Newman–Keuls procedure.

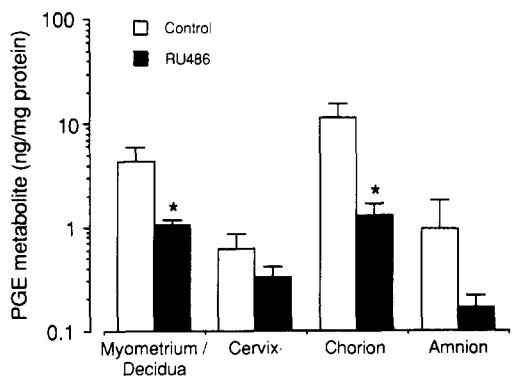

Fig. 1. The metabolic activity of guinea-pig tissues from animals treated either with vehicle alone or with 10 mg RU486 for 24 h. Metabolism by tissue from RU486-treated animals differed significantly from metabolism by tissue from vehicle-treated animals ($P < 0.02$). Mean protein concentrations were 11.3 ± 1.1 mg/ml (myometrium/decidua), 7.1 ± 1.2 mg/ml (cervix), 5.8 ± 0.8 mg/ml (chorion) and 4.3 ± 0.5 mg/ml (amnion). Control incubations without tissue gave less than 18 pg PGE metabolite. 13,14-dihydro-15-keto PGE levels are derived from added substrate (300 ng) using homogenised tissue and added cofactors.

RESULTS

Guinea-pigs of 50–55 days gestation had a mean fetal weight of 34.7 ± 3.7 g (mean ± SEM) and mean placental weights of 4.2 ± 0.2 g.

Metabolism experiments

Maximum production of PGE metabolite (13,14-dihydro-15-keto PGE) from incubations with 300 ng PGE substrate was 22.9 ng/incubate representing <8% conversion of the substrate. A comparison of vehicle and RU486-treated animals is shown in Fig. 1, F metabolite levels were also measured in these incubates and found to be less than 0.05 ng per mg protein in all tissues.

Synthetic potential

The incubation of the 1:10 dilution of the supernatant gave no significant differences between the control and treated groups of animals although there were marked differences between the tissues. Total PG production (the sum of PGE, PGF, Thromboxane B_2 and 6-oxo$PGF_1\alpha$) was myo/decidua 1.385 ± 0.289 ng; cervix 0.562 ± 0.096 ng; chorion 0.382 ± 0.053 ng; ammonium 5.127 ± 2.342 ng and in RU486-treated animals myo/decidua 1.43 ± 0.315 ng; cervix 0.95 ± 0.380 ng; chorion 0.30 ± 0.105 ng and amnion 2.27 ± 1.038 ng. It can be seen that the majority of production was in the amnion and the next highest was the myometrium/decidua with the minimum production by the chorion (Fig. 2). Some very high levels of TxB_2 and 6-oxo$PGF_1\alpha$ occurred in the control samples which were not present in the RU486-treated group but there were no significant differences.

Although conditions for metabolism were not ideal in these incubations (there was no addition of exogenous cofactors) the levels of metabolites are shown in Table 1. The levels of PGF metabolite (13,14-dihydro-15-keto PGF) were higher in the control group when compared to the RU486 group in both chorion and amnion.

Table 1. Metabolite concentrations in incubations without cofactors

Tissue	DHKE	±SEM	DHKF	±SEM	DHK6KF	±SEM
Vehicle						
Myo/D	46	17	156	42	126	10
Cervix	48	13	121	34	73	12
Chorion	40	8	81	16	65	8
Amnion	343	265	195	58	397	302
RU486						
Myo/D	59	8	83	28	127	27
Cervix	62	16	94	35	63	18
Chorion	36	6	40*	5	49	10
Amnion	51	8	56*	11	75	18

DHKE = 13,14-dihydro-15-keto PGE_2, DHKF = 13,14-dihydro-15-keto $PGF_2\alpha$ and DHK6KF = 13,14-dihydro-6,15-keto $PGF_1\alpha$.
*Significantly less than control values $P = < 0.05$.

DISCUSSION

We have tested the catabolic activity of tissues from the pregnant guinea-pig and shown that treatment with the progesterone antagonist RU486 results in a significant loss of metabolism of PGs to their inactive dihydro-15-keto form. Such a reduction in PG inactivation would account for the increase in sensitivity of myometrium to exogenous PG which has been observed directly in guinea-pig studies [13] and which is inferred from clinical data in the human where the efficacy of RU486 is greatly enhanced by the subsequent administration of otherwise sub-threshold doses of synthetic prostaglandins [1–4]. The period of gestation chosen in these experiments (days 50–55) corresponds to a time at which catabolism is about to decline as a natural feature of the guinea-pig pregnancy [24].

The reduced conversion of PG to its metabolite initiated by antiprogestin would

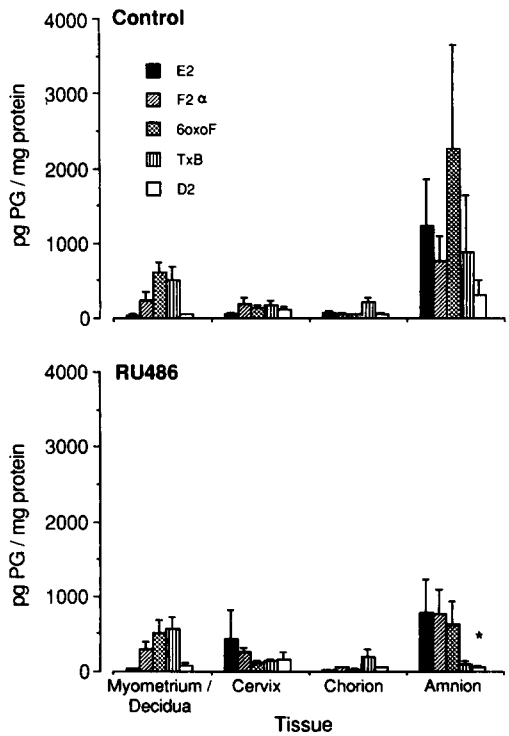

Fig. 2. The synthesis of prostaglandins by homogenates of tissue from animals treated either with vehicle alone or with 10 mg RU486 for 24 h. Production of PGD was significantly reduced in amnion tissue obtained from animals treated with RU486 ($P < 0.05$).

also account for the observed reduction in PGF metabolite observed in the uterine vein blood from guinea-pigs treated with antiprogestins [19, 20]. It is clear that a reduction of metabolite in the venous drainage would not be a valid measure of prostaglandin production within the uterus if changes in catabolism are also involved. Since prostaglandins are considered to exert their action locally, the control over breakdown as well as synthesis must be considered as an appropriate regulator of function. In the human uterus the ability to catabolise PG is high early in pregnancy [25] and maintained throughout gestation. This is understandable protection against prostaglandin production causing an inappropriate increase in myometrial activity during the pregnancy. Such a protective catabolism would obviously have to be reversed prior to induction of abortion or indeed prior to normal parturition. Extensive research into the role of prostaglandins in normal parturition has shown that the amnion is the main source of PG production in the term uterus [26]. The amnion has little ability to metabolise prostaglandin but the adjacent chorion produces little prostaglandin but has extensive catabolic capacity [27]. Thus prostaglandin produced by the amnion is largely inactivated before reaching the myometrium. The above results also show that in the chorion of the pregnant guinea-pig there is little prostaglandin production but massive catabolism of prostaglandin and this catabolism is reduced significantly (9-fold reduction) by treatment with antiprogestin *in vivo* for 24 h. Thus the antiprogestin treatment would render any endogenous prostaglandin production effective in activating myometrial contractility. A circular effect of prostaglandins on the pregnant myometrium has been described in which stretch of the uterus causes prostaglandin production and prostaglandins result in myometrial tension [28]. A permissive mechanism for the action of antiprogestin as described above would reduce the threshold above which prostaglandin synthesis would result in a sustained cycle of myometrial contractions and endogenous prostaglandin synthesis. In that situation myometrial contractility would appear low (as observed in the guinea-pig [12]) or variable (as seen in the human [29, 30]). In both cases an enhanced sensitivity to exogenous PG would be induced as has been observed [12, 13, 1–4].

Although a 5.7-fold increase in PGE production by tissue from the cervix was observed in this study (Fig. 2), this increase was not significant because of a very variable response. In the amnion a decrease of synthesis of prostaglandin synthesis was observed, which again was not significant. This may be due to a stimulation of PG production *in vivo* prior to tissue harvesting, which would lead to reduced precursor availability.

The significance of these findings is that the role of antiprogestins and progesterone in governing PG catabolism may prove to be an important regulator of myometrial contractility and arguably more important than an induced increase in synthesis which would merely result in transiently elevated levels which would be rapidly lowered by catabolic activity. The reduction in prostaglandin catabolism would also facilitate prostaglandin migration between compartments in the uterus and since the amnion is thought to be the major producer of prostaglandin to start labour and the myometrium thought to be the target for this prostaglandin, it is essential for the highly active catabolism by the intervening chorion to be reduced before labour can be initiated. Although the action of RU486 in inducing abortion is usually seen as an antiprogestin effect, the ability of the drug to sensitize the myometrium to exogenous prostaglandin may depend in part on its antiglucocorticoid effects since studies in the guinea-pig by Elger and colleagues have shown that a well characterised antiprogestin with reduced antiglucorticoid effect (ZK98,734) is relatively inactive in priming myometrium with increased sensitivity to exogenous PG [12]. Further work is therefore needed to investigate the relative contribution of antiglucocorticoid and antiprogestin effects in reducing prostaglandin catabolism.

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