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HORMONAL AND RELATED FACTORS AFFECTING THE RELEASE OF PROSTAGLANDIN $F_{2\alpha}$ FROM THE UTERUS*

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SUMMARY

While evidence is accumulating that prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is the uterine luteolytic factor in several sub-primate species, factors controlling the release of $PGF_{2\alpha}$ from the uterus are not fully documented. The present study utilizes two models consisting of either the *in situ* or the autotransplanted uterus of the sheep. Four factors affecting the release of $PGF_{2\alpha}$ from the uterus are described. (a) *Ovarian steroid hormones*: Spontaneous peaks of estradiol-17β (E_2-17β) occur throughout the estrous cycle but it is not until the time of corpus luteum regression that peaks of $PGF_{2\alpha}$ release from the uterus become associated with peaks of E_2-17β secretion. When physiological amounts of E_2-17β are infused into the arterial supply of the autotransplanted uterus, $PGF_{2\alpha}$ is released only late in the luteal phase suggesting that a priming effect of progesterone may also be necessary for $PGF_{2\alpha}$ synthesis. (b) *Oxytocin*: When physiological amounts of oxytocin are infused into the arterial supply of the *in situ* uterus, uterine tonus and amplitude of contractions increase immediately and are associated with a simultaneous increase in $PGF_{2\alpha}$ release, an effect which varied with the steroid status of the animal. Indomethacin inhibits the oxytocin-induced release of $PGF_{2\alpha}$ but not uterine contractions even although infusions of $PGF_{2\alpha}$ alone mimic this effect of oxytocin. It is possible that $PGF_{2\alpha}$ released during oxytocin action may have other important physiological functions such as altering uterine blood flow or changing cervical tone. (c) *Mechanical stimulation*: When the *in situ* uterus is mechanically stimulated by massaging it for 10 min, a rapid and sustained release of $PGF_{2\alpha}$ occurs only very early and very late in the cycle suggesting a dependence of this stimulus on the steroid hormone status of the animal. Peripheral blood levels of oxytocin were found to be elevated during massage at certain stages of the cycle, suggesting that at least some of the massage-induced release of $PGF_{2\alpha}$ may be mediated through oxytocin action on the uterus. (d) *Bacterial endotoxin*: Endotoxin has long been known to cause abortion in women as well as in lower species. Recent studies in pregnant mice have implicated uterine $PGF_{2\alpha}$ as the mediator of the abortifacient action of endotoxin. Minute quantities of endotoxin injected into the arterial supply of the uterus produce a marked and immediate increase in $PGF_{2\alpha}$ release, an effect which is abolished by Indomethacin. These studies at the organ level confirm and extend the evidence that the abortifacient action of endotoxin is mediated *via* the release of $PGF_{2\alpha}$ from the uterus.

In conclusion the response of the uterus to a variety of physiological and pathological stimuli appears to center invariably on the release of $PGF_{2\alpha}$ (and possibly other PGs) and evidence exists that this event is wholly or partially dependent on the endogenous steroid status of the animal.

INTRODUCTION

It has been known for many years that the uterus plays an important role in corpus luteum (CL) regression in a number of mammalian species, primates being notable exceptions (See Review, 1). Recent studies [2–4] indicate that a substance of uterine origin, namely prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is the uterine luteolytic factor in the sheep and good evidence exists for a similar role for $PGF_{2\alpha}$ in the guinea-pig [5]. The identity of $PGF_{2\alpha}$ as a luteolytic hormone in the sheep is based on the finding that the onset of CL regression coincides with a marked increase in $PGF_{2\alpha}$ concentration in uterine vein blood as determined by g.l.c./MS [6, 7], by bioassay [8], and by radioimmunoassay [4]. When an amount of $PGF_{2\alpha}$ equivalent to the peak luteolytic release (25 μg/h) is infused into one uterine vein, premature CL regression is induced, an effect which is not observed during systemic infusions of $PGF_{2\alpha}$ [2]. These findings support the existence of a mechanism for the local transport of $PGF_{2\alpha}$ from the uterus to the ovary and in addition support the role of $PGF_{2\alpha}$ as a luteolytic hormone in the sheep.

Knowledge of the factors affecting the synthesis and/or release of $PGF_{2\alpha}$ from the uterus is incomplete. The present study utilizes two model systems consisting either of the *in situ*, or the autotransplanted uterus of the sheep. Using these models, four different, but inter-related factors affecting the release of $PGF_{2\alpha}$

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Table 1. Concentration of prostaglandin $F_{2\alpha}$ and estradiol-17β in utero-ovarian venous blood sampled from the *in situ* uterus once/day late in the estrous cycle of (From: 27)

Day of cycle	No. of ewes	Mean $PGF_{2\alpha}$ (ng/ml ± S.E.)	Mean E_2-17β (pg/ml ± S.E.)
12	3	3·0 ± 1·6	15·7 ± 3·9
13	4	2·9 ± 1·4	10·8 ± 5·0
14	4	4·7 ± 2·2	54·6 ± 8·8
15	4	18·2 ± 7·3	178·6 ± 13·1
16	3	2·8 ± 1·3	21·3 ± 5·6

from the uterus will be considered, *viz.* 1. ovarian steroid hormones, 2. the polypeptide, oxytocin, 3. mechanical stimulation in the form of uterine massage and 4. bacterial endotoxin.

EXPERIMENTAL RESULTS

1. *Ovarian steroid hormones*

(a) *Endogenous secretion of estradiol-17β, progesterone, prostaglandin $F_{2\alpha}$, and plasma LH levels in the cycling and early pregnant ewe.*

Sheep with an autotransplanted uterus and ovary were used in this study because these preparations permit the continuous sampling of utero-ovarian venous blood in the conscious, undisturbed animal over long periods of time [3, 9]. For comparative purposes several normal cycling and early pregnant sheep were prepared with a cannula inserted into a utero-ovarian vein *in situ* to permit short term sampling of blood from the uterus over a period of several days. Blood samples were analyzed for $PGF_{2\alpha}$, progesterone (P), estradiol-17β (E_2-17β), and luteinizing hormone (LH) using sensitive radioimmunoassay methods. Initially the animals with a cannula in the *in situ* utero-ovarian vein were sampled once per day during the late luteal phase and the plasma was analyzed for $PGF_{2\alpha}$ and E_2-17β concentration. The results are shown in Table 1, where it can be seen that the mean level of $PGF_{2\alpha}$ was significantly higher on day 15 (18·2 ng/ml) than the mean levels at the other times sampled (3·4 ng/ml). In addition, the concentration of E_2-17β on day 15 was also significantly elevated (178·6 pg/ml) compared to the other days examined (25·6 pg/ml). However with this approach, the precise temporal relationships between E_2-17β secretion and $PGF_{2\alpha}$ release could not be established.

In an attempt to resolve the above problem, samples of utero-ovarian vein blood were collected at frequent intervals during the estrous cycle in two sheep bearing utero-ovarian transplants. In the first animal, samples were collected every 8 h from day 13 of one cycle to day 1 of the following cycle (estrous = day 0). With this sampling frequency, $PGF_{2\alpha}$ was observed to be released in what appeared to be a biphasic peak on day 15 at which time E_2-17β concentration rose from a mean of 66 pg/ml to a mean

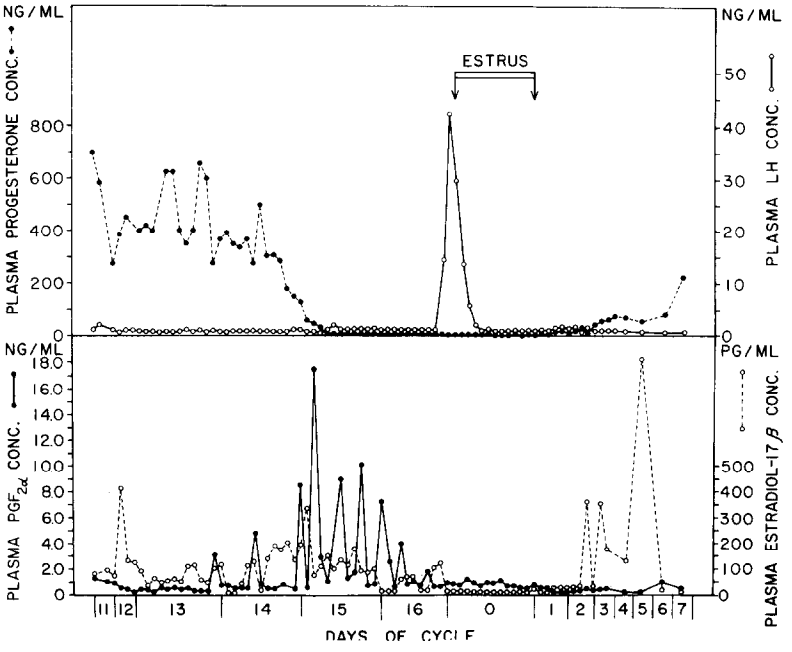

Fig. 1. Utero-ovarian vein plasma concentration of $PGF_{2\alpha}$ (ng/ml), progesterone (ng/ml), LH (ng/ml), and E_2-17β (pg/ml) in a sheep bearing a utero-ovarian transplant sampled every 2 h during the cycle. (From: 27).

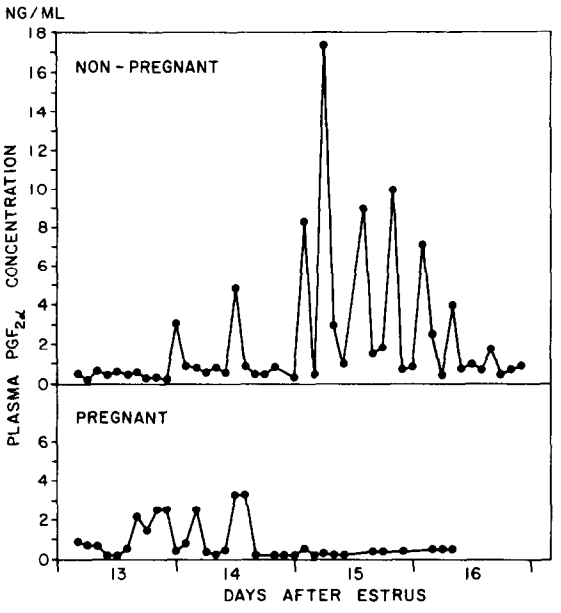

Fig. 2. Peaks of $PGF_{2\alpha}$ in utero-ovarian vein plasma collected *in situ* from a non-pregnant and a pregnant ewe during the time of CL regression. While the smaller peaks on days 13 and 14 were observed in both animals, the larger peaks on days 15 and 16, associated with CL regression, were absent in the pregnant animal. (From: 27).

of over 200 pg/ml. In the second ewe sampling frequency was increased to 12 times per day (every 2 h) and the results are shown in Fig. 1. It can be seen that although peaks of E_2-17β occurred through the cycle (including the day 3–4 peak), it was not until day 13 and 14 that small peaks of $PGF_{2\alpha}$ became associated with the peaks of E_2-17β. Larger peaks of $PGF_{2\alpha}$ lasting 2 h or less were observed on day 15 and were associated with the completion of CL regression as evidenced by a fall in P concentration.

It is known that the embryo must be present in the ovine uterus on day 12 or 13 if the CL is to be converted into the CL of pregnancy [10]. Normal ewes were mated to a fertile ram and the utero-ovarian vein adjacent to the CL was cannulated on cycle day 12. Samples were collected at frequent intervals for several days. It can be seen (Fig. 2) that the large peaks of $PGF_{2\alpha}$ observed on days 15 and 16 in the non-pregnant ewe were absent in the pregnant animal in which a pregnancy was confirmed by the recovery of an embryo. The release of $PGF_{2\alpha}$ in a non-pregnant ewe is shown in Fig. 2 for comparison. This absence of peaks in the early pregnant ewe is similar to recent results from other laboratories [11, 12] and suggests that the presence of an embryo may effectively inhibit the major luteolytic release of $PGF_{2\alpha}$ and thus prevent the onset of CL regression.

(b) *The effect of exogenous E_2-17β and Indomethacin on the release of $PGF_{2\alpha}$ from the uterus*

Recent studies have shown that exogenous E_2-17β treatment increases the release of $PGF_{2\alpha}$ from the uterus of the guinea-pig [5] and raises the level of "PGF" in peripheral blood of the sheep [13]. However, in these studies the E_2-17β was given by intramuscular injection in pharmacological doses and hence it was not established whether this effect of E_2-17β on the uterus was direct or indirect.

To establish whether the observed association during the late luteal phase between peaks of E_2-17β and $PGF_{2\alpha}$ was significant and also to determine whether the effect of E_2-17β on $PGF_{2\alpha}$ release was direct or indirect, physiological amounts of E_2-17β were infused in autologous plasma into the arterial supply of the autotransplanted uterus or into the systemic circulation at different stages of the cycle. In four ewes the mean release of $PGF_{2\alpha}$ during the control period was 1·40 (±0·6 S.E.) μg/h on day 14 of the cycle. After the infusion of E_2-17β at 1·0 ng/min the release of $PGF_{2\alpha}$ rose to 102·2 (±10·7 S.E.) μg/h between 60 and 90 min after beginning the infusion. In contrast, as shown in Fig. 3, E_2-17β infusions performed on days 6 and 10 of the cycle produced no increase in the basal release of $PGF_{2\alpha}$. To determine whether E_2-17β acted directly or indirectly on the uterus, a similar infusion was given into the systemic circulation on day 14 of the cycle. No change in the basal secretion of $PGF_{2\alpha}$ was observed after the infusion, indicating that E_2-17β acts directly on the uterus and not by an indirect mechanism such as *via* the pituitary.

Indomethacin is known to be a potent inhibitor of $PGF_{2\alpha}$ synthesis [14]. To determine whether the E_2-17β-induced release of $PGF_{2\alpha}$ represented *de novo* synthesis or the release of stored material, Indomethacin (2·5 μg/min) was infused for 60 min superimposed on the infusion of E_2-17β into the uterus. The result of this type of experiment is shown in Fig. 3 where it can be seen that Indomethacin markedly depressed the E_2-17β-induced release of $PGF_{2\alpha}$ on day 14. The finding that local infusions of E_2-17β into the uterus released $PGF_{2\alpha}$ only late in the cycle suggested that a prior period of progesterone is probably necessary

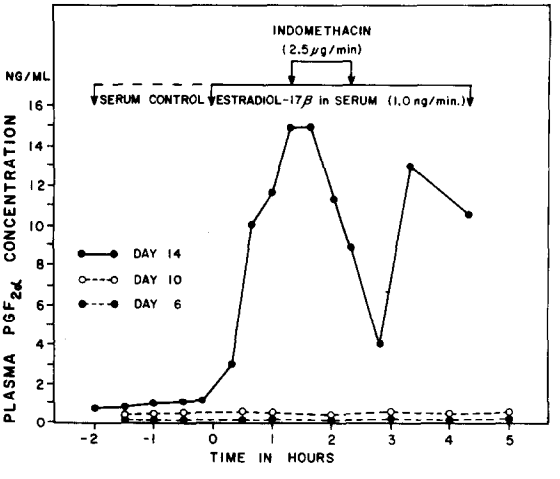

Fig. 3. The effect of a 1 h intra-arterial infusion of Indomethacin (2·5 μg/min) on the E_2-17β-induced release of $PGF_{2\alpha}$ from the autotransplanted uterus of the sheep. On days 6 and 10 (early luteal phase) no $PGF_{2\alpha}$ was released. In contrast, on day 14, E_2-17β infusion produced a release of $PGF_{2\alpha}$ which was temporarily suppressed by Indomethacin. (From: 27).

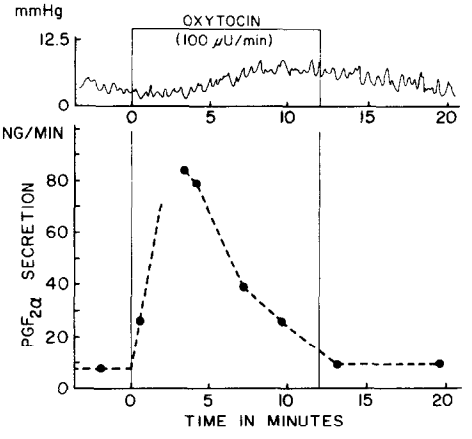

Fig. 4. The effect of oxytocin infused *via* the uterine artery *in situ* on intrauterine pressure and on the secretion of $PGF_{2\alpha}$ into uterine venous blood (day 13 of the cycle). (From: 28).

before E_2-17β can elicit a release of $PGF_{2\alpha}$, a conclusion which is supported by the correlation observed between the endogenous spontaneous secretion of E_2-17β and $PGF_{2\alpha}$ in the normal cycling ewe (Fig. 1).

2. *Oxytocin*

Because Indomethacin and Meclofenamate prevented oxytocin-induced contractions of the rat uterus *in vitro*, Vane and Williams [15] recently proposed that oxytocin promotes the synthesis of uterine PGs which in turn may induce uterine contractions. Under physiological conditions *in vivo* we find that oxytocin can increase the rate of secretion of $PGF_{2\alpha}$ from the *in situ* sheep uterus in advance of noticeable changes in uterine motility (Fig. 4).

The concentration of oxytocin in the blood of sheep often reaches 100 μU/ml during vaginal stimulation [16]. Since the flow through one uterine artery approximates 5 ml/min during the luteal phase, infusions of oxytocin into the uterine circulation at rates as high as 500 μU/min would appear to be within physiological limits. In Fig. 4 it can be seen that an infusion rate 5 times lower than this amount elevated the secretion of $PGF_{2\alpha}$ into the uterine vein at least 10-fold. A substantial increase in the secretion rate occurred before intra-uterine pressure changed.

On the other hand, we observed that while Indomethacin inhibits the oxytocin-induced synthesis of uterine $PGF_{2\alpha}$, it does not necessarily prevent oxytocin-induced uterine contractions (Fig. 5). However, infusions of small amounts of $PGF_{2\alpha}$ into the uterine artery were able to mimic both the increase in uterine pressure and uterine contractions characteristically produced by the infusion of oxytocin.

Our observations thus support the view that the PG-synthetic mechanism can be extremely sensitive to oxytocin but our results argue against, not only the hypothesis that PGs mediate oxytocin's effect on uterine motility, but also against its converse, *viz.* that uterine motility must increase before PGs are generated in response to oxytocin. Oxytocin thus appears to have two effects on the uterus: it stimulates PG synthesis and it increases myometrial activity. It would seem that the uterine contracting effect of $PGF_{2\alpha}$ and oxytocin are quite independent of each other but, since $PGF_{2\alpha}$ is normally released during oxytocin stimulation, $PGF_{2\alpha}$ may act synergistically with this polypeptide. In addition the release of $PGF_{2\alpha}$ during oxytocin action on the uterus may have other important physiological effects such as altering uterine blood flow or influencing tonus of the cervix.

Although further study is needed, the preliminary results presented in Fig. 6 indicate that the responsiveness of the PG-synthetic mechanism to oxytocin varies with the estrous cycle, suggesting at least a partial dependence of the system on the gonadal steroid status of the animal [17].

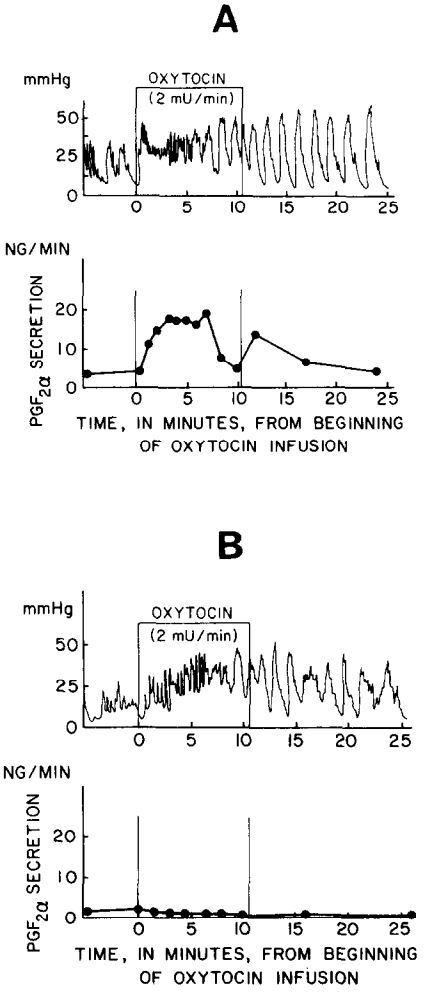

Fig. 5. Effect of Indomethacin on uterine activity and on secretion of $PGF_{2\alpha}$ into uterine venous blood during infusion of oxytocin *via* the uterine artery *in situ* (day 3 of the cycle). (A) Prior to Indomethacin, oxytocin infused at 2 mU/min caused the secretion of $PGF_{2\alpha}$ to increase to a peak of 20 ng/min from a control level of 3 ng/min. (B) At T = 0, Indomethacin had been infused into the uterine artery for 90 min at 12 μg/min. Intrauterine pressure responses were similar before and after Indomethacin treatment. (From: 28).

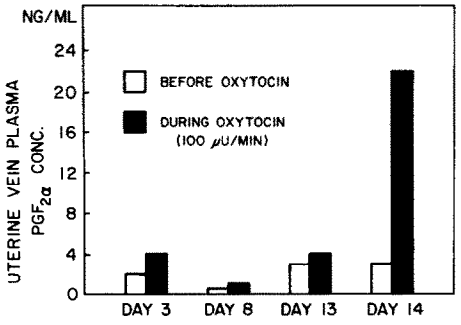

Fig. 6. Peak $PGF_{2\alpha}$ concentration in the uterine vein of *in situ* uterus during the intra-arterial infusion of oxytocin (100 μU/min) on different days of the cycle. The greatest release of $PGF_{2\alpha}$ occurred during the late part of the cycle. (From: 28).

3. *Mechanical stimulation*

It has been known for some time that mechanical stimulation, such as massage of the isolated guinea-pig lung[18] or the distension of the guinea-pig uterus [19], will rapidly cause the release of PGs. In addition the values reported for $PGF_{2\alpha}$ in uterine vein blood from different laboratories have shown very marked variations, a fact which was suspected to be due to differences in the manipulation of the uterus during sample collection. Accordingly, experiments were conducted in which the *in situ* uterus was mechanically stimulated on different days of the cycle by hand massaging it gently for 10 to 15 min during which time uterine venous blood was continuously collected over ice [20]. The effect of massage on $PGF_{2\alpha}$ release is shown in Fig. 7. It can be seen that the uterus is most responsive in the late luteal phase, pregnant animals being notable exceptions. We were aware that oxytocin was a potent stimulus to $PGF_{2\alpha}$ release (See Figs. 4 and 6), and that stimulation of the female tract can elicit oxytocin release [16]. Accordingly, using a sensitive mammary strip bioassay, the endogenous levels of oxytocin were measured

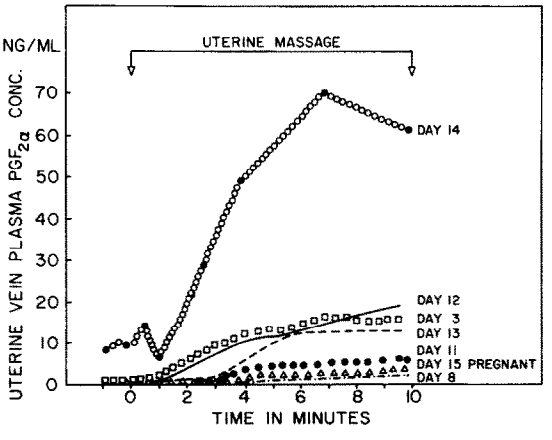

Fig. 7. The effect of mechanical stimulation (10 min massage) of the uterus on the release of $PGF_{2\alpha}$ into the uterine vein in non-pregnant and pregnant ewes at various times after estrus. As the cycle progressed the response became more pronounced. Early pregnant animals showed a diminished response. (From: 20).

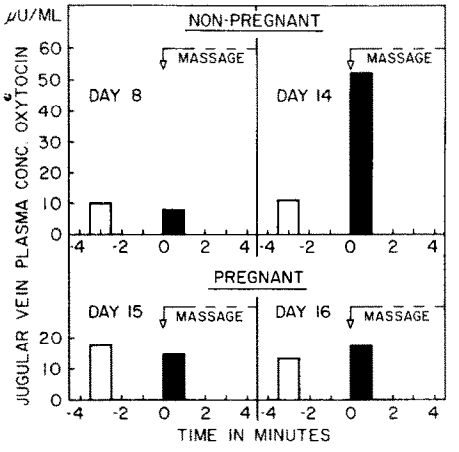

Fig. 8. The concentration of oxytocin (μU/ml) in jugular vein plasma before and one minute after mechanical stimulation (massage) of the uterus on day 8 and 14 of the cycle. The response was greatest on day 14 and appeared to be diminished in early pregnancy. (From: 20).

in jugular blood immediately before and one minute after begining uterine massage. The results are shown in Fig. 8 where it can be seen that a 5-fold increase in oxytocin occurs on day 14 but not on day 8 or in early pregnancy.

The ability of mechanical stimulation to release $PGF_{2\alpha}$ from the uterus appears to be dependent on two very distinct, but simultaneously operating, variables: (i) the variation of the oxytocin releasing reflex during the cycle and (ii) the variation in the ability of the uterus to release $PGF_{2\alpha}$ in response to oxytocin during the cycle. It is likely that both parameters are related to the steroid status of the animal. It would thus appear that great care must be taken during experiments on the female reproductive tract if spurious peaks of $PGF_{2\alpha}$ are to be avoided, particularly at times when the oxytocin releasing reflex is operable. It is worthy of note that both the oxytocin-induced release of $PGF_{2\alpha}$ (See Fig. 5) and the massage-induced

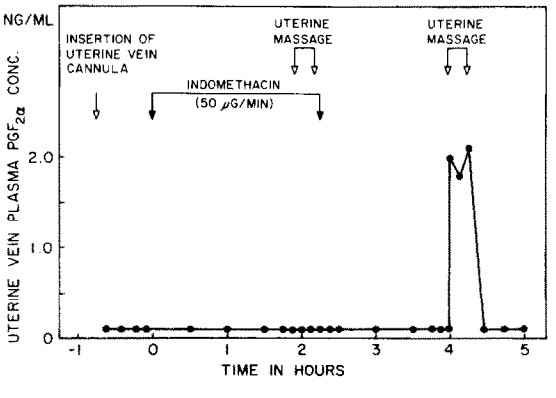

Fig. 9. Suppression of the mechanically-induced (10 min uterine massage) release of $PGF_{2\alpha}$ from the ovine uterus by pre-infusing Indomethacin (50 μg/min) into the uterine artery for two hours on cycle day 14. Two hours after stopping the Indomethacin, the normally marked response to massage (see. Fig. 7) appeared to be returning. (From: 20).

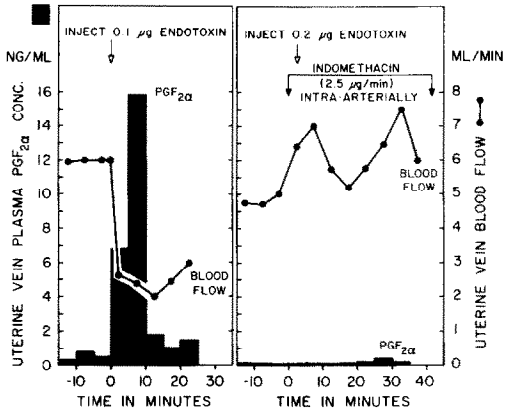

Fig. 10. The effect of endotoxin, given as a single injection (0·1 to 0·2 μg) into the arterial supply of the *in situ* ovine uterus, on $PGF_{2\alpha}$ release and uterine blood flow. Endotoxin caused an immediate and characteristic drop in blood flow and was accompanied by a major release of $PGF_{2\alpha}$. During Indomethacin administration into the uterine artery, endotoxin did not release $PGF_{2\alpha}$ and blood flow did not drop below control values. (From: 29).

release of $PGF_{2\alpha}$ are abolished by Indomethacin pretreatment of the uterus.

4. *Endotoxin*

It is known that experimental animals show greater sensitivity to bacterial endotoxins during pregnancy and the endotoxemia may induce abortion and fetal death in humans and lower animals [21, 22]. In recent papers [23, 24] evidence was presented that the abortifacient action of endotoxin in day 16 pregnant mice is mediated by $PGF_{2\alpha}$, the latter arising from the uterine endometrium in response to a single, small dose of toxin. In addition, the results suggested that intrauterine fetal death (as distinguished from abortion) and maternal diarrhea are mediated by PGs of the E series. All three endotoxic manifestations were abolished by pretreatment with the PG synthetase inhibitor, Indomethacin.

In the present study we examined the effect of a minute dose of endotoxin (prepared from *Salmonella enteritidis*) on $PGF_{2\alpha}$ release from the *in situ* uterus of ewes in the late luteal phase. An intra-arterial injection of 0·1 μg of endotoxin evoked an immediate drop in uterine blood flow concomitant with the release into the uterine venous effluent of a relatively high concentration of $PGF_{2\alpha}$ (Fig. 10). $PGF_{2\alpha}$ was measured in unextracted plasma (heparinized) according to a radioimmunoassay procedure described by Stylos, *et al.* [25]. The intra-arterial infusion of Indomethacin in saline (2·5 μg/ml/min) prior to and following injection of endotoxin effectively blocked the uterine blood flow response and the release of $PGF_{2\alpha}$. These results at the local organ level thus confirm and extend earlier findings in the intact animal. We intend to investigate the effects of hormonal conditioning on the uterine response to endotoxin utilizing sheep in various stages of the cycle.

DISCUSSION

Four factors affecting the release of $PGF_{2\alpha}$ from the uterus have been examined in an attempt to explore the nature of this process. From the data obtained during the study of the spontaneous secretion of ovarian steroids and from the E_2-17β infusion experiments, it would appear that both E_2-17β and progesterone can influence the release of $PGF_{2\alpha}$. This conclusion is in keeping with the results of earlier workers in this field who found that the administration of both exogenous E_2-17β and exogenous progesterone shortened the estrous cycle in the sheep.

While oxytocin infusions caused a pronounced release of $PGF_{2\alpha}$ from the uterus at certain stages of the cycle, it is not as yet clear whether this observation bears any significance in terms of luteolysis. Previous studies have shown that E_2-17β sensitizes the oxytocin releasing reflex but E_2-17β *per se* does not cause an increase in circulating blood levels of oxytocin. The fact that ewes bearing utero-ovarian autotransplants show normal spontaneous peaks of $PGF_{2\alpha}$ during luteal regression suggests that the oxytocin releasing reflex may not be in itself important, since the uterus is denervated during transplantation. However the ability of rising levels of E_2-17β to sensitize the uterus to standing levels of oxytocin would still have to be considered. A recent report [26] suggests that E_2-17β treatment may indeed increase the $PGF_{2\alpha}$ releasing effect of oxytocin in the ovine uterus.

The results obtained in the mechanical stimulation studies indicate the importance of careful handling of the female reproductive tract if misleading peaks of $PGF_{2\alpha}$ are to be avoided. It is not yet clear whether oxytocin is responsible for all of the observed release of $PGF_{2\alpha}$ during uterine massage. The known release of $PGF_{2\alpha}$ directly by mechanical stimulation of the isolated lung and uterus suggests that there may be a contribution from trauma in our experiments. However it should be possible to determine the relative contributions of oxytocin and trauma to the $PGF_{2\alpha}$ release by comparing the results obtained in massage experiments on the *in situ* uterus with those obtained in the autotransplanted uterus. Since the latter preparation is denervated there should be no reflex release of oxytocin.

Endotoxin has long been known to cause abortion in women as well as in other species. The results reported here with the *in situ* uterus support the mediating role of $PGF_{2\alpha}$ in the abortifacient effect previously reported in the pregnant mouse. The release of $PGF_{2\alpha}$ from the sheep uterus, which may be hormonally dependent, was abolished by Indomethacin indicating that endotoxin causes *de novo* synthesis of $PGF_{2\alpha}$. The results at the local organ level thus confirm and extend earlier findings in the intact animal.

In conclusion the response of the uterus to a variety of physiological and pathological stimuli appears to center invariably on the release of $PGF_{2\alpha}$ and this event may be wholly or partially dependent on the

steroid status of the animal. By comparing and contrasting the $PGF_{2\alpha}$ releasing effect of these various stimuli, it is hoped that a greater understanding will be achieved of the mechanisms involved in the synthesis and release of $PGF_{2\alpha}$ from the uterus and the apparent modulating role which ovarian steroid hormones may play.

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