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Does Prostaglandin $F_{2\alpha}$ Released from the Uterus by Oxytocin Mediate the Oxytocic Action of Oxytocin?

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ABSTRACT

The role of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in the oxytocic response to oxytocin (OT) was investigated by infusing OT directly into the uterine artery of the sheep and recording changes in both the pattern of uterine motility and the rate of secretion of $PGF_{2\alpha}$ at different stages of the estrous cycle. On Day 3 (estrus = Day 0), OT infused from 0.1 to 5.0 mU/min produced a dose-related increase in the rate of secretion of $PGF_{2\alpha}$ but increased intrauterine pressure (IUP) and the frequency of contractions only at intermediate (1 mU/min) or high (5 mU/min) doses. Although exogenous $PGF_{2\alpha}$ infused at 15 μg/h mimicked OT, the myometrium responded normally to OT when the production of endogenous $PGF_{2\alpha}$ was suppressed with indomethacin. On Day 8, an intermediate dose of OT changed neither the pattern of uterine motility nor the rate of secretion of $PGF_{2\alpha}$. On Day 14, OT infused at rates as low as 0.025 mU/min increased the secretion of $PGF_{2\alpha}$ 4-fold but induced no change in IUP or frequency of contraction. At 0.1 mU/min, OT increased the rate of secretion of $PGF_{2\alpha}$ nearly 10-fold within 3 min but changed IUP only slightly and after a delay of 5 min. Even at higher doses, OT invariably elevated $PGF_{2\alpha}$ secretion but did not consistently raise IUP. We conclude that while increased contractile activity is not a *sine qua non* for OT-induced synthesis of $PGF_{2\alpha}$, neither is increased synthesis of $PGF_{2\alpha}$ an essential intermediate step in the activation of the myometrium by OT.

INTRODUCTION

After Karim and coworkers (Karim and Devlin, 1967; Karim and Sharma, 1971) suggested that prostaglandins (PGs) may be physiological oxytocics, considerable support developed for the view that PGs released from the uterus as a consequence of OT-induced contractions act synergistically with OT to change myometrial activity (Brummer, 1971; Beazley, 1971; Fuchs and Fuchs, 1973; Liggins, 1973). In 1973, however, Vane and Williams made the novel suggestion, based on work with an *in vitro* system, that the primary action of OT is to stimulate PG synthesis in uterine tissue. PG, they postulated, rather than OT, is the proximate cause of uterine contraction. We have reexamined this hypothesis *in vivo*, using the *in situ* uterus of the nonpregnant, cycling sheep as an experimental model. Although our results support the view that OT can stimulate uterine synthesis of PG, they argue against not only the hypothesis that uterine motility must increase before PGs are generated in response to OT but also against its converse, *viz.*, that PGs mediate the oxytocic action of OT.

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MATERIALS AND METHODS

Cycling Merino ewes on Day 3 (N=6), 8 (N=1) or 14 (N=3) of the estrous cycle (day of estrus = 0) were anesthetized with thiopental and maintained under anesthesia with Fluothane and oxygen in a semiclosed system. The uterus was reflected out of the abdominal cavity through a midline incision to expose the vasculature contained in the broad ligament. The common hypogastric artery was cannulated with a 20 gauge Teflon cannula filled with heparin in saline (100 IU/ml). The cannula was directed into either the right or left hypogastric artery and hence into either the right or left uterine artery. Other branches of the hypogastric artery were clamped with serraphins to ensure that the infusate reached only the uterus. The cannula was secured with a purse-string suture to permit blood to flow into the uterus continuously. The right or left uterine vein was cannulated at a point 2–3 cm distal to the uterus with a heparin-filled Teflon cannula approximating the diameter of the vein itself (3–5 mm) and secured with encircling ligatures so that the entire uterine venous effluent of the horn could be collected. Between collections, venous drainage continued via the substantial collateral circulation to the cervix and contralateral uterine horn. A fluid-filled catheter with a balloon (undistended volume = 1 ml) at its tip was inserted into the body of the uterus via a small incision and passed along the uterine lumen until the balloon reached a point approximately 2 cm from the apex of the horn opposite the ovary bearing the corpus luteum. When all surgical manipulations were complete, the animal was given 8000 IU of heparin intravenously.

Collections of the uterine venous effluent, timed with the event marker of a Grass polygraph (Model 7B), were made into conical centrifuge tubes graduated in divisions of 0.2 ml. The distal end of the cannula was held at a fixed level throughout each experiment. Frequent collections were made before, during and after solutions of $PGF_{2\alpha}$ (as the water-soluble tromethamine salt) or OT in saline or indomethacin in autologous blood plasma were infused with a constant-flow pump (Harvard Apparatus, Model 600-910/920) at a rate of 0.05 ml/min into the local uterine circulation via the arterial cannula. Each infusion of $PGF_{2\alpha}$ or OT lasted approximately 10 min and successive infusions were separated by an interval of 30 min to more than an hour if necessary until basal contractile activity reestablished itself. Substances were administered in the following sequence: saline, $PGF_{2\alpha}$, OT in increasing amounts except in the experiments with indomethacin where a constant amount was administered repeatedly. The $PGF_{2\alpha}$ was a gift of the Upjohn Company. Purified synthetic oxytocin (400 IU/mg) without preservatives was supplied by Sandoz, Switzerland. Indomethacin was provided by Merck and Co.

Pressure in the intrauterine balloon was recorded continuously on the Grass polygraph using a Statham pressure transducer (P23AC) and a low-level DC preamplifier. By carefully accounting for dead space in the arterial and venous cannulae, each period of blood collection and each period of infusion were adjusted in time (± less than a min) with the trace of intrauterine pressure (IUP).

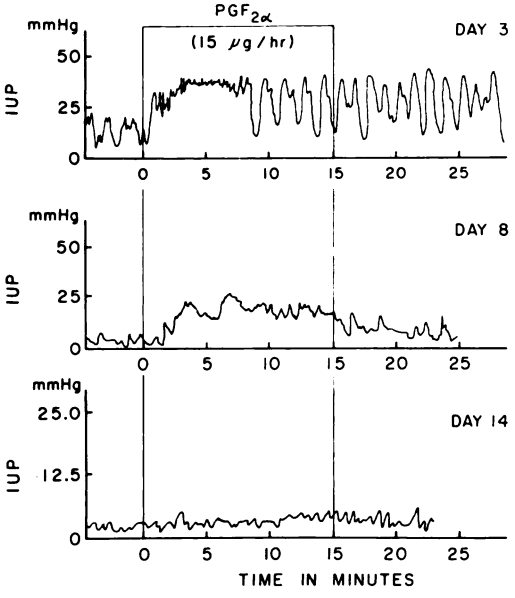

FIG. 1. Changes in intrauterine pressure (IUP) during the infusion of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) into the uterine artery of sheep on Day 3, Day 8 and Day 14 of the estrous cycle (Estrus = 0). Note the expanded scale for IUP in the bottom panel. A rate of infusion of 15 μg/h was chosen as a standard after preliminary experiments with animals on Day 3 of the cycle showed a barely detectable response at 5 μg/h and persistent hypertonus at 100 μg/h.

Blood samples (1–4 ml) were collected in heparinized tubes over ice. Plasma was separated after centrifugation at 4°C and frozen at −20°C. $PGF_{2\alpha}$ was measured directly in unextracted plasma using the radioimmunoassay procedure described by Van Orden and Farley (1973) with an antibody prepared according to the method of Stylos et al. (1972). The standard curve and cross-reactivity data were similar to those reported by Van Orden and Farley but two of the metabolites of $PGF_{2\alpha}$ were also tested (13,14-dihydro-15-keto-$PGF_{2\alpha}$ and 13,14-dihydro-$PGF_{2\alpha}$). Only the latter showed slight cross-reactivity (1.5 percent). When $PGF_{2\alpha}$ was added in doubling dilutions ranging from 0.5–0.06 ng/tube to 200 μl aliquots of a pool of arterial plasma (known to contain undetectable amounts of endogenous $PGF_{2\alpha}$), recoveries averaged 110.8 ± 2.4 percent (± SEM) and showed no systematic variation with dose. Moreover, the recovery of $PGF_{2\alpha}$ (0.6 ng/ml) added to uterine venous plasma from each of the experiments reported herein was quantitative when corrected for endogenous levels and showed no systematic variation with phase of the estrous cycle. The inter-assay coefficient of variation calculated from these recoveries was 9.2 percent. The intra-assay coefficient of variation for 12 replicates of a single sample containing standard $PGF_{2\alpha}$ (0.6 ng/ml) was 3.1 percent. Ten pg of $PGF_{2\alpha}$ reduced the binding of labeled $PGF_{2\alpha}$ in the assay significantly at the 95 percent level of confidence. Thus, when 200 μl aliquots of plasma were assayed, levels of $PGF_{2\alpha}$ as low as 50 pg/ml were detectable. For comparison, the lowest concentration recorded in uterine venous plasma throughout this study was 97 pg/ml.

RESULTS

Effect of Exogenous $PGF_{2\alpha}$

The contractile response of the uterus to infusions of $PGF_{2\alpha}$ appeared to vary with the estrous cycle (Fig. 1). *Day 3.* When infused for 10 min at a rate of 15 μg/h, the uterus responded immediately with a period of hypertonus and high frequency contractions. During the course of the infusion, this pattern changed abruptly to one of large amplitude, low frequency contractions that persisted, in some cases, for more than an hour after the infusion had ended. The results presented are typical of those observed in 3 separate experiments performed on Day 3 of the cycle. *Day 8.* The uterus exhibited little spontaneous activity, but exogenous $PGF_{2\alpha}$ induced hypertonicity (Fig. 1B). No large-amplitude contractions developed, however. *Day 14.* Spontaneous activity was again minimal and $PGF_{2\alpha}$, infused at 15 μg/h in 2 separate experiments, had almost no effect on frequency, amplitude or tonus (Fig. 1C). A moderate increase in tonus (to 20 mmHg) was recorded in one case (not shown)

but the infusion rate was 100 μg/h.

Effect of Exogenous OT

The response of the uterus to local infusions of OT also appeared to vary with the estrous cycle. *Day 3.* The myometrium was spontaneously active, exhibiting a pattern of high-amplitude contractions with superimposed high-frequency activity. OT, infused at 0.1 mU/min, caused no obvious change in this pattern of uterine motility but, as the infusion continued, the rate of secretion of $PGF_{2\alpha}$ increased to a peak after about 8 min (Fig. 2). At a rate of 1 mU/min (Fig. 3), the infusion of OT still caused no immediate change in myometrial activity but the rate of secretion of $PGF_{2\alpha}$ reached a peak after only 3–4 min. At a still higher rate of infusion (5 mU/min, Fig. 4), OT led to a hypertonic response and, within 2 min, to a 10-fold increase in the rate of secretion of $PGF_{2\alpha}$. This dose-effect relationship between OT and $PGF_{2\alpha}$ secretion was observed in each of 3 animals on Day 3 of the cycle (Table 1). The increase in secretion of $PGF_{2\alpha}$ was apparently not related to the rate of blood flow since the latter, at most, only doubled during infusions of OT. It should be noted that, in this entire study, uterine venous blood flow ranged only from 4.1 to 12.2 ml/min. For the most part, therefore, variations in the concentration of $PGF_{2\alpha}$ account for the observed variations in rate of secretion.

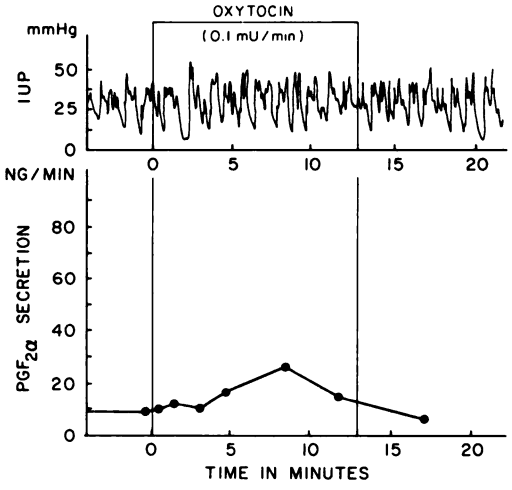

FIG. 2. The effect of oxytocin (OT), infused into the uterine artery at a rate of 0.1 mU/min, on IUP and on the rate of secretion of $PGF_{2\alpha}$ into uterine venous blood. Day 3 of the cycle.

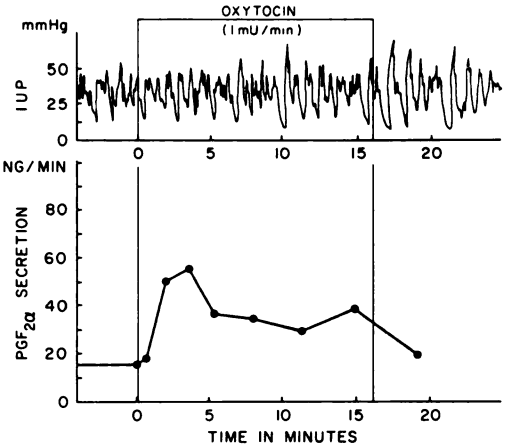

FIG. 3. A continuation of the experiment depicted in Fig. 2 with a higher dose of OT.

Day 8

OT had no detectable effect on uterine motility except at the highest dose (Fig. 5) and in no case did the extremely low rate of secretion of $PGF_{2\alpha}$ change in any consistent way.

Day 14

As on Day 8, exogenous OT had little effect on IUP except at high doses (Table 1) but, in contrast to Day 8, OT delivered to the Day 14 uterus at 0.025 and 0.1 mU/min (in separate experiments) produced 6-fold and 11-fold increases, respectively, in the secretion of $PGF_{2\alpha}$

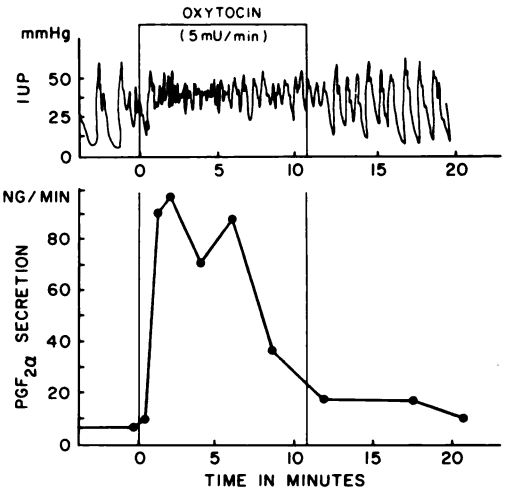

FIG. 4. A continuation of the experiment shown in Figs. 2 and 3 with OT infused at 5 mU/min.

TABLE 1. Effects of OT and $PGF_{2\alpha}$ on intrauterine pressure and on uterine venous secretion of $PGF_{2\alpha}$.

Day of cycle	Treatment[a]	1st Replicate			2nd Replicate			3rd Replicate		
		Basal[b]	Peak[c]	Δ_{IUP}[d]	Basal	Peak	Δ_{IUP}	Basal	Peak	Δ_{IUP}
Day 3	0.9% Saline	5.8 ± 1.6 (4)	4.7	0	2.5 ± 0.4 (4)	2.4	+12	2.5 ± 0.1 (5)	2.2	-21
	$PGF_{2\alpha}$, 15 μg/hr	5.3 ± 0.8 (3)	73.2	+95	1.9 ± 0.2 (3)	83.2	+39	1.9 ± 0.1 (4)	128.3	+79
	OT, 0.1 mU/min	9.1 ± 0.8 (4)	21.8	+5				1.4 ± 0.1 (3)	3.0	-6
	OT, 1.0 mU/min	11.2 (2)	56.4	+10	1.3 ± 0.2 (5)	5.3	+38	0.9 (1)	5.7	+48
	OT, 5.0 mU/min	6.7 ± 0.3 (4)	97.0	+61	1.8 ± 0.3 (5)	17.8	+84	1.2 ± 0.3 (2)	9.5	+99
Day 3	OT, 2 mU/min	3.0 ± 0.2 (5)	18.7	+48	1.8 ± 0.3 (4)	4.8	+178	1.5 (2)	10.8	+96
	OT, 2 mU/min (after 45 min indo)[e]	1.9 ± 0.3 (3)	2.0	+84	1.3 ± 0.2 (4)	1.2	+205	2.2 ± 0.3 (4)	1.3	+92
	OT, 2 mU/min (after 90 min indo)	2.4 ± 0.3 (3)	2.8	+67				0.7 ± 0.1 (4)	0.6	+96
Day 8	$PGF_{2\alpha}$, 15 μg/hr	1.1 (2)	> 75.0	+310						
	OT, 0.1 mU/min	0.7 ± 0.2 (4)	1.2	+4						
	OT, 1.0 mU/min	1.2 (2)	1.2	+5						
	OT, 10.0 mU/min	0.9 (1)	1.1	+850						
Day 14	$PGF_{2\alpha}$, 15 μg/hr	6.3 ± 0.4 (4)	102.2	+15						
	OT, 0.025 mU/min	5.3 ± 0.7 (6)	29.9	+8						
	OT, 0.25 mU/min	4.5 ± 0.4 (6)	12.6	+22						
	OT, 2.5 mU/min	2.3 ± 0.2 (3)	8.2	-19						
Day 14	0.9% Saline	9.9 ± 0.8 (5)	8.5	+12						
	$PGF_{2\alpha}$, 15 μg/hr	6.3 ± 1.5 (3)	47.6	+6						
	OT, 0.1 mU/min	8.2 (1)	90.2	+16						
	OT, 1.0 mU/min	8.0 (2)	16.2	-14	8.4 ± 0.4 (4)	46.9	+8			
	OT, 5.0 mU/min	7.2 (2)	31.4	+30	6.0 ± 0.2 (3)	23.4	+55			

[a]Sterile saline (0.9%), $PGF_{2\alpha}$ and oxytocin (OT) were infused for 10 min.

[b]Mean secretion rate of $PGF_{2\alpha}$ (ng/min ± SEM) into uterine venous blood during the 10–30 min period immediately preceding infusion. Numbers in parentheses indicate number of blood samples collected.

[c]Highest rate of secretion of $PGF_{2\alpha}$ (ng/min) recorded during infusion.

[d]Percent change in total intrauterine pressure determined by planimetry. Total area under pressure trace during the 10 min period immediately preceding infusion (A_1) was compared to the area during infusion (A_2) by the relation $\Delta_{IUP} = (A_2 - A_1)/A_1 \times 100$.

[e]Indomethacin (indo) was infused into the uterine artery for 45 min at a rate of 12 μg/min before the infusion of oxytocin began. In 2 experiments the infusion was repeated after 45 min more of indomethacin treatment.

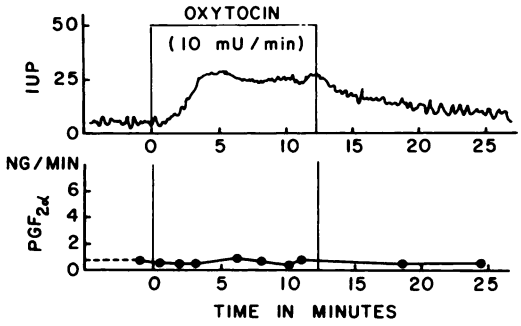

FIG. 5. The effect of OT infused into the uterine artery at 10.0 mU/min on IUP and on the rate of secretion of $PGF_{2\alpha}$ into uterine venous blood. Day 8.

(Fig. 6). In every experiment performed on Day 14, however, the lowest dose, always given first, was at least as effective as any higher dose administered subsequently.

Effect of Indomethacin

In 3 animals exhibiting OT-induced changes in both uterine motility and secretion of $PGF_{2\alpha}$ (Day 3 of the cycle), indomethacin was infused continuously into the uterine artery at 12 μg/min (Table 1). Figure 8 depicts the results of one of these experiments. After 45 min, when the treatment had caused the basal rate of secretion of $PGF_{2\alpha}$ to decline significantly ($P<0.01$) from 3.1 ± 0.1 mg/min to 1.3

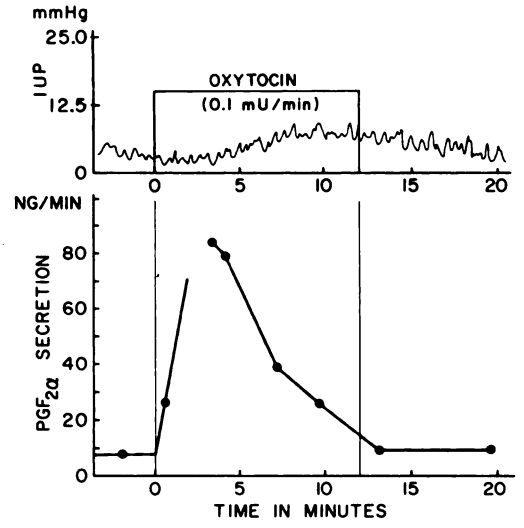

FIG. 6. The effect of OT, infused into the uterine artery at 0.1 mU/min, on IUP and on the rate of secretion of $PGF_{2\alpha}$ into uterine venous blood. Day 14.

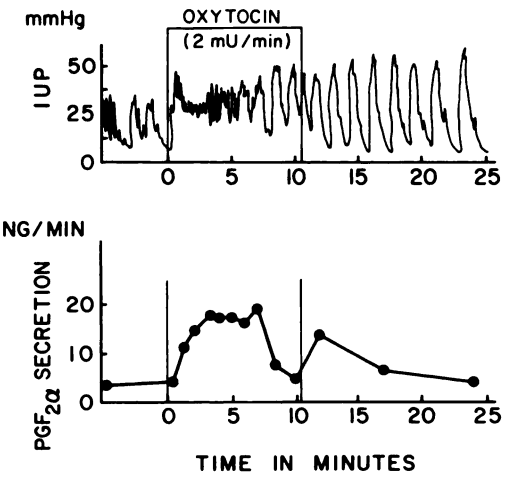

FIG. 7. The effect of OT, infused into the uterine artery at a rate of 2 mU/min, on IUP and on the rate of secretion of $PGF_{2\alpha}$ into uterine venous blood. Day 3.

± 0.2 ng/min, an infusion of OT (2 mU/min) was superimposed on the infusion of indomethacin. Even though both the uterine venous concentration and the rate of secretion of $PGF_{2\alpha}$ declined further during the course of the OT infusion, the change in uterine activity was almost identical to that induced by OT before indomethacin was infused (Fig. 7).

DISCUSSION

The background of exposure of the uterus to ovarian steroids is critically different on each of

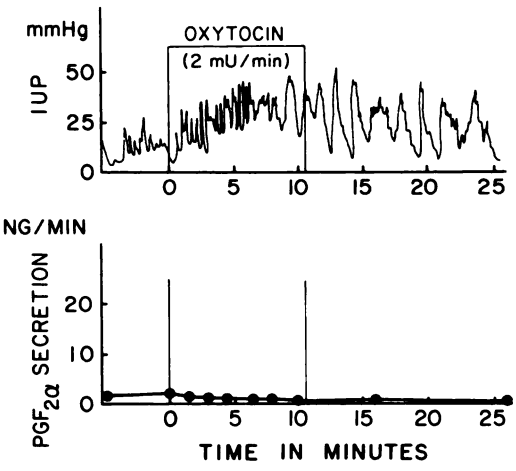

FIG. 8. A continuation of the experiment depicted in Fig. 7. The same dose of OT was infused but, prior to the infusion, indomethacin had been infused into the uterine artery for 45 min at a rate of 12 μg/min.

the 3 days of the estrous cycle chosen for study in these experiments. On Day 3, progesterone levels have been minimal for 5–6 days but a transient, postestrus surge of estrogen occurs at this time (Cox et al., 1971; Barcikowski et al., 1974). By Day 8, the ovary is beginning to secrete progesterone at a maximal rate (Thorburn et al., 1973; Cox et al., 1973) but the production of estrogen is relatively low (Barcikowski et al., 1974; Cox et al., 1973). On Day 14, when the luteal phase is nearly at an end, circulating progesterone begins to fall and estrogen tends to increase (Cox et al., 1971; Barcikowski et al., 1974). No substantial, spontaneous increases in uterine venous $PGF_{2\alpha}$ are observed on Day 3 or 8 but small increases have been seen occasionally on Day 14 (Barcikowski et al., 1974; Cox et al., 1973).

The myometrium appeared to be more sensitive to intermediate doses of OT (1–5 mU/min) on Day 3 than on Day 14, and myometrial sensitivity to $PGF_{2\alpha}$ followed a similar trend. The PG-releasing response of the uterus to OT varied with the cycle in a different way. OT led to a dose-related increase in the secretion of $PGF_{2\alpha}$ from the uterus on Day 3 but, instead of being ineffective on Day 14, OT caused almost immediate increases in $PGF_{2\alpha}$ secretion with little or no associated increase in uterine motility. We interpret these results to mean that increased myometrial activity is not a prerequisite to OT-induced release of $PGF_{2\alpha}$ from the uterus. Indeed, we have recently found that endometrial tissue *in vitro*, entirely isolated from the myometrium, can produce large amounts of $PGF_{2\alpha}$ when incubated with OT at concentrations as low as 10 μU/ml (unpublished observations).

Although this result strongly supports the assertion of Vane and Williams (1973) that OT has a direct effect on the synthesis of uterine $PGF_{2\alpha}$, the further suggestion of these authors that OT may cause myometrial contractions only through the agency of PGs remains in question. This latter proposal, based on the finding that uterine strips exposed to indomethacin respond to PGs but not to OT, does not conform with our observation that OT increases myometrial activity in the indomethacin-treated uterus of the sheep. Vane and Williams did report that OT induces contractile responses in indomethacin-treated uterine strips from pregnant rats. They suggested that in this special case an undetectable increase in the production of PGs may have occurred. We cannot categorically reject this interpretation but we have observed that the rate of secretion of $PGF_{2\alpha}$ from the indomethacin-treated uterus of the sheep does not increase and may even decline slightly when OT is infused. We therefore favor the view that PGs are not necessarily involved in OT's effect on the myometrium. However, it is possible that $PGF_{2\alpha}$ might increase in highly circumscribed regions of the uterus and escape detection in the venous effluent. Also, even though available evidence suggests that $PGF_{2\alpha}$ is the most prevalent uterine PG in the sheep (Wilson et al., 1972; Harrison et al., 1972), we cannot entirely rule out the possibility that OT may have stimulated the synthesis of a different PG in the indomethacin-treated uterus.

The accumulating evidence of a direct effect of OT on uterine synthesis of PGs permits the speculation that OT may play a fundamental role in the episodes of PG release which cause luteolysis in sheep (Barcikowski et al., 1974). Since the flow of blood through one uterine artery is at least 5 ml/min (Anderson and Hackshaw, 1974), the infusion of OT into the uterine circulation at 0.1 mU/min could result in a concentration in uterine blood of no more than 20 μU/ml, only about 2–3 times the resting level (Roberts and Share, 1969). Thus, small elevations in circulating OT appear capable of increasing the rate of secretion of $PGF_{2\alpha}$. Estrogens, which enhance the synthesis of $PGF_{2\alpha}$ by the uterus (Barcikowski et al., 1974), rise at the time of luteolysis (Cox et al., 1971; Barcikowski et al., 1974; Cox et al., 1973; McCracken et al., 1970) and, most significantly, amplify OT's effect on uterine synthesis of $PGF_{2\alpha}$ in the sheep (Sharma and Fitzpatrick, 1974), may condition the uterus to synthesize PGs in response to minimal elevations of OT or even to standing levels of the hormone. Implicit in this speculation is the intriguing prospect that the central nervous system, acting through the agency of OT, may contribute to the regulation of luteolysis.

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