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Relationship Between the Uterotonic Action of Oxytocin and Prostaglandins: Oxytocin Action and Release of PG-Activity in Isolated Nonpregnant and Pregnant Rat Uteri[1]

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ABSTRACT

The relationship between the uterotonic action of oxytocin (OT) and prostaglandins (PG) was studied on isolated rat uteri. We found that specific OT antagonist had no effect on the uterine response to PG under similar condition when the response to an equi-potent dose of OT was completely suppressed. Effective PG synthetase inhibitory doses of indomethacin and diclofenac sodium had no significant effect on the uterine response to OT or PG. These findings indicate that the myometrial receptors for OT and PG are functionally separate and distinct and that the uterotonic action of OT is independent of PG participation. Parturient uterus is extremely sensitive to OT action. One possible mechanism for this sensitization may be associated with an enhanced PG synthesis and release induced by OT. We measured the basal PG content and release in isolated uteri from nonpregnant, nonpregnant and estrogen treated rats, and from rats in the last four days of pregnancy. We found that estrogen increased the PG content and release in nonpregnant rat uteri. The absolute levels, however, remained low, less than 50 ng/uterus and 25 ng/uterus/hr. In late pregnant uteri, PG synthesizing capacity increased progressively reaching an enormous peak, an increase of more than 1000 percent on the expected day of delivery. OT, 1.0 mU/ml, caused contractions in both nonpregnant and pregnant uteri. The PG content and release of the nonpregnant uteri were not significantly affected by OT. This is consistent with our finding that the uterotonic action of OT is independent of PG. In the pregnant uteri, PG release was further markedly enhanced by OT. The PG content, however, was not significantly altered. Thus, our findings support the hypothesis that the marked increase in sensitivity of the parturient uterus to OT is related to an enhanced PG synthesis and release induced by OT. The possibility that the uterotonic and PG-releasing actions of OT are two separate functions of OT, each acting on a different receptor site and/or mechanism is discussed.

INTRODUCTION

The uterotonic action of oxytocin (OT) has been known for many decades. The hormone is believed to play a functional role in parturition. Over the past few years, there is a growing body of evidence that prostaglandins (PGs) also play an important role in parturition. Clinically, both OT and PGs (PGE_2 and $PGF_{2\alpha}$) are proven effective oxytocics. The mechanism of action and the interrelationship between these two groups of uterotonic agents, physiologically or pharmacologically, are poorly understood. Vane and Williams (1973) first suggested that the uterine stimulating activity of OT might be mediated through uterine PGs. Hertelendy (1973) showed that oxytocin-induced parturition in the term pregnant rabbit could be blocked by indomethacin, an inhibitor of PG biosynthesis. Because of the fundamental significance of this implication, it is imperative that direct evidence be sought to ascertain whether uterine contractions induced by OT are linked to a PG production or release. We, therefore, measured PG release from isolated rat uterus in response to OT stimulation. Our preliminary studies showed that OT indeed could induce the release of PG-like activity from isolated rat uterus into the bathing medium (Chan, 1974a, b, 1976).

In this paper, we report in greater detail our studies of the PG-releasing activity of OT and the relation to its uterine stimulating action. We also studied the PG-releasing activity of OT on isolated uteri from pregnant rats in the last four days of gestation to determine whether the marked increase in sensitivity of the parturient uterus to OT may be associated with an enhanced PG release to OT stimulation.

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MATERIALS AND METHODS

Uterotonic Activities of OT and PG

The uterine stimulating activities of OT and $PGF_{2\alpha}$ were studied on isolated uteri from adult, virgin Wistar albino rats in natural estrus. The estrous stage of the rat was determined in the morning by microscopic examination of the vaginal smear. The uterine horns were removed and suspended in an organ bath (6–8 ml) in van Dyke-Hastings (1928) solution, continuously aerated with 95 percent O_2 and 5 percent CO_2, pH 7.4 and maintained at 30°C. The van Dyke-Hastings solution is one of the commonly used bathing media for the rat oxytocic assay of neurohypophysial polypeptides. It differs from the Krebs-bicarbonate solution principally in its lowered Ca^{++} (0.5 mM), Mg^{++} (0.5 mM) and glucose (0.1 percent) concentrations. In this bathing medium, uterine horn retains a good sensitivity to OT and seldom exhibits spontaneous contractions. After an equilibration period of at least 60 min, the uterine contractile responses to single injections (contact time either 1 min or 5 min depending on experimental requirement) of OT or $PGF_{2\alpha}$ were measured with an isotonic or isometric transducer and transcribed on a polygraph. The effects of OT inhibitor or PG synthetase inhibitors on the uterine response to OT or $PGF_{2\alpha}$ were investigated in this isolated rat uterine preparation. OT inhibitor was introduced into the bath also as single injections, 5–10 seconds prior to the injection of OT or $PGF_{2\alpha}$. PG synthetase inhibitor was added to the bathing medium at least 30 min before the testing began. The following concentrations of PG synthetase inhibitor were used: 2.5, 5.0, 10 and 20 μM.

PG Release in Nonpregnant Uterus

Virgin, Wistar albino rats weighing between 180 and 200 g were used. Uterine horns were removed from rats either in diestrus, determined by microscopic examination of the vaginal smear, or that had been treated with diethylstilbestrol dipropionate (DES) 10 μg/100 g s.c. 24 h prior to the experiment. From each rat, one horn was used as the control and the contralateral horn as the experimental counterpart subject to OT stimulation. Thus, the release of PG from the OT stimulated horn was always compared against its matched-pair control. Six to eight rats were used in each experiment. The control horns and the experimental horns were pooled respectively and placed in 15 ml of van Dyke-Hastings solution, pH 7.4 at 30°C and aerated continuously with 95 percent O_2 and 5 percent CO_2. After a 60 min equilibration period during which time the bathing fluid was changed every 10 min, the incubation period began. The bathing medium for the experimental horns was switched to one which contained in addition 1.0 mU/ml of OT (Syntocinon). The bathing fluid was withdrawn and a fresh portion replaced at 15 min intervals until a total incubation time of 60 or 90 min was attained. At the end of each experiment, the uterine tissues were immediately placed in an ice-cold bathing medium. The tissues were blotted lightly on filter paper and the wet weights of the two tissue groups were taken. The tissue samples were then stored in 15 ml of isopropyl alcohol at −20°C until used for PG extraction. The samples of bathing fluid from the control and the experimental horns were pooled respectively, acidified to pH 3 with 1.0 N HCl and extracted twice with an equal volume of ethyl acetate. The ethyl acetate phases were pooled respectively and evaporated to a small volume with a rotatory evaporator at 40°C under reduced pressure. The final drying step was carried out under nitrogen at atmospheric pressure. The residue was stored at −20°C until used for bioassays.

Extraction of tissue PG was carried out by homogenizing the tissue in isopropyl alcohol with a glass tissue grinder. An equal volume of phosphate buffer, pH 7.4, was added to the homogenate and mixed in a shaker for 30 min. After centrifugation, the alcoholic supernatant was withdrawn and washed twice with an equal volume of n-hexane to remove neutral fats and lipids. It was then extracted twice with an equal volume of chloroform. The chloroform phase was evaporated to dryness as described above for the bathing fluid sample. The residue was stored at −20°C until used for bioassays.

PG Release in Pregnant Uterus

PG release was determined in isolated pregnant uterus following the protocol as described for the nonpregnant uterus. Because of the greater uterine mass, only two rats were needed in each experiment. Four groups of rat, 19-days, 21-days, 22-days and 1-day postpartum, were used. All pregnant rats were mated in our animal care facilities. The day of appearance of sperms in the vaginal swab was taken as Day 1 of pregnancy. On the designated day, the uterine horns were removed. Each horn was cut open longitudinally and the fetuses and placentae were removed. In rare cases, when only one horn was gravid, the gravid horn was divided into halves longitudinally along the placental sites. Matched-pair control from the same animal was therefore obtained in all cases. The uterine horn was set up in a 30 ml organ bath for isotonic or isometric contraction recording throughout the entire experiment. The uterine horn was then allowed to stabilize till either the tissue became quiescent or the contractions became regular. This equilibration period took two to three hours.

TLC Separation and Bioassay of Extracts

The PG extracts from most experiments were assayed without further purification. The dry residue was reconstituted with 0.5 ml Krebs-bicarbonate solution, pH 7.4, and assayed on the rat fundal (stomach) strip (Vane, 1957) superfused with Krebs-bicarbonate solution at 38°C. Contractions of the fundal strip were measured with an isotonic transducer and displayed on a polygraph. Bracket assay was performed using authentic $PGF_{2\alpha}$ as standard and the values expressed as $PGF_{2\alpha}$ equivalents. The experimental sample and its matched-pair control were always assayed on the same preparation (matched-pair assays). For TLC runs, the PG extracts from two to three experiments were pooled. The dry residue was dissolved in 0.25 ml ethanol and applied to a precoated silica gel TLC plate, 0.25 mm thickness, with preabsorbent area (Kontes TLC plate). The TLC was developed in the AI solvent of Green and

Samuelsson (1961). A marker plate with authentic PGE_2 and $PGF_{2\alpha}$, 10 μg each, was run simultaneously with the sample plate. The marker plate was visualized with 10 percent phosphomolybdic acid in ethanol. The corresponding zones on the sample plate were scraped off and eluted with 2 ml ethanol. The ethanol was evaporated to dryness. The residue was prepared for bioassay as described above. All bioassay values were corrected for extraction and/or TLC recovery loss, determined with the aid of ^{3}H-$PGF_{2\alpha}$.

Materials

The oxytocin used was Syntocinon (Sandoz). Oxytocin inhibitor was [1-L-penicillamine]-oxytocin (Chan et al., 1967, 1974a). PGE_2 and $PGF_{2\alpha}$ ($PGF_{2\alpha}$-THAM) were generously supplied by Dr. John Pike of The Upjohn Company. The PG synthetase inhibitors used were diclofenac sodium (Voltaren) kindly supplied by Dr. William D. Cash of Ciba-Geigy and indomethacin (Indocin), a gift from Dr. Harry J. Robinson of Merck Sharp and Dohme. Multiple labelled ^{3}H-$PGF_{2\alpha}$ was purchased from New England Nuclear.

RESULTS

Effects of OT Inhibitor and PG Synthetase Inhibitors on Uterine Response to OT and PG

Effects of OT Inhibitor. [1-L-Penicillamine] oxytocin ([Pen] OT) is a specific competitive inhibitor of oxytocin and its structurally related analogues. We had shown that with appropriate doses, it antagonized the uterine response to OT in both *in vitro* and *in vivo* rat preparations (Chan et al., 1967; Vavrek et al., 1972; Chan et al., 1974a). As we had reported earlier, this OT inhibitor has no effect on the uterine response to PGE_2 and $PGF_{2\alpha}$ (Chan et al., 1974b). Figure 1 shows one representative experiment on an isolated uterus from a rat in estrus. The response to 4 mU of OT was completely blocked by 1.0 μg of [Pen] OT. The uterine response to an equi-potent dose of $PGF_{2\alpha}$, 0.6 μg, however, was not inhibited even by doubling the [Pen] OT dose. In fact, the response to $PGF_{2\alpha}$ was slightly potentiated in the presence of [Pen] OT.

Effects of PG Synthetase Inhibitors. The effects of two potent PG synthetase inhibitors, indomethacin (Vane, 1971) and diclofenac sodium (Ku et al., 1975) on the uterine response to OT were studied each on four uterine horns from rats in estrus. The concentrations of the PG synthetase inhibitor employed were 2.5, 5, 10 and 20 μM, in and exceeding the effective dose range for PG synthetase inhibition (Ku et al., 1975). Indomethacin up to 10 μM had only a marginal inhibitory effect on the uterine response to OT. The higher dose with diclofenac sodium, 20 μM, produced a partial inhibition of the OT response. As expected, the response to $PGF_{2\alpha}$ was not affected by these PG synthetase inhibitors. Figure 2 shows the

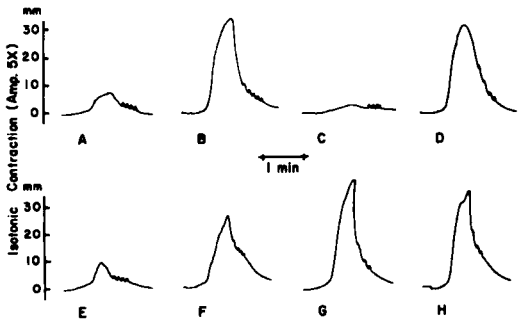

FIG. 1. Effects of [Pen] OT, an OT inhibitor, on the uterotonic responses to OT and $PGF_{2\alpha}$. Upper tracings A to D show the inhibition of the response to OT by [Pen] OT. At A, 2 mU OT; B, 4 mU OT; C, 1 μg [Pen] OT + 4 mU OT; D, 4 mU OT. Bottom tracings E to H show the potentiation of the response to $PGF_{2\alpha}$ by [Pen] OT. At E, 0.3 μg $PGF_{2\alpha}$; F, 0.6 μg $PGF_{2\alpha}$; G, 2 μg [Pen] OT + 0.6 μg $PGF_{2\alpha}$; H, 0.6 μg $PGF_{2\alpha}$. Tracings A to H were all from the same uterine horn. Time intervals between each injection were 6 min. The tissue was washed at the falling phase of each contraction. The bath volume was 6 ml and the temperature 30°C.

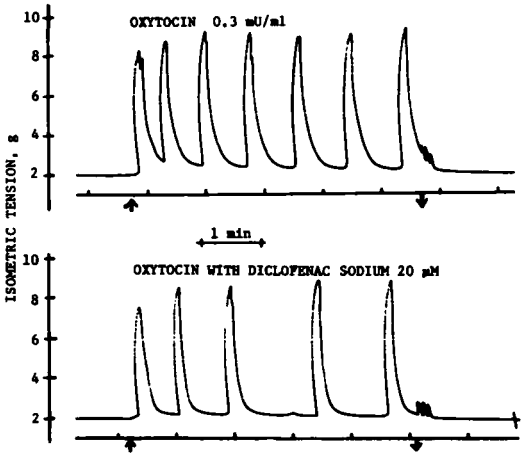

FIG. 2. Effect of PG synthetase inhibitor on the uterotonic response to OT. The upper panel shows the uterine contractions to 0.3 mU/ml OT. Drug contact time was 5 min as marked by arrows. The bath temperature was 37°C. The lower panel shows the uterine contractions of the same uterine horn to the same dose of OT after the tissue had been bathed in a medium containing 20 μM of diclofenac sodium, a potent PG synthetase inhibitor, for 45 min.

typical responses from one experiment with diclofenac sodium.

Effects of OT on Uterine Release and Content of PG

Nonpregnant Uterus. The effect of OT, 1.0 mU/ml, on PG release from nonpregnant rat uterus was studied in three groups of rats in diestrus and in three groups of rats that had been treated with DES, 10 μg/100 g 24 h prior to the experiment. Each group consisted of 6 rats. Table 1 shows that DES treated uterine horns had a higher release rate of $PGF_{2\alpha}$-like activity than uterine horns in diestrus under basal conditions as shown by the control horns. Addition of 1.0 mU/ml of OT to the incubation medium caused a marked shortening (contraction) of the uterine horn which was evident on visual inspection. There was an apparent increase in the PG release rate in the oxytocin stimulated horns. The difference, however, was not statistically significant.

Pregnant Uterus. The effect of OT, 1.0 mU/ml, on the release of PG-like activity from uteri taken in the last four days of pregnancy and the first day of postpartum was studied. Four groups of rats, 19-days pregnant, 21-days pregnant, 22-days pregnant and 1-day postpartum, were used. Three experiments were performed on each group consisting of two rats. Table 1 shows the average values from these experiments. It is apparent that the basal release of $PGF_{2\alpha}$-like activity from the isolated pregnant uteri increased during the last 4 days of pregnancy, reaching the highest level on the 22nd day of pregnancy, the expected day of delivery. This observation confirms the earlier report of such a relationship by Vane and Williams (1973). In this series of experiments, contractility of the uterine horn was monitored throughout the entire experiment. In agreement with Harney et al. (1974), we also found that spontaneous uterine contractility was associated with a high basal release of PG activity. The 19-days pregnant uteri had a low basal PG release. These uteri generally became quiescent during the equilibration period. The 22-days pregnant uteri had a high basal PG release and rarely attained a quiescent state despite the long equilibration period of more than three hours. The 21-days pregnant uteri occupied an intermediate position in basal PG release and spontaneous contractions. The basal PG release dropped sharply in the 1-day postpartum uteri. The postpartum uteri were also less spontaneously active.

OT stimulation, 1.0 mU/ml, caused a marked increase in PG release in all three groups of pregnant uteri and a smaller increase in the 1-day postpartum uteri. In the quiescent uteri, this dose of OT caused strong and phasic contractions. In the active uteri, a sustained contraction (contracture) was produced.

In two experiments, the 22-days pregnant uteri were quiescent following the equilibration period. The basal PG release rate during the quiescent state was determined. The quiescent uteri were then made to contract spontaneously by increasing the Ca^{++} concentration in the bathing medium from 0.5 mM to 2.5 mM and by raising the bath temperature from 30°C to 37°C. Figure 3 shows the marked PG release in a 30 min sample obtained during the peak of spontaneous activity. The average PG release rates from these two experiments over a 60 min period were 18 ± 3 ng/g/h during the quiescent state and 140 ± 11 ng/g/h during the contract-

TABLE 1. Release of $PGF_{2\alpha}$-like activity in ng/g wet wt/hr ± S.E.

	Control horn	Stimulated horn oxytocin 1 mU/ml	t-test for paired difference	
Non-pregnant diestrous	37 ± 13	54 ± 7	Not significant	(n = 6)
Non-pregnant DES-treated	64 ± 20	51 ± 19	Not significant	(n = 9)
19-days pregnant	12 ± 3	30 ± 4	P<0.01	(n = 6)
21-days pregnant	41 ± 12	68 ± 12	P<0.01	(n = 10)
22-days pregnant	84 ± 14	113 ± 13	P<0.05	(n = 8)
1-day postpartum	18 ± 3	22 ± 4	P≈0.05	(n = 9)

The values shown represent the average values of at least three experiments in each group ± S.E. of the mean. n shows the number of matched-pair assays.

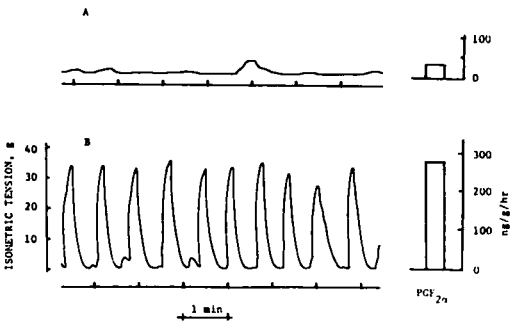

FIG. 3. Uterine contractions and PG release in an isolated 22-days pregnant rat uterus. Upper panel shows the quiescent state and PG release in $PGF_{2\alpha}$ equivalents of a 22-days pregnant uterine horn in a low Ca^{++} and low temperature medium. Lower panel shows the same uterine horn in active contracting state and PG release in a normal Ca^{++} and normal temperature medium. See text for details.

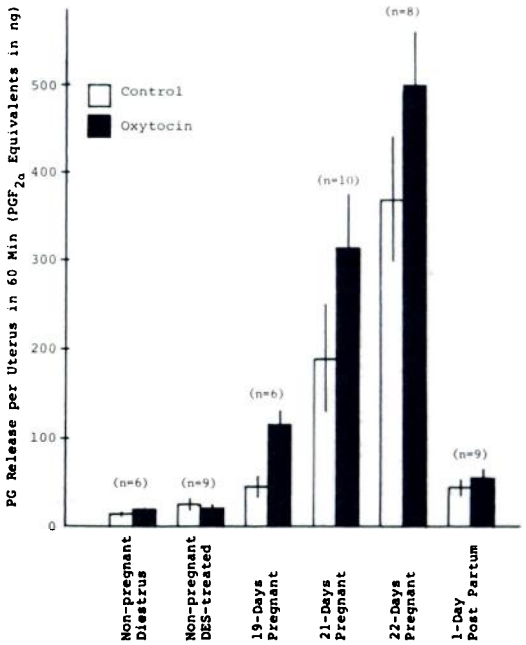

FIG. 4. Release of PG-like activity from nonpregnant and pregnant uteri during basal state and during OT stimulation. Open bars show the PG release from the control horns representing the basal state. Black bars show the PG release from the OT stimulated horns. The values shown are the average values of at least three experiments in each group. The vertical line within each bar shows the S.E. of the mean. n shows the number of matched-pair assays. The difference between the stimulated horn and its matched-pair control horn is not statistically significant for the nonpregnant uteri; highly significant for the pregnant uteri with $P<0.01$; significant for the postpartum uteri with $P\sim0.05$. The OT concentration used in all experiments was 1.0 mU/ml. The incubation was carried out in van Dyke-Hastings (1928) solution at 30°C.

ing state. The difference was highly significant with $P<<0.01$ and n=4.

The total amount of PG release per uterus was also determined. Figure 4 shows the total amount of PG release per uterus in a 60 min period from nonpregnant and pregnant uteri during basal condition and during OT stimulation. The least amount was found in diestrus, 12 ± 4 ng under basal condition and 17 ± 1 ng under OT stimulation. The highest amount was found in 22-days pregnant uteri, 376 ± 70 ng under basal condition and 502 ± 66 ng under OT stimulation.

The tissue content of PG activity was also measured. Table 2 shows both the tissue concentration of PG and the total PG content per uterus. In nonpregnant uteri, DES treatment increased the tissue PG content two fold. In pregnant uteri, the PG content rose sharply during the last two days of gestation and remained at a high level on the 1st day of postpartum. The peak level was on the expected day of delivery, and was more than 10 times that was found in the diestrous uterus.

OT stimulation although markedly increased the PG release rate had no significant effect on the tissue PG content as shown by the tissue PG concentrations of the OT stimulated horns and their matched-pair control horns.

DISCUSSION

The principal aims of this investigation were to determine 1) whether the uterotonic action of OT is mediated by or linked to a uterine PG mechanism and 2) whether the marked increase in sensitivity of the parturient uterus to OT is the result of an enhanced PG release induced by OT.

Our study on the nonpregnant uterus with OT, [Pen] OT, $PGF_{2\alpha}$ and PG synthetase inhibitors, indomethacin and diclofenac sodium clearly shows that the uterotonic action of OT is largely independent of PG participation. The failure of [Pen] OT, a specific OT antagonist, to inhibit the uterine response to $PGF_{2\alpha}$ suggests that the myometrial receptors for OT and PG are functionally separate and distinct. We had previously presented evidence that OT and PG may each activate a different receptor-response

TABLE 2. Tissue content of $PGF_{2\alpha}$-like activity.

	ng/g wet wt ± S.E.		
	Control horn	Stimulated horn oxytocin 1 mU/ml	Basal state ng/uterus ± S.E.
Non-pregnant diestrous	70 ± 30	97 ± 41	22 ± 9 (n = 6)
Non-pregnant DES-treated	128 ± 50	84 ± 33	48 ± 18 (n = 6)
19-days pregnant	9 ± 1	11 ± 1	34 ± 6 (n = 6)
21-days pregnant	36 ± 4	44 ± 5	165 ± 25 (n = 14)
22-days pregnant	62 ± 11	59 ± 7	272 ± 47 (n = 9)
1-day postpartum	94 ± 17	100 ± 14	232 ± 40 (n = 9)

The values shown represent the average values of at least three experiments in each group ± S.E. of the mean. n shows the number of matched-pair assays. The difference between the stimulated horn and its matched-pair control was not significant statistically. The cause for the lower value in the OT-stimulated horn of the DES-treated rat was not apparent to us.

mechanism in the uterus (Chan et al., 1974b). That the uterotonic action of OT is largely independent of PG release is shown by our finding that indomethacin and diclofenac sodium, two potent PG synthetase inhibitors (Vane, 1971; Ku et al., 1975) had little effect on the uterine response to OT. Even at a very high dose, 20 μM, only a partial inhibition of the OT response was observed. This apparent discrepancy between our finding and that of Vane and Williams (1973), who first reported the indomethacin inhibition of OT and suggested that the OT action may be mediated through PG release, may be explained by the observation reported by Baudouin-Legros et al. (1974). The latter investigators found that indomethacin suppressed the increased sensitivity to angiotensin and OT of the rat uterus in diestrus and proestrus. The response of metestrous uterus, however, was not affected by indomethacin. They attributed the increase in sensitivity to angiotensin and OT in diestrus and proestrus to a heightened synthesis of PG and was therefore susceptible to indomethacin inhibition. Vane and Williams did not determine the stage of estrus of the uteri used in their experiments. In random selection, the majority of the rats would fall in diestrus and proestrus. In our experiments, only uteri from rats in estrus were used in this portion of the study.

It is noteworthy that Vane and Williams also found that indomethacin had no effect on the pregnant uterus' response to OT. Roberts and McCracken (1976) measured OT-induced PG release in uterine venous blood and uterine contractions in the sheep and found that indomethacin suppressed PG secretion but not the myometrial contractions induced by OT. Thus, our findings here and those of others cited above (Baudouin-Legros et al., 1974; Roberts and McCracken, 1976) do not support the hypothesis that the uterotonic action of OT may be mediated by stimulating PG synthesis and release, but rather they indicate that OT produces uterine contractions by its own action.

Although the uterotonic action of OT is not dependent on PG synthesis, OT does have a PG-releasing property. We first demonstrated that OT caused the release of PG-like activity from isolated rat uterus (Chan, 1974a, b). OT has since been shown to cause the release of $PGF_{2\alpha}$ in the uterine venous blood of sheep (Sharma and Fitzpatrick, 1974; Mitchell et al., 1975; Roberts and McCracken, 1976) and also from the ovine endometrium *in vitro* (Roberts et al., 1976).

In the present experiments, we found that isolated rat uteri released PG-like activity into the medium on incubation under control conditions. This basal PG release was influenced by estrogen and by the stage of pregnancy. Ham et al. (1975) showed that the uterine PGF level was elevated in normal cycling rats at proestrus when estrogen level was high, and in ovariectomized rats treated with estrogen. Castracane and Jordan (1975) also reported a similar finding in ovariectomized rats. We, too, found that DES-treated nonpregnant rat uteri had a higher basal PG release rate and tissue PG content than uteri from rats in diestrus. OT, 1.0 mU/ml, caused strong contractions in these

isolated uteri, but did not significantly alter either the release or the tissue content of PG. The lack of a correlation between the uterotonic action of OT and PG release is consistent with our findings on OT, PG and PG synthetase inhibitors which indicate that the uterotonic action of OT is largely independent of PG participation.

The basal PG release rate from 19-days pregnant uteri was low. The rate increased sharply on Day 21 and reached a peak on Day 22, the expected day of delivery. With the increase in basal PG release, spontaneous uterine contractions also became more prominent. On the first day of postpartum, basal PG release rate had dropped to the 19-days pregnant level. Spontaneous uterine contractions also declined. Our observations on PG release from the late pregnant rat uteri are very similar qualitatively to those reported earlier by Aiken (1972) and Harney et al. (1974). The quantity of PG release, however, was far less in our experiments. The low PG release rate probably was inherent in the system we used. In order to study the effects of OT, it was essential that the uteri be in a quiescent or relatively quiescent state when OT was not added to the bathing medium. This was achieved by the use of a low Ca^{++} (0.5 mM) medium, a low incubation temperature (30°C) and a long equilibration period (3 h or longer). In two experiments, when spontaneous contractions were induced by raising the medium Ca^{++} concentration to 2.5 mM and the temperature to 37°C, the rate of PG release was greatly increased, approaching those reported by others as shown in Fig. 3.

A most significant difference between the pregnant uteri and the nonpregnant uteri is their PG release in response to OT. OT 1.0 mU/ml, caused marked uterine contractions and a marked increase in PG release in the late pregnant uteri when compared to their respective matched-pair controls. In nonpregnant uteri, OT caused contractions but had no significant effect on PG release. Mitchell et al. (1975) also reported similar findings in their *in vivo* study on pregnant sheep.

The PG release rates expressed in ng/g tissue wet wt/h (Table 1) show that the nonpregnant uteri had a higher PG release rate than 19-days pregnant uteri and a similar rate as the 21-days pregnant uteri. Only the 22-days pregnant uteri had a higher release rate of PG than the nonpregnant uteri. Rates based on values per gram of tissue wet weight, however, do not describe the total picture because they do not take into account the dilution effect due to the increase in water content and the greatly increased mass of the gravid uterus. The average wet weight of the nonpregnant uteri used in our experiment was 0.38 ± 0.02 g and for the 22-days pregnant uteri was 4.40 ± 0.12 g. When the PG release rate was expressed on a per uterus basis as shown in Fig. 4, the very marked increase in PG synthesizing capacity of the pregnant uterus becomes apparent. This is also reflected in the total PG content per uterus as shown in Table 2.

It is important to note that in the pregnant uteri, the total quantity of PG released per uterus during the incubation period was greater than the total PG content of the uterus. This suggests a *de novo* synthesis and rapid release of PG from the tissue as is generally believed (Biti, 1975). The fact that OT markedly increased the release of PG but had little effect on the tissue PG content also supports this concept of *de novo* PG synthesis.

In conclusion, our findings show that the uterotonic action of OT is independent of PG synthesis in the uterus. In the late pregnant uterus, especially on the last day of pregnancy, the PG synthesizing capacity is remarkably increased. The PG-release mechanism also becomes sensitive to OT stimulation. Oxytocin causes uterine contractions as well as PG release from the late pregnant uteri. Since OT and PG have a synergistic action on the uterus (Chan et al., 1974b), this PG-releasing action of OT may be one of the mechanisms responsible for the marked increase in sensitivity of the parturient uterus to OT. It seems likely that the PG-releasing action of OT is separate and distinct from the uterotonic action of OT. Evidence for this is seen in our study with [Pen] OT, in which we observed that this specific OT antagonist not only failed to inhibit the uterine response to PG, but on the contrary, potentiated the response to PG when large doses of [Pen] OT were used. This suggests that [Pen] OT, though it does not show agonistic activity on the myometrium, may retain the PG-releasing activity of OT and hence potentiate the response of injected PG. Other investigators also have recently presented evidence suggesting such dual actions of OT (Roberts and McCracken, 1976; Roberts et al., 1976).

Figure 5 represents our scheme of the interactions between OT and PG in the uterus. We have already shown that OT and PG each

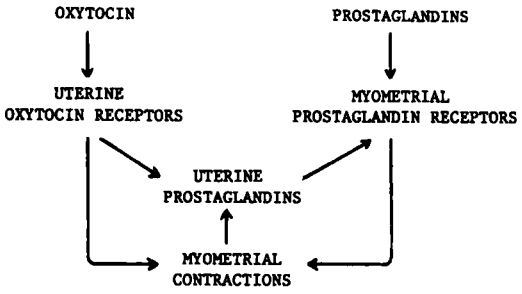

FIG. 5. A schematic representation of interactions between OT and PG in the uterus.

activates a separate and different receptor for myometrial contractions. In the uterus, OT may act on two different receptors, one leading to myometrial contractions and the other leading to PG release. In addition, OT may also cause PG release in a nonspecific manner secondary to its myometrial contracting effect. This working hypothesis is now being tested in our laboratory.

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