Effects of oxytocin and uterine and luteal prostaglandins on the functional regression of the corpus luteum in pseudopregnant rats

L. Cao and W. Y. Chan*

Department of Pharmacology, Cornell University Medical College, New York, NY 10021, USA

The effects of oxytocin on luteal regression in the pseudopregnant rat and whether the luteolytic effect of oxytocin could be blocked by an oxytocin receptor antagonist were investigated. We determined the temporal relationship between the effects of oxytocin on the duration of pseudopregnancy and concentrations of progesterone in plasma, and uterine and luteal prostaglandin concentrations. Pseudopregnancy was induced in normal cyclic rats by gonadotrophin treatment. On day 6 of pseudopregnancy, rats were assigned to one of three groups. One group was given oxytocin, 500 mU s.c., twice a day for three days. A control group was given saline injections. A third group was given the same dose of oxytocin and an oxytocin receptor antagonist, [Pen¹,Phe(Me)²,Thr⁴,Orn⁸]oxytocin, 300 µg day⁻¹, delivered by micro-osmotic pumps for the same three-day period. Rats were either observed to determine the duration of pseudopregnancy, or killed on days 2, 6, 7, 9, 11 and 13 of pseudopregnancy for measurements of plasma concentrations of progesterone and luteal and uterine PGF_{2α} and PGE₂ concentrations by radioimmunoassays. Oxytocin injections shortened the duration of pseudopregnancy from the mean of 13.5 ± 0.3 days for the control group to 11.5 ± 0.3 days for the oxytocin-treated group (*P* < 0.01). The oxytocin-induced shortening of pseudopregnancy was associated with a premature functional regression of the corpus luteum. Both luteal and uterine PGF_{2α} concentrations were found to increase with luteolysis and reached peak values before the return of oestrus. However, only uterine PGF_{2α} synthesis was stimulated by oxytocin treatment. There was no significant increase in uterine or luteal PGE₂ synthesis during luteolysis. The effect of oxytocin on shortening the duration of pseudopregnancy was blocked by the co-administration of the oxytocin receptor antagonist. Thus, our findings show that oxytocin is luteolytic in pseudopregnant rats. The close temporal association between oxytocin-induced functional luteal regression and oxytocin-stimulated uterine prostaglandin production suggests that the oxytocin-induced luteolysis is mediated via uterine PGF_{2α} as has been shown in domestic ruminants. The luteolytic action of oxytocin was mediated by the oxytocin receptor, as it was blocked by a specific oxytocin receptor antagonist.

Introduction

There is substantial evidence linking oxytocin to luteolysis in large domestic ruminants (Flint and Sheldrick, 1983; and reviews: Richardson, 1986; Silvia *et al.*, 1991; Bazer, 1992). It is generally considered that ovarian oxytocin secreted by the corpus luteum is the endogenous oxytocin involved in luteolysis (Flint and Sheldrick, 1983; Wathes, 1984; Flint *et al.*, 1990). The luteolytic action of oxytocin depends on a functioning uterus. It is thought that luteal oxytocin stimulates the secretion of PGF_{2α} from the uterus which is responsible for the luteolysis (Flint and Sheldrick, 1983; Flint *et al.*, 1990). PGF_{2α} also stimulates oxytocin secretion from the corpus luteum (Flint and Sheldrick, 1982; Watkins and Moore, 1987; Lamsa *et al.*, 1989). Because endogenous pulses of PGF_{2α} occur synchronously with pulses of oxytocin during luteolysis, it has been suggested that luteal

oxytocin and uterine PGF_{2α} exist in a positive feedback loop (Fairclough *et al.*, 1980, 1984; Webb *et al.*, 1981; Hooper *et al.*, 1986; Moore *et al.*, 1986; Flint *et al.*, 1990). This positive feedback loop or the uterine prostaglandin secretory response to oxytocin becomes functional only late in the oestrous cycle, leading to luteolysis (Fairclough *et al.*, 1984; Silvia and Taylor, 1989; Silvia *et al.*, 1992). A similar luteolytic mechanism has also been reported in pigs (Kieborz *et al.*, 1991).

The corpus luteum of humans and rats also synthesizes PGF_{2α} (Challis *et al.*, 1977; Swanston *et al.*, 1977; Weems, 1979). The relative importance of the uterine and luteal prostaglandin components in luteal maintenance has not been elucidated. In pregnant and pseudopregnant rats, a high correlation between luteal PGF_{2α} contents and the demise of luteal function has been reported, suggesting a role for luteal PGF_{2α} in luteolysis at least in rats (Olofsson and Selstam, 1988; Olofsson *et al.*, 1990).

It has not been shown whether oxytocin is luteolytic in rats. It has also not been shown whether the luteolytic action of

*Correspondence.

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oxytocin is mediated by the classical oxytocin receptors and could be blocked by specific oxytocin receptor antagonists. In the study reported here we determined whether oxytocin caused functional regression of the corpus luteum in pseudo-pregnant rats, investigated the temporal relationships between oxytocin-induced luteolysis and luteal and uterine prostaglandin production, and determined whether oxytocin-induced luteolysis can be blocked by a specific oxytocin receptor antagonist. Preliminary results of this study were reported at the 24th Annual Meeting of the Society for the Study of Reproduction, Vancouver, Canada (1991).

Materials and Methods

Materials

The oxytocin antagonist used was $[\text{Pen}^1, \text{Phe}(\text{ME})^2, \text{Thr}^4, \text{Orn}^6]$ -oxytocin synthesized by V. J. Hruby (University of Arizona, Tucson, AZ) and characterized in our previous studies (Chan *et al.*, 1986, 1990). It is a selective oxytocin- and V_{1a} -receptor antagonist with no V_2 -receptor activity. The oxytocin used was Pitocin (Parke-Davis, Morris Plains, NJ). Diclofenac sodium was purchased from Sigma (St Louis, MO). Multilabelled $[\text{^3H}]\text{PGF}_{2\alpha}$ and $[\text{^3H}]\text{PGE}_2$ were purchased from NEN DuPont (Boston, MA). Anti- $\text{PGF}_{2\alpha}$, anti- PGE_2 and prostaglandin standards were obtained from Advanced Magnetics (Cambridge, MA) and the Coat-A-Count Progesterone RIA kit from Diagnostic Products Corp. (Los Angeles, CA).

Animals and experimental protocol

Wistar rats, 200–225 g, were purchased from Hilltop Laboratories (Scottsdale, PA). Only rats showing regular oestrous cycles were used. Pseudopregnancy was induced by gonadotrophin treatment followed by cervical stimulation as described by Rao and Gibor (1987). At metoestrus, 50 iu PMSG wash injected s.c.; this was followed 56 h later by 50 iu hCG. Sixteen hours after hCG injection, cervical stimulation was applied with a glass rod. When the vaginal smear showed primarily leucocytic infiltration on the next day, the rat was considered at day 1 of pseudopregnancy. Vaginal smears were taken and examined every morning for each rat. The day before the next emergence of oestrus was considered as the last day of pseudopregnancy. On day 6 of pseudopregnancy, rats were randomly assigned to one of three groups. (i) The oxytocin-treated group was given 500 mU oxytocin (Pitocin: 10 U ml^{-1} , diluted in saline), injected s.c. twice a day at 09.00 h and 17.00 h on days 6, 7 and 8. (ii) The control group was given saline injections at the same time. Four of the controls also carried a saline-filled osmotic pump as described below. (iii) The oxytocin and oxytocin antagonist-treated group was given the same dose of oxytocin but was also given a continuous infusion of an oxytocin antagonist, $[\text{Pen}^1, \text{Phe}(\text{ME})^2, \text{Thr}^4, \text{Orn}^6]$ -oxytocin, delivered by an Alzet micro-osmotic pump, model 1003D (Alza, Palo Alto, CA), implanted s.c. over the neck and shoulder region, at a rate of $300 \mu\text{g day}^{-1}$ from days 6 to 8. The oxytocin antagonist was dissolved and diluted in 0.25% acetic acid. The micro-osmotic pump had a delivery capacity of 3–3.5

days and was not removed on day 9. Twelve rats from each of the oxytocin-treated and control groups, and four rats from the oxytocin and oxytocin antagonist-treated group were observed to monitor the duration of pseudopregnancy. In addition, three or more rats from the oxytocin-treated and the control groups were killed on each of days 2, 6, 7, 9, 11 and 13 between 09.00 and 10.00 h to obtain tissue and blood specimens for determinations of luteal and uterine prostaglandin concentrations and plasma progesterone concentrations. Under surgical depth anaesthesia with 4% isoflurane (Anaquest, Madison, WI), the uterus and ovaries were quickly removed and a heparinized blood sample was obtained by cardiac puncture. The animal was then killed by an anaesthetic overdose. The uterus and ovaries were placed in ice-cold 0.9% NaCl containing $10 \mu\text{g}$ of diclofenac sodium ml^{-1} , a potent cyclooxygenase inhibitor, to suppress prostaglandin synthesis. The uterus was dissected clean, blotted on a filter paper and its weight determined. It was then frozen in liquid nitrogen until used for prostaglandin extractions. The ovaries were dissected at ice-cold temperature under a dissecting microscope and the large corpora lutea of pseudopregnancy were harvested as described by Olofsson and Selstam (1988). The wet weight of the harvested corpora lutea was determined and the tissue frozen in liquid nitrogen until used for prostaglandin extraction. The blood sample was centrifuged at $4000 g$ for 20 min at 4°C and the plasma stored frozen until used for progesterone determination by radioimmunoassay.

Determinations of progesterone and prostaglandins

Plasma progesterone concentration was determined directly without extraction by radioimmunoassay with a Coat-A-Count progesterone radioimmunoassay kit. The progesterone RIA kit was highly specific for progesterone with low crossreactivities, $< 2.0\%$ for 20α -dihydroprogesterone and deoxycorticosterone and $< 1.0\%$ for other steroids normally in the plasma. The radioimmunoassay kit was validated in our laboratory with two concentrations of progesterone in plasma from male rats. The recovery was between 110 and 120%. Luteal and uterine $\text{PGF}_{2\alpha}$ and PGE_2 contents were extracted and eluted through a Sep-Pak C_{18} cartridge (Waters, Milford, MA) with an acidic ethyl acetate system before radioimmunoassays. Extractions of tissue prostaglandin were carried out by homogenizing the tissue in 50% isopropyl alcohol in phosphate buffer, pH 7.4, with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY). An equal volume of phosphate buffer was added to the homogenate and mixed in a shaker for 10 min. The homogenate was then centrifuged at $4000 g$ for 30 min. The supernatant was acidified to pH 3–4 with 10% formic acid for extraction with a Sep-Pak C_{18} cartridge. The Sep-Pak was washed with 2.0 ml methanol and conditioned with the acidified alcoholic buffer, pH 3–4. The supernatant was then passed through the Sep-Pak twice; washed with 5.0 ml water and 2.0 ml hexane, and then eluted with 5.0 ml ethyl acetate. The resultant prostaglandin extract was dried at 40°C under nitrogen. The residue was stored at -20°C until used for radioimmunoassay. Details of this extraction method and the prostaglandin radioimmunoassay system have been described by Chan (1987). Each sample was measured in duplicate and at two different dilutions. The antisera were highly specific for PGE_2 or $\text{PGF}_{2\alpha}$ with low cross-reactivities: for the anti- PGE_2 , $< 1.5\%$ with $\text{PGF}_{2\alpha}$ and $< 1.0\%$

Fig. 1. Concentrations of progesterone in plasma of (○) control and (●) oxytocin-treated rats during the course of pseudopregnancy. Each point represents the mean $\pm$ SEM of four pseudopregnant rats (except days 2 and 11, $n = 3$). Oxytocin-treated rats were given oxytocin 500 mU s.c. twice a day from day 6 to day 8. Control rats were given saline. Day 11 was the last day of pseudopregnancy for the oxytocin-treated rats. *Significantly different from the corresponding control ($P < 0.05$); **significantly different from the corresponding control ($P < 0.01$).

for other prostaglandins relevant to the uterus; for the anti-PGF_{2α}, $<0.5\%$ with PGE₂ and $<0.1\%$ for other relevant prostaglandins. The sensitivity of the radioimmunoassay for progesterone was 50 pg ml⁻¹ and for the prostaglandins 15 pg ml⁻¹. The intra-assay and interassay coefficients of variation were $<10\%$ and $<15\%$, respectively.

Statistical analysis

All data are expressed as sample or group means $\pm$ SEM and analysed by analysis of variance. Difference between group means were analysed by Student's *t* test and considered significant when $P < 0.05$.

Results

Effects of oxytocin on the duration of pseudopregnancy

Pseudopregnancy was induced with gonadotrophin treatment followed by cervical stimulation in normal cyclic rats with more than 90% success rate. In 12 controls, the mean duration of pseudopregnancy was 13.5 ± 0.3 days. There was no difference between the eight saline injection only controls and the four injection plus pump controls. Oxytocin injections, 500 mU s.c. twice a day, from day 6 to day 8 of pseudopregnancy reduced the duration of pseudopregnancy. In 12 oxytocin-treated rats, the mean duration of pseudopregnancy was 11.5 ± 0.3 days. The difference between the two groups was statistically significant ($P < 0.01$).

The oxytocin-induced shortening of pseudopregnancy was associated with a premature fall in concentrations of progesterone in plasma, occurring two days earlier than in the control group. An initial increase in plasma progesterone concentrations following oxytocin injection was observed before the rapid fall in plasma progesterone (Fig. 1).

Fig. 2. Concentrations of PGF_{2α} in uterus of (□) control and (■) oxytocin-treated rats during the course of pseudopregnancy. Each bar represents the mean $\pm$ SEM of four rats (except day 6, $n = 3$). Oxytocin-treated rats were given 500 mU oxytocin s.c. twice a day from day 6 to day 8. Control rats were given saline. *Significantly different from the corresponding control ($P < 0.05$).

Effects of oxytocin on uterine and luteal prostaglandin contents

Uterine PGF_{2α} concentrations increased during the second half of pseudopregnancy. There was a gradual increase in uterine PGF_{2α} concentrations when measured on day 6, day 7 and day 9 and a marked increase on day 11 and day 13 (Fig. 2). The PGF_{2α} concentrations on day 11 and day 13 were four times higher than concentrations on day 6. Oxytocin treatment stimulated uterine PGF_{2α} synthesis. Uterine PGF_{2α} concentrations on day 7 and day 9 of oxytocin-treated rats were significantly higher than the corresponding controls ($P < 0.05$).

Luteal PGF_{2α} concentrations on day 6 were slightly higher than the corresponding uterine PGF_{2α} concentrations, but the difference was not statistically significant. Luteal PGF_{2α} concentrations remained relatively stable when determined on day 6, day 7 and day 9. On day 11, the PGF_{2α} concentration was twice the day 6 value (Fig. 3). Oxytocin treatment had no significant effect on luteal PGF_{2α} concentrations.

As expected, uterine PGE₂ concentrations were low. Luteal PGE₂ concentrations were high and comparable to the PGF_{2α} values. Neither uterine nor luteal PGE₂ concentrations underwent significant changes during luteolysis (Fig. 4). Oxytocin treatment had no effect on PGE₂ synthesis, except on day 11 when luteal PGE₂ concentrations of the oxytocin-treated rats were significantly higher than the corresponding control values ($P < 0.05$).

Effect of oxytocin antagonist

In four pseudopregnant rats, [Pen¹,Phe(Me)²,Thr⁴,Orn⁸]-oxytocin, a specific oxytocin receptor antagonist, was administered with oxytocin. Oxytocin 500 mU was injected s.c. twice a day from day 6 to day 8. The oxytocin antagonist (300 μg day⁻¹) was delivered by a micro-osmotic pump also from day 6 to day 8. The oxytocin antagonist protected the oxytocin-treated rats from the luteolytic action of oxytocin. The mean

Fig. 3. Luteal concentrations of PGF_{2α} in (□) control and (■) oxytocin-treated rats during the course of pseudopregnancy. Each bar represents the mean ± SEM of four rats (except day 6, *n* = 3). Oxytocin-treated rats were given 500 mU oxytocin s.c. twice a day from day 6 to day 8. Control rats were given saline.

duration of pseudopregnancy of the rats treated with oxytocin and oxytocin antagonist was not different from that of the normal pseudopregnant rats (Table 1).

Discussion

There is substantial experimental evidence suggesting that oxytocin has a role in luteolysis in domestic ruminants. It is not known whether oxytocin has a similar role in rodents. In the study reported here, we demonstrated that oxytocin was also luteolytic in the pseudopregnant rat. Injections of oxytocin from day 6 to day 8 of pseudopregnancy resulted in a premature termination of pseudopregnancy. The mean duration of pseudopregnancy of the normal controls in our study was 13.5 days. In the oxytocin-treated rats, it was shortened to 11.5 days. We did not administer oxytocin treatment until day 6 of pseudopregnancy, since newly formed corpora lutea are known to be resistant to the luteolytic action of PGF_{2α} (Behrman *et al.*, 1979). In ewes, it has also been shown that the responsiveness of the uterine PGF secretory system to oxytocin developed only shortly before the onset of luteal regression (Fairclough *et al.*, 1984; Silvia *et al.*, 1992). If oxytocin treatment could be initiated earlier in the pseudopregnant rats, it could produce a more dramatic effect.

The oxytocin-induced termination of pseudopregnancy was associated with a functional regression of the corpus luteum as reflected by the decline in plasma progesterone concentrations. In oxytocin-treated rats, there was also a premature fall in concentrations of progesterone in plasma by 2 days. Oxytocin treatment induced an initial increase in circulating progesterone before the precipitous fall. The mechanism of this stimulation is unknown. As the increase in progesterone secretion of the control pseudopregnant rats appeared to reach a plateau between day 6 and day 9, the day 7 progesterone value of the oxytocin-treated rats might, in fact, be within the same range. However, other investigators have reported that oxytocin stimulates progesterone release in luteal cells *in vitro* (Jarry *et al.*, 1990; Musah

et al., 1990; Miyamoto and Schams, 1991) and in pigs at midoestrous cycle (Kieborz *et al.*, 1991). The physiological significance of this oxytocin action is unclear to us. It has been hypothesized that oxytocin may be a luteotrophic factor stimulating progesterone secretion during the early oestrous period, but is luteolytic in the late luteal period (Homeida and Khalafalla, 1987; Miyamoto and Schams, 1991; Homeida and Al-Ekhnah, 1992).

Measurements of uterine and luteal prostaglandin contents in our experiments provide evidence that oxytocin-induced luteolysis in rats probably also involves uterine PGF_{2α} as the luteolysin. Uterine PGF_{2α}, but not PGE₂, was found to increase with spontaneous luteolysis. Oxytocin treatment stimulated uterine PGF_{2α} synthesis. Increase in uterine PGF_{2α} concentrations in the oxytocin-treated rats was advanced by 2 days, the same duration by which pseudopregnancy was shortened. Thus, there was a close temporal correlation between oxytocin-induced termination of pseudopregnancy and oxytocin-induced stimulation of uterine PGF_{2α} synthesis. Luteal PGF_{2α} concentrations measured on day 6, 7 and 9 were found to be in a similar range to uterine PGF_{2α}. On day 11, the PGF_{2α} concentration was twice the day 6 value. Oxytocin treatment had no significant effect on luteal PGF_{2α} concentrations. Thus, our findings suggest that oxytocin induces luteolysis in pseudopregnant rats, probably indirectly via uterine PGF_{2α} release, as in the ruminants. Our observations are consistent with earlier studies that have suggested a role for the uterus and PGF_{2α} in the regulation of luteolysis in rats. Hysterectomy and inhibition of prostaglandin synthesis by indomethacin were found to increase the duration of pseudopregnancy in rats (Melampy *et al.*, 1964; Lau *et al.*, 1975). Measuring peripheral plasma concentrations of PGE₂, PGF_{2α} and progesterone in pseudopregnant rats, Saksena *et al.* (1974) concluded that an increase in the plasma concentration of PGF_{2α} on day 7 was the cause of the gradual decline in peripheral progesterone concentrations. These earlier studies also found no correlation between PGE₂ and peripheral progesterone. Thus, our findings together with those earlier studies strongly suggest that, in rats, as in ruminants, uterine PGF_{2α} is the prime initiator of luteolysis. Oxytocin induces luteolysis in pseudopregnant rats indirectly via its action on releasing uterine PGF_{2α}. This interpretation is consistent with findings by Mukhopadhyay *et al.* (1984) and Olofsson *et al.* (1992) which showed that oxytocin had no effects on luteal progesterone and prostaglandin secretion *in vitro*.

Luteal PGE₂ concentrations were four to five times higher than that found for the uterus and were in the same concentration range as were luteal PGF_{2α}. There was no clear increase in uterine or luteal PGE₂ synthesis during luteolysis. However, on day 11, there was an increase in luteal PGE₂ concentration of the oxytocin-treated rats. The difference between the oxytocin-treated rats and the control rats was statistically significant (*P* < 0.05). The luteal prostaglandin profiles during pseudopregnancy observed in our study were similar to those reported by Olofsson and Norjavaara (1990). The high concentrations of luteal PGE₂ and its function in luteolysis are not understood. The authors have suggested that concentrations of luteal PGE₂ are kept high to ensure further PGF_{2α} formation in the regressing corpus luteum via the PGE₂-9-keto-reductase pathway. These same authors also concluded from their study that the increase in luteal PGF_{2α} during luteolysis can be intrinsically

Fig. 4. Uterine and luteal concentrations of PGE₂ in (□) control and (■) oxytocin-treated rats during the course of pseudopregnancy. (a) uterine PGE₂; (b) luteal PGE₂. Each bar represents the mean $\pm$ SEM of four rats (except day 6, $n = 3$). Oxytocin-treated rats were given 500 mU oxytocin s.c. twice a day from day 6 to day 8. Control rats were given saline. *Significantly different from the corresponding control ($P < 0.05$).

Table 1. Antagonism of the luteolytic action of oxytocin by a specific oxytocin receptor antagonist in pseudopregnant rats

Treatment	<i>n</i>	Duration of pseudopregnancy (days)
Control	12	11.3 $\pm$ 0.3
Oxytocin	12	11.5 $\pm$ 0.3*
Oxytocin antagonist and oxytocin	4	14.0 $\pm$ 0.2

Values shown are means $\pm$ SEM; n = number of rats in group.

*Significantly different from the control and the OT antagonist plus OT groups ($P < 0.01$).

accomplished by the corpus luteum and that luteal PGF_{2 α} could be a physiological signal for luteolysis in rats. However, Weems (1979) showed that in pseudopregnant rats with bilateral decidualomata, luteal maintenance was extended for approximately the same time as the duration of gestation with no inhibition of PGF_{2 α} secretion. The author concluded that extended luteal function by decidualomata occurred by some mechanism other than an inhibition of PGF_{2 α} secretion. This finding would suggest that uterine or luteal PGF_{2 α} is important only in luteolysis of nonpregnant rats.

The oxytocin-induced luteolysis, reducing of the duration of pseudopregnancy, appeared to be mediated by the oxytocin receptor, as the action was blocked by the co-administration of a specific oxytocin receptor antagonist. The doses of oxytocin used in this study were pharmacological concentrations. Whether oxytocin is normally involved in luteolysis in rats is not known. For domestic ruminants, it is generally believed that ovarian oxytocin secreted by the corpus luteum is the endogenous oxytocin involved in luteolysis (Flint and Sheldrick, 1983; Wathes, 1984; Flint *et al.*, 1990). In goats, intra-arterial

administration of an oxytocin antagonist between days 12 and 20 of the oestrous cycle delay luteal regression (Homeida and Khalafalla, 1987). Human and rat ovaries also produce oxytocin (Wathes *et al.*, 1982; Khan-Dawood and Dawood, 1983; Dawood and Khan-Dawood, 1986; Wolf *et al.*, 1986; Ho and Lee, 1992). However, the physiological functions of ovarian oxytocin in primates and rats are not understood. Wolf *et al.* (1986) found a decreased secretion rate of ovarian oxytocin at pro-oestrus and oestrus in rats when blood concentration of prolactin was increased. Ho and Lee (1992), however, found that the ovarian content of oxytocin was tenfold higher at oestrus than at other phases. These two authors suggested that rat ovarian oxytocin may participate in the ovulatory process as well as in the luteolytic process. Concentrations of oxytocin in plasma show no significant changes during the oestrous cycle. This finding suggests that ovarian oxytocin, not pituitary oxytocin, is probably involved in the regulation of luteal functions. The physiology of ovarian oxytocin in luteal functions merits further study.

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