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Identification of functional high and low affinity states of the prostaglandin F2 alpha receptor in the ovine corpus luteum *in vivo* and their role in hormone pulsatility

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Equivocal evidence has accumulated for the presence of high and low affinity receptors for $PGF_{2\alpha}$ in the corpus luteum based on binding affinities of ^3H-$PGF_{2\alpha}$ to cell membranes or separated whole cells. Some studies report only high affinity sites, while others report the occurrence of both high and low affinity sites. We have previously demonstrated, using subluteolytic levels of $PGF_{2\alpha}$, the existence of functional high affinity luteal $PGF_{2\alpha}$ receptors which show desensitization and recovery after 6 to 9 h. The present study, using direct intra-arterial infusions of $PGF_{2\alpha}$ into the autotransplanted ovary in conscious sheep, was designed to probe for the existence of functional high and low affinity states of the $PGF_{2\alpha}$ receptor in the corpus luteum *in vivo*. Subluteolytic and luteolytic concentrations of $PGF_{2\alpha}$ (100 pg/min and 2500 pg/min, respectively) were infused sequentially, each for 2 h, into the ovary during the luteal phase ($n = 7$ sheep). The same low and high concentrations of the inactive metabolite of $PGF_{2\alpha}$ (PGFM) were given over the same time periods as negative controls ($n = 4$ sheep). During the 2 h intra-arterial infusion of 100 pg/min of $PGF_{2\alpha}$ the secretion rate of oxytocin increased ($P < 0.01$) while the secretion rate of progesterone was unaffected. In contrast, during the 2 h intra-arterial infusion of 2500 pg/min of $PGF_{2\alpha}$, secretion rate of oxytocin increased ($P < 0.01$) and secretion rate of progesterone now began to decline ($P < 0.05$). During the 2 h infusions of identical concentrations of PGFM, the secretion rate of oxytocin and progesterone remained unchanged. These results indicate the existence of functional high and low affinity states of the $PGF_{2\alpha}$ receptor within the ovine corpus luteum *in vivo*.

Keywords: prostaglandin $F_{2\alpha}$ receptors; receptor desensitization; receptor affinity; oxytocin; corpus luteum; hormone pulsatility

Introduction

Although the systemic administration of $PGF_{2\alpha}$ (Watkins & Moore, 1987) or cloprostenol (Flint & Sheldrick, 1982), to sheep during the luteal phase of the cycle caused the secretion of oxytocin (OT) from the corpus luteum, it was not known whether this effect was direct or indirect. However, the intra-arterial infusion of subluteolytic levels of $PGF_{2\alpha}$ (25-250 pg/min) into the ovary of conscious sheep during the luteal phase of the estrous cycle, immediately evoked a significant increase in ovarian OT secretion without any effect on progesterone (P) secretion (Lamsa *et al.*, 1989). This latter study indicated that the ovine corpus luteum is sensitive to low levels of $PGF_{2\alpha}$ presumably acting via high affinity receptors, which caused the selective release of luteal OT from the large granulosa-derived luteal cells, the sole source of luteal OT (Rodgers *et al.*, 1983; Theodosis *et al.*, 1986). We subsequently demonstrated that the high affinity $PGF_{2\alpha}$ receptors mediating the release of OT from the large luteal cells became desensitized after 1 h of a continuous infusion of $PGF_{2\alpha}$ (100 pg/min) and that a rest period of 6 to 9 h was required to restore the function of the high affinity receptors (Lamsa *et al.*, 1992). Since others had shown that the large luteal cells of the ovine corpus luteum secretes most of the basal output of P (Harrison *et al.*, 1987), it occurred to us that, in order to suppress P secretion (which requires relatively high concentrations of $PGF_{2\alpha}$; (McCracken *et al.*, 1984)) the large luteal cells, in addition to having high affinity $PGF_{2\alpha}$ receptors, would most likely also possess low affinity receptors for $PGF_{2\alpha}$. If so, one might expect that after desensitization of the high affinity receptor, activation of such putative low affinity $PGF_{2\alpha}$ receptors on large luteal cells would also evoke the secretion of OT as well as suppressing P secretion. The present report demonstrates such a result which indicates the existence of functional high and low affinity states of the $PGF_{2\alpha}$ receptor on the large luteal cells of the ovine corpus luteum.

Results

To test the hypothesis that the large granulosa-derived luteal cells of the ovine corpus luteum possessed both high and low affinity receptors for $PGF_{2\alpha}$, the high affinity receptor was first desensitized (as shown by the cessation of OT secretion) with a two hour intra-arterial infusion of a subluteolytic level $PGF_{2\alpha}$ (100 pg/min) into the ovary. Immediately after terminating such a desensitizing infusion, a two hour intra-arterial infusion of a luteolytic level of $PGF_{2\alpha}$ (2500 pg/min) was begun. As shown in Figure 1, the infusion of 2500 pg/min $PGF_{2\alpha}$ immediately evoked an additional wave of OT secretion. As with the low level infusion rate of $PGF_{2\alpha}$, the high infusion rate of $PGF_{2\alpha}$ also resulted in desensitization after 1 h of infusion as demonstrated by a cessation of OT secretion. These results indicate that the large luteal cells of the ovine corpus luteum possess both functional high and low affinity receptors for $PGF_{2\alpha}$ both of which become desensitized after 1 h of administration of the appropriate concentration of ligand. As shown in Figure 2, during the low infusion rate of $PGF_{2\alpha}$ (100 pg/min) the secretion rate of P was unchanged while during the high infusion rate of $PGF_{2\alpha}$ (2500 pg/min), the secretion rate of P began to decline ($P < 0.05$). This is consistent with the rate of decline in the secretion rate of P observed previously when a luteolytic level of $PGF_{2\alpha}$ was given alone (McCracken *et al.*, 1984). An example from an individual animal showing both the secretion rate of P (μg/min) and OT (ng/min) during the high and low infusion rates of $PGF_{2\alpha}$ is depicted in Figure 3. In contrast, PGFM infused at the same concentrations for the same time periods had no effect on either the secretion rate of OT (Figure 4) or P (Figure 5).

Discussion

Receptor subtypes are known to exist for PGE, and possibly thromboxanes as well, while only one receptor type for $PGF_{2\alpha}$ is currently reported to exist (Coleman *et al.*, 1990;

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Sakamoto *et al.*, 1994; Sugimoto *et al.*, 1994). However, equivocal evidence has accumulated for the presence of high and low affinity receptors for $PGF_{2\alpha}$ based on binding affinities of ^3H-$PGF_{2\alpha}$ to cell membranes or separated whole cells from corpora lutea or liver. Some studies indicate only a single high affinity binding site for $PGF_{2\alpha}$ (Bussmann, 1989; Weipz *et al.*, 1992; Sakamoto *et al.*, 1994) while others report the occurrence of both high and low affinity binding sites (Rao, 1975; Balapure *et al.*, 1989; Neuschäfer-Rube *et al.*, 1993). However, specific functions for these postulated high and low affinity receptors for $PGF_{2\alpha}$ have not been assigned. The present report, based on the ability of intra-arterial infusions of $PGF_{2\alpha}$ to evoke the secretion of OT from the ovine corpus luteum *in vivo*, indicates the existence of functional high and low affinity states of the $PGF_{2\alpha}$ receptor on the large luteal cells of the ovine corpus luteum, the sole source of luteal OT in the sheep. Of the ^3H-$PGF_{2\alpha}$ binding studies cited above, the report by Balapure *et al.* (1989) most closely agrees with our results. These workers found low affinity sites on small luteal cells and both high and low affinity $PGF_{2\alpha}$ binding sites on large luteal cells. However, they considered that the low affinity site on large luteal cells resulted from contamination with small luteal cells.

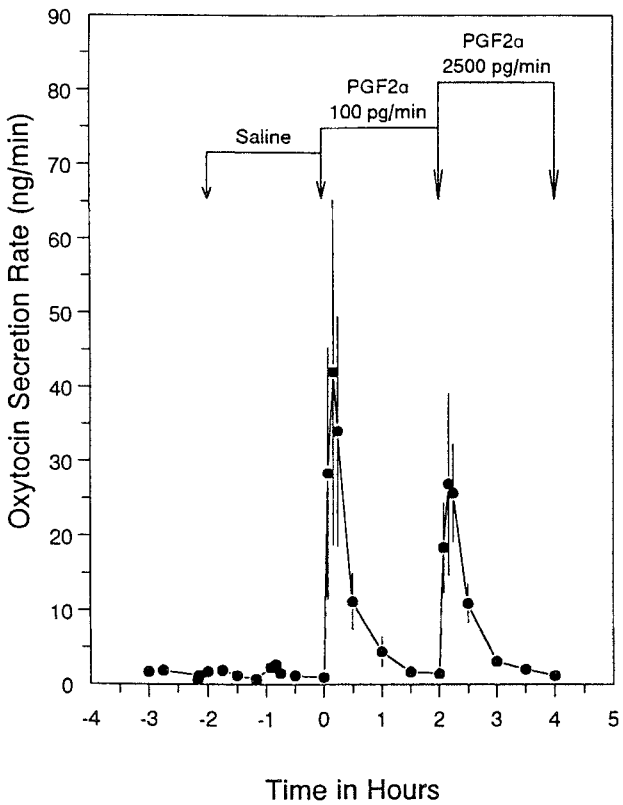

Figure 1 Secretion rate of OT (mean ± SE; $n = 7$ sheep) during the control period and during 2 h intra-arterial infusions of saline, 100 and 2500 pg/min $PGF_{2\alpha}$

Although our results establish that two desensitizable $PGF_{2\alpha}$ receptors of different affinities exist on large luteal cells, there could be two populations of OT-containing large luteal cells, one of which possesses high affinity $PGF_{2\alpha}$ receptors and another which possesses low affinity $PGF_{2\alpha}$ receptors. Thus, it is possible that two separate releases of OT could occur in response to low and high infusion rates of $PGF_{2\alpha}$ from two subtypes of large luteal cells. In the bovine corpus luteum, there is preliminary evidence that two populations of large luteal cells may exist which are distinguishable by the distribution of endosomes and by the selective degranulation of each large cell type by different levels of $PGF_{2\alpha}$ given systemically (Michael J. Fields, personal communication). Alternatively, rather than two discrete types of $PGF_{2\alpha}$ receptors there could be only one basic $PGF_{2\alpha}$ receptor protein which may be converted from a high affinity state to a low affinity state via ligand activation of a G protein as has been shown for the leukotriene B_4 receptor in human myeloid cells (Slipetz *et al.*, 1993). While recent studies indicate the presence of a single major mRNA species for the $PGF_{2\alpha}$ receptor in ovine luteal tissue (Eldering *et al.*, 1995; Niswender *et al.*, 1995; Reuda *et al.*, 1995), alternate splicing of $PGF_{2\alpha}$ receptor mRNA could give rise to $PGF_{2\alpha}$ receptor subtypes as has been shown to occur for the PGE receptor (Negishi *et al.*, 1993). Regardless of the explanation, the present study indicates that two different functional affinity states for the $PGF_{2\alpha}$ receptor exist within the large luteal cell population. The observed desensitization of both high and low affinity receptors, each after one hour, could be due either to receptor saturation or to desensitization of second messenger systems. Presently it is not known whether the two

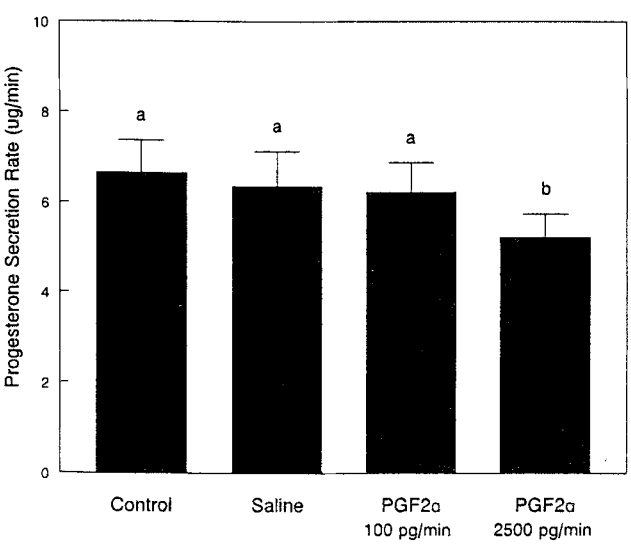

Figure 2 Secretion rate of P (mean ± SE; $n = 7$ sheep) during the control period and during 2 h intra-arterial infusions of saline, 100 and 2500 pg/min $PGF_{2\alpha}$. Bars with different letters differ at $P < 0.05$

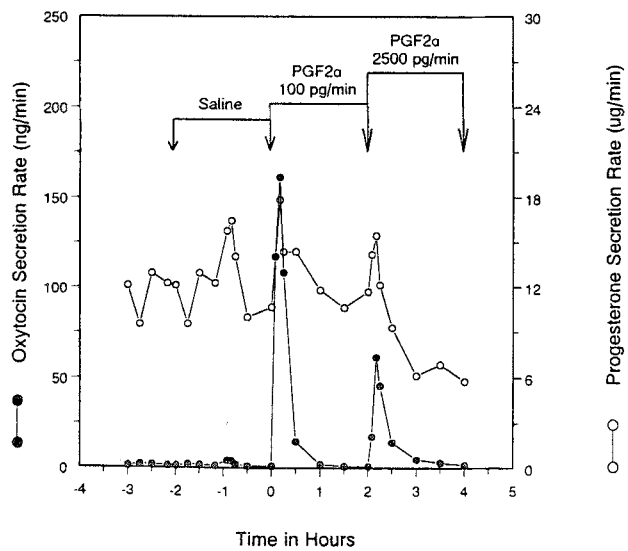

Figure 3 Example of one animal from Figure 1 showing individual response to $PGF_{2\alpha}$ infusions. P secretion rate is also illustrated in this graph. The secretion rate of P begins to decline only during the infusion of the luteolytic level of $PGF_{2\alpha}$ (2500 pg/min)

affinity states of the $PGF_{2\alpha}$ receptor identified in this study on large luteal cells are coupled to the same or different second messenger systems. If the two affinity states exist in the same large cell type there could still be two separate second messenger systems, as has been shown for glucagon receptors on liver cells (Wakelam *et al.*, 1986).

Under physiological conditions, we propose that basal plasma OT of pituitary origin, (McCracken *et al.*, 1995) interacting with rising levels of OT receptors in the endometrium towards the end of the estrous cycle (McCracken *et al.*, 1984), will cause low levels of $PGF_{2\alpha}$ to be secreted by the uterus. Such low levels of $PGF_{2\alpha}$ acting on the high affinity $PGF_{2\alpha}$ receptors on the large luteal cells will stimulate luteal OT secretion which, in turn, will increase the secretion of uterine $PGF_{2\alpha}$. $PGF_{2\alpha}$ secretion from the uterus will now be high enough to have two effects on the corpus luteum via the low affinity $PGF_{2\alpha}$ receptor and now will cause (a) the inhibition of P secretion and (b) additional OT to be released from the large luteal cells (hence reinforcing uterine $PGF_{2\alpha}$ secretion). Such effects will continue until both $PGF_{2\alpha}$ receptor types are desensitized after 1 h, thus curtailing luteal OT secretion and terminating the production of each luteolytic pulse of $PGF_{2\alpha}$ from the uterus. When $PGF_{2\alpha}$ receptors on the luteal cells recover after 6 to 9 h, similar luteolytic pulses of $PGF_{2\alpha}$ from the uterus will occur until the corpus luteum regresses under the local influence of at least five intermittent luteolytic pulses of $PGF_{2\alpha}$ (McCracken *et al.*, 1984). However in the present study, by using separate low and high infusion rates of $PGF_{2\alpha}$ into the ovarian autotransplant model (which is separated from the uterus), we have demonstrated two discrete peaks of OT secretion from the corpus luteum which normally would occur in unison in the intact animal to produce each luteolytic pulse of $PGF_{2\alpha}$ from the uterus.

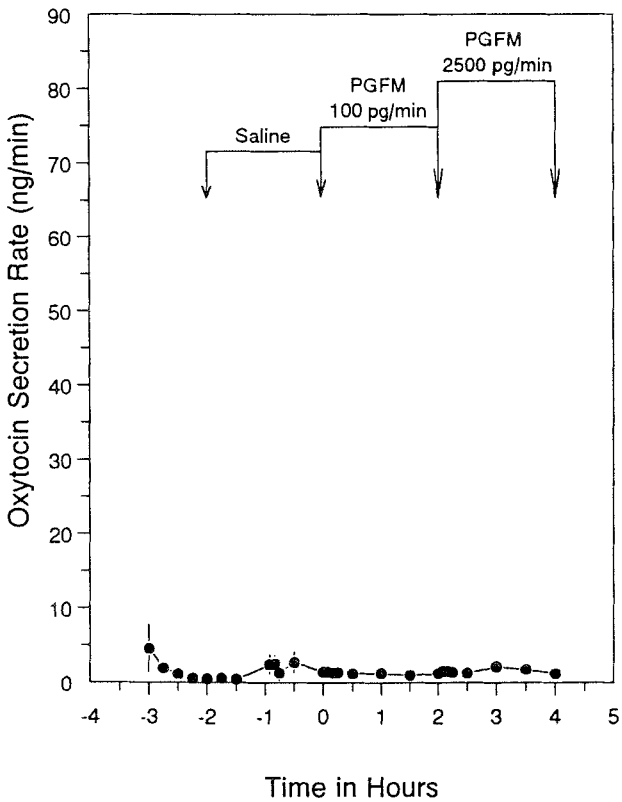

Figure 4 Secretion rate of OT (mean ± SE, $n = 4$ sheep) during the control period and during 2 h intra-arterial infusions of saline, 100 and 2500 pg/min PGFM

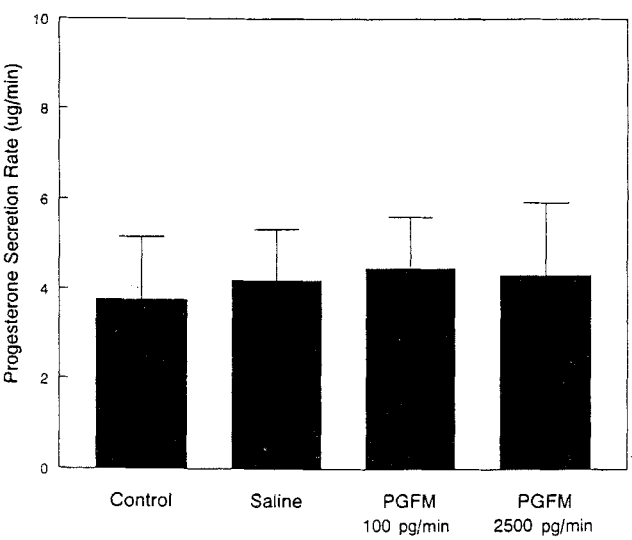

Figure 5 Secretion rate of P (mean ± SE, $n = 4$ sheep) during the control period and during 2 h intra-arterial infusions of saline, 100 and 2500 pg/min PGFM

Materials and methods

Experimental animals

Intra-arterial infusions into the ovary of conscious sheep were conducted using animals with the left ovary autotransplanted with vascular anastomoses into a jugulo-carotid loop and with the right overy removed (Goding *et al.*, 1967; McCracken *et al.*, 1971). Each animals received two intramuscular injections of 5 mg of $PGF_{2\alpha}$ (Lutalyse, Upjohn Co., Kalamazoo, MI) at an interval of 4 h to synchronize estrus. Estrus (day 0 of the cycle) which was determined by vasectomized rams, occurred 48–72 h after $PGF_{2\alpha}$ administration. The two infusion paradigms described below were always carried out on day 12 after estrus in order to mimic conditions existing in cycling intact sheep 1 or 2 days before the onset of luteolysis. Animals were randomly assigned to one of two infusion paradigms: (1) sequential 2 h intra-arterial infusions of saline, 100 and 2500 pg/min of $PGF_{2\alpha}$ ($n = 7$), or (2) sequential 2 h intra-arterial infusions of saline, 100 and 2500 pg/min of 13,14,-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM), an inactive metabolite of $PGF_{2\alpha}$ ($n = 4$). Each of the above series of intra-ovarian infusions was carried out in a separate animal on day 12 of the cycle.

Intra-arterial infusions of the ovary in conscious sheep

Twenty-four hours before each infusion experiment, the left carotid artery and jugular vein contained within the isolated jugulo-carotid loop were cannulated as described by McCracken *et al.* (1971). On the day of each infusion experiment, stock solutions (1 mg/ml) of $PGF_{2\alpha}$ (tromethamine salt) and PGFM (Cayman Chemical Co., Ann Arbor, MI) were prepared in 100% ethanol. The concentration was then adjusted for infusions with sterile, pyrogen and preservative-free aqueous 0.9% sodium chloride (Abbott Laboratories, North Chicago, IL). During each infusion experiment, 10 000 i.u. of heparin was given i.v. as an anticoagulant at −3 h and at +1 h. To insure direct infusion of $PGF_{2\alpha}$ or PGFM into the arterial supply of the ovary, a sphygmomanometer cuff was inflated on the cranial side of the jugulo-carotid loop to 300 mmHg. Substances were administered by means of an infusion pump (Harvard Apparatus, Cambridge, MA) set to deliver 0.1 ml/min (saline, 100 or 2500 pg/min of $PGF_{2\alpha}$ or PGFM). These infusions rates of $PGF_{2\alpha}$ were shown previously to be subluteolytic (Lamsa *et al.*, 1989) and luteolytic (McCracken *et al.*, 1984), respectively.

Collection of ovarian venous blood

Timed samples of ovarian venous blood (5 ml) were collected during a 1 h control period and during the sequential 2 h intra-arterial infusion of saline, 100 and 2500 pg/min of $PGF_{2\alpha}$ or PGFM. The timed samples were collected via a jugular vein catheter into tubes over ice by inflating the sphygmomanometer cuff on the cranial side of the transplanted ovary and by manual occlusion of the jugular vein on the cardiac side of the transplanted ovary. Immediately after collection, each blood sample was centrifuged at 1000 *g* at 4°C for 30 min and the hematocrit and plasma volume measured. This yielded plasma flow (ml/min), and hence allowed calculation of ovarian secretion rate of OT and P (mass/unit time). Plasma was removed promptly and stored at −20°C until OT and P were determined by RIA.

Radioimmunoassay

The concentration of OT in ovarian vein plasma was measured by RIA as described by Lamsa *et al.* (1989), and the values were corrected for extraction losses. RIA for P was performed on unextracted ovarian venous plasma after dilution (50:1) with assay buffer. A solid phase RIA was used to measure P (Diagnostic Products, Los Angeles, CA) as previously described by Holt *et al.* (1989). The sensitivities of the OT and P assays were 1 and 10 pg/tube, respectively.

Statistical analyses

Secretion rates of OT and P were analysed by one-way analysis variance. Difference between means was determined by Tukey's HSD test. Values of $P<0.05$ were considered significant.

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