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Animal Reproduction Science 51 (1998) 81–96

ANIMAL REPRODUCTION SCIENCE

Oestrogenic effects of ICI 182,780, a putative anti-oestrogen, on the secretion of oxytocin and prostaglandin $F_{2\alpha}$ during oestrous cycle in the intact ewe

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Accepted 2 February 1998

Abstract

The effect of ICI 182,780, oestrogen antagonist, on the concentrations of oxytocin and uterine $PGF_{2\alpha}$ was investigated in intact Border Leicester Merino cross ewes during the late oestrous cycle. Twelve cyclic ewes ($n = 6$ per group) were randomly assigned to receive, at 6 h intervals, intra-muscular injection of either peanut oil or ICI 182,780 (1.5 mg kg^{-1} day^{-1}) in oil for 2 days, starting at 1900 h on day 13 until 1300 h on day 15 post-oestrus. Hourly blood samples were collected via a jugular catheter from 0800 h on day 14 for 37 h and then daily over days 16, 17 and 18 post-oestrus. Peripheral plasma concentrations of oxytocin, the metabolite of prostaglandin $F_{2\alpha}$, 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$, (PGFM) and progesterone were measured by radioimmunoassay. All ewes treated with ICI 182,780 exhibited functional luteal regression as indicated by a marked reduction in plasma progesterone concentrations to less than 1000 pg/ml over the period of 18–36 h during sampling period on days 14 and 15 of the oestrous cycle. In five of six vehicle-treated ewes, progesterone concentrations declined between day 16 and day 18 post-oestrus. In the remaining control ewe, progesterone concentrations reach less than 1000 pg/ml within 36 h of the commencement of the sampling period. During the frequent sampling period, the number of oxytocin pulses in the ICI 182,780 treated ewes was significantly higher compared to control ewes (2.7 ± 0.3 vs. 0.8 ± 0.3). The mean amplitude of oxytocin pulses observed was also greater (70.4 ± 19.5 pg/ml) in ewes treated with ICI 182,780, but was not

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PII S0378-4320(98)00068-2

significantly different from control ewes (33.5 ± 12.9 pg/ml). Oxytocin pulses may however have occurred following the initial two ICI 182,780 injections but before commencing blood sampling. The oxytocin pulses were detected at a mean of 3.2 ± 0.2 h following each injection with ICI 182,780 during blood sampling. In the ICI 182,780-treated ewes, the pulsatile pattern of plasma PGFM in jugular blood samples over the 37 h sampling period on days 14 and 15 post-oestrus had a higher amplitude (512.9 ± 158.9 vs. 121.7 ± 78.7 pg/ml) and pulse area (618.1 ± 183.3 vs. 151.5 ± 102.9 (pg/ml)τ) compared to the vehicle-treated ewes ($P < 0.05$) respectively. The average number of PGFM pulses observed per ewe was 3.0 ± 0.7 in the ICI 182,780-treated group and was significantly ($P < 0.02$) higher than the number of pulses (0.5 ± 0.3) observed in ewes treated with vehicle alone. The PGFM pulses were detected at 4.2 ± 0.6 h following each injection with ICI 182,780 during blood sampling. The percentage of PGFM pulses that occurred coincidently with a significant elevation of oxytocin concentrations was 44.4% in ICI 182,780-treated compared to 66.6% in control ewes. We conclude that administration of oestrogen antagonist ICI 182,780 accelerated development of the luteolytic mechanism by enhancing pulsatile secretion of oxytocin and PGFM which suggests that ICI 182,780 acts as an agonist for oxytocin and prostaglandin $F_{2\alpha}$ release in intact ewes when administered at 1.5 mg/kg/day over Day 13 to 15 post-oestrus.

Keywords: Oestrogen antagonist; Oxytocin; Prostaglandin; Sheep endocrinology

1. Introduction

Considerable evidence now exists indicating that oxytocin can induce endometrial prostaglandin $F_{2\alpha}$ (PG)$F_{2\alpha}$ secretion from the uterus (Sharma and Fitzpatrick, 1974; Roberts et al., 1976; Flint et al., 1990) and that $PGF_{2\alpha}$ can stimulate luteal oxytocin secretion (Flint and Sheldrick, 1982; Watkins and Moore, 1987; Lamsa et al., 1989). Pulsatile release of $PGF_{2\alpha}$ by the endometrium may be more effective than continuous secretion since Schramm et al. (1983) have demonstrated that exogenous administration of $PGF_{2\alpha}$ is more potent as a luteolysin when administered in a series of pulses rather than as a continuous infusion. During luteolysis, oxytocin pulses, or its associated neurophysin, occur simultaneously with pulses of $PGF_{2\alpha}$ (Fairclough et al., 1980; Webb et al., 1981; Flint and Sheldrick, 1983; Moore et al., 1986; Hooper et al., 1986). Oestrogen also appears to play a role in regulation of uterine function since administration of relatively high doses of oestradiol to ewes during the mid-luteal phase (Ford et al., 1975; Hixon and Flint, 1987) or to ewes and cows on day 12 (Spencer et al., 1995; Brunner et al., 1969) resulted in premature luteal regression and caused a premature decline in luteal weight (Hawk and Bolt, 1970). Oestrogen also appears to affect the timing, magnitude and pattern of $PGF_{2\alpha}$ response to oxytocin (McCracken, 1980; Zhang et al., 1991; Beard and Lamming, 1994). The mechanism by which oestrogen interacts with oxytocin and $PGF_{2\alpha}$ at luteolysis is not clear, although time course studies indicate that it takes several hours to act.

In a preliminary report, Fairclough et al. (1988) administered oestradiol 17-β to intact ewes on days 12, 13 and 14 of the oestrous cycle and found an associated increase in plasma oxytocin–neurophysin and/or PGFM with a maximum concentration occurring at 4–8 h after oestrogen treatment. Furthermore, Mann and Lamming (1995) have reported that in ovariectomized cows there was a reduction in the strength of the

luteolytic signal over the later stages of the luteal phase following removal of an oestradiol implant on day 11 of the simulated cycle. In heifers, Jacobs et al. (1988) have also shown that the increase in secretion of endogenous $PGF_{2\alpha}$ that follows induction of luteolysis with the $PGF_{2\alpha}$ agonist cloprostenol is suppressed by intravenous injections of tamoxifen, an oestrogen antagonist. These data indicated that the $PGF_{2\alpha}$ secretion at the time of luteolysis is dependent on oestradiol.

Oestradiol-17β is also involved in regulation of the corpus luteum and influences luteolysis. In ewes, oestrogen may act directly on the corpus luteum through an intra-ovarian mechanism because injection of oestrogen directly into the corpus luteum at day 10 post-oestrus resulted in regression of the injected corpus luteum but had little effect on the contralateral corpus luteum (Cook et al., 1974). Furthermore, removal of the main source of oestrogen by destruction of ovarian follicles extended the cycle length in the ewe (Karsch et al., 1970; Ginther, 1971; Zhang et al., 1991) and cattle (Fogwell et al., 1985; Villa-Godoy et al., 1985). The mechanism by which oestrogen acts to stimulate oxytocin and uterine $PGF_{2\alpha}$ is uncertain.

ICI 182,780 is thought to be a pure steroidal oestrogen antagonist which blocks oestrogen action by competing with endogenous oestrogen for oestrogen receptors present in the nuclei of oestrogen responsive tissue (Baker and Jaffe, 1996). ICI 182,780 has been found to be devoid of agonist activity in vivo in the immature and mature rat (Wakeling et al., 1991) and blocked the production of the 250 kDa protein which was associated with the stimulatory action of oestrogen in ovariectomized rat (Yu et al., 1996). Recently, Dibbs et al. (1995) have shown that administration of ICI 182,780 to ovariectomized rat subcutaneously (1.5 mg/kg daily for 3 days) blocked the effect of oestrogen on the uterine oxytocin receptor within 24 h after the final dose. However, Wakeling (1993) reported that ICI 182,780 showed no effect on bone density analysis in adult female rats, whereas ovariectomy significantly reduced bone density. In guinea-pigs the administration of 4 mg of the same putative oestrogen antagonist (equivalent to 4.7–6.15 mg/kg), once a day on days 11–14 of the cycle, significantly reduced the output of $PGF_{2\alpha}$ from superfused uterine horn in vitro on day 15 post-oestrus (Poyser, 1993). In this case, the ICI 182,780 compound may be acting by increasing receptor turnover and reducing the cellular content of oestrogen receptors (Parker, 1993). Administration of ICI 182,780 (12 mg) daily for 7 days in women with normal menstrual cycles, decrease the incidence of the mid-cycle LH surge and had an anti-proliferative effect on endometrial thickness as measured by ultrasound (Thomas et al., 1994).

This study was undertaken to investigate the inhibitory action of the purported oestrogen receptor antagonist (ICI 182,780) on oxytocin, uterine $PGF_{2\alpha}$ production, and on luteal function in intact ewes.

2. Materials and methods

2.1. Animals

All protocols were approved by the Animal Experimentation Ethics Committees of Victoria University of Technology (AEEC 95/025) and Monash University.

Twelve 3-year-old Border Leicester Merino cross ewes, weighing 42–55 kg, were used in this study during the breeding season. The oestrous cycles of the ewes were synchronised by the insertion of an intra-vaginal controlled internal drug release (CIDR) device impregnated with 300 mg progesterone (EAZI-breed CIDR G, Riverina Artificial Breeders, Albury, Australia) for 14 days and by an intra-muscular injection of Serum Gonadotrophin (400 IU) to each ewe following removal of the CIDR. Ewes were housed in an open barn together with a crayon-bearing vasectomized rams. Ewes were checked twice daily for behavioural oestrus. The day that the ewe display oestrus behaviour was designated to be Day 0. One week before experimentation, the ewes were maintained in individual pens with access to water and fed lucerne chaff, oats and ewe and lamb pellets ad libitum.

On day 13 post-oestrus, cannulation was performed under local anaesthesia (10% Lignocaine Hydrochloride Spray: Xylocaine). The jugular vein of each ewe was cannulated by insertion of an intracath (Intervenous Catheter Placement Unit; 16 GA; Desert Medical, Becton Dickinson, Sandy, UT). The exteriorised end of the catheter was connected to a 3 way stopcock. The catheters were secured to the ewes by placing Setonet (Elastic net bandage, size 5; Seton Products, England) around the neck over the cannula. Cannulae were then maintained for the duration of the experiment by filling with heparinised saline (50 IU/ml) and used for the collection of blood samples.

2.2. Experimental protocol

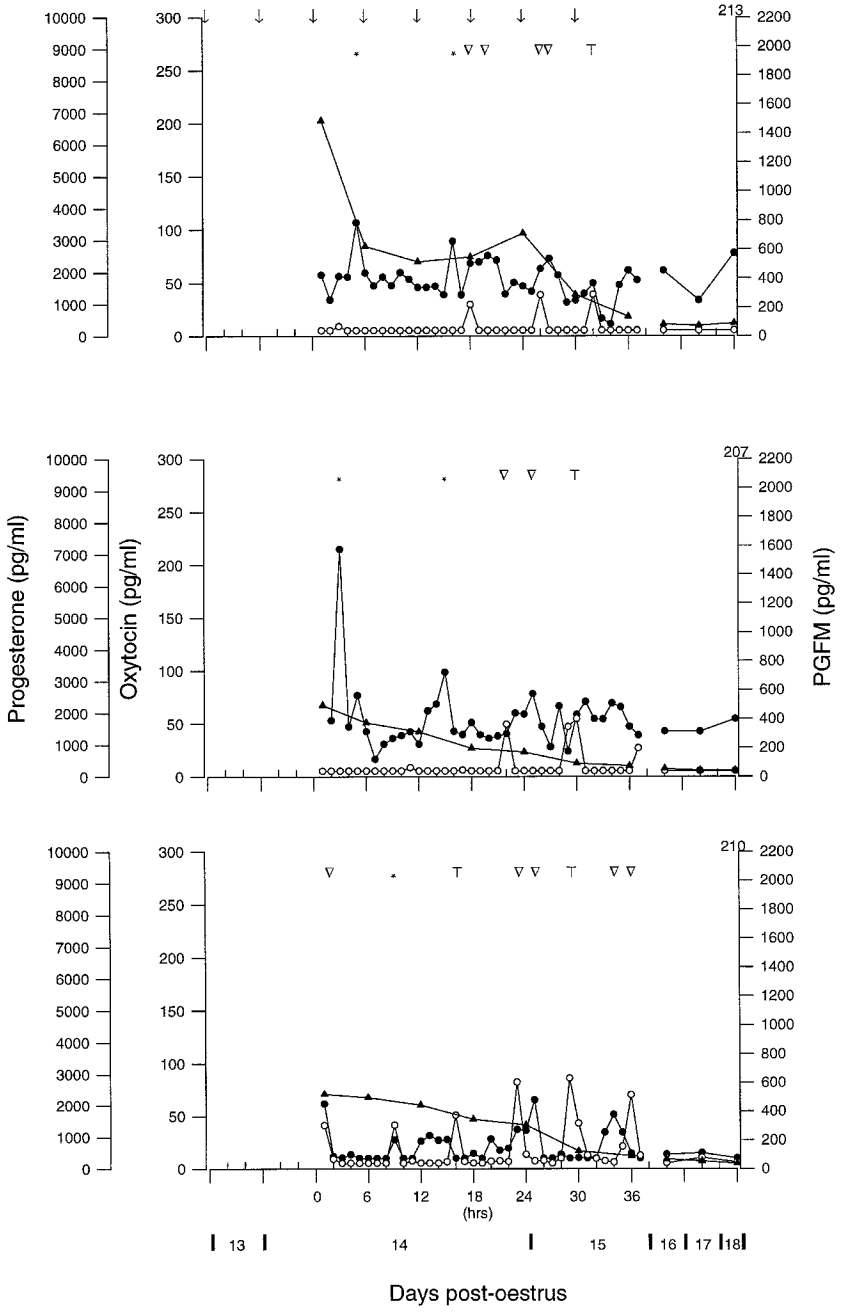
Ewes were randomly assigned to two groups ($n = 6$ per group). One group was treated with intra-muscular injections of 1.5 mg kg^{-1} day^{-1} ICI 182,780 [7α-[9-(4,4,5,5,5-pentafluoro-pentylsulphinyl)nonyl]estra-1,3,5(10)-triene-3,17β-diol] (a gift from ICI Pharmaceuticals, Australia) in peanut oil while the other group was treated with peanut oil alone (controls), at 6 h intervals at 1900 h on day 13 up until 1300 h on day 15 post-oestrus. The ICI 182,780 (8.5 mg in 1 ml) was dissolved in absolute ethanol to a concentration of 5% (v/v) of the final volume. The exact volume of peanut oil was then added and the ethanol was evaporated using mild heat (40°C) and under constant stirring with a magnetic stirrer.

2.3. Blood sampling

Blood samples (5 ml) were collected over days 14 and 15 of the oestrous cycle at hourly intervals starting at 0800 h (12 h after commencement of injection of antagonist) for 37 h and then daily over days 16, 17 and 18 post-oestrus. Blood samples were

Fig. 1. Effect of ICI 182,780 on oxytocin (●), PGFM (○), and progesterone (▲) concentrations in peripheral plasma in intact ewes late in the oestrous cycle ($n = 6$; ewe 212, 217, 222, 213, 207, 210). ICI 182,780 was injected at 1900 h on Day 13 post-oestrus. ICI 182,780 (1.5 mg kg^{-1} day^{-1}) was administered intra-muscularly every 6 h up until 1300 h on Day 15 post-oestrus. (↓) represent time of injection. Zero time (0) h represents 0800 h on Day 14. Blood samples were collected hourly starting at (0) h time up to 37 h during Days 14 and 15 post-oestrus and, thereafter, once a day on Days 16, 17 and 18 post-oestrus. Statistically significant pulses in the oxytocin and PGFM concentrations are identified by (*) and (⊤), respectively; inverted triangles (▽) identify synchronous pulses of both compounds.

collected into polypropylene tubes containing aspirin and ethylenediaminetetraacetic acid (EDTA), 0.7 and 0.5 mg/ml blood respectively. The catheter was refilled with heparinised saline (50 IU/ml) during frequent blood sampling. Blood samples were then



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