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Oxytocin induction of pulses of a prostaglandin metabolite and luteolysis in mares

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ABSTRACT

A procedure for oxytocin (OT) administration on Day 13 postovulation was developed in mares for stimulation of a pulse of PGFM (a PGF2α metabolite) that mimics a natural PGFM pulse during luteolysis. Bolus treatment with each of five OT doses (1–10 IU/mare, n = 3) stimulated a burst of PGFM that was maximum in 4 minutes and was unlike a natural pulse. A 2-hour OT infusion of 1.25, 2.5, or 5 IU/100 kg (n = 4) induced a PGFM pulse similar to reported pulses; lower doses did not. The peak of an induced pulse (approximately 260–380 pg/mL) seemed similar to reported natural peaks (approximately 200–300 pg/mL), using the same assay system. The interval from nadir to nadir was 6.6 ± 0.2 hours. Percentage decrease in progesterone (P4) within 8 hours was greater ($P < 0.05$) for doses of 1.25, 2.5, or 5 IU/100 kg (43%–50%) than that for a vehicle group (11%). Treatment with flunixin meglumine (1.0 mg/kg), a cyclooxygenase inhibitor, decreased ($P < 0.008$) P4 concentration, but treatment 2 hours before the beginning of OT infusion (2.5 IU/100 kg) did not prevent the OT-induced PGFM pulses and the decrease in P4. In conclusion, a PGFM pulse was simulated by infusion of OT during 2 hours but not by a single OT bolus, and an OT-simulated PGFM pulse stimulated a decrease in P4 that was not prevented by a cyclooxygenase inhibitor. These are the first firm demonstrations that OT in mares as in other species has a role in luteolysis.

1. Introduction

The regression of the CL or luteolysis is a pivotal reproductive event during the estrous cycle in farm species, including mares. Luteolysis represents a decrease in progesterone (P4) from the response of the CL to the secretion of PGF2α by the endometrium (review [1]). In the mare, luteolysis begins on average on Day 14 (Day 0 = ovulation) and lasts 23 hours on the basis of hourly blood sampling [2]. The end of luteolysis is defined as a P4 decrease to less than 1 ng/mL [3]. The main plasma PGF2α metabolite is 15-keto-13,14-dihydro-prostaglandin F2α (PGFM) [4]. The metabolite has a longer half-life and is often used to represent circulating concentrations of PGF2α [5]. In many species, including mares, complete luteolysis requires secretion of multiple pulses of PGF2α by the nongravid uterus [6]. The interval between the peaks of sequential PGFM pulses in mares is 9 hours, and the interval from nadir to nadir at the PGFM base is 5 hours [6]. Inhibition of PGF2α secretion at the expected time of luteolysis with a cyclooxygenase inhibitor (flunixin meglumine [FM]) induces a delay in the beginning of luteolysis [7].

On the basis of hourly blood sampling, the transition into the luteolytic period in mares is manifested within

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1 hour [8]. Pulses of PGFM are relatively small before luteolysis (e.g., peak 30 ng/mL) compared with that during luteolysis (e.g., 190 ng/mL) [8]. Each PGFM pulse that occurs during luteolysis is temporally associated with a pulse of oxytocin (OT) [9]. The small transitional pulse at the hour of the initiation of luteolysis is also associated with a pulse of OT, whereas previous small PGFM pulses are not [10]. Oxytocin therefore may play a role in the effectiveness of a PGF2α pulse during luteolysis and the effectiveness of the small transitional pulse at the initiation of luteolysis in mares (review [2]).

Oxytocin is synthesized in the hypothalamus, stored in the posterior pituitary, and secreted in pulses [11,12]. The CL also synthesizes and secretes OT during the estrous cycle in domestic ruminants [13,14], but the CL apparently does not secrete OT in mares [15]. Circulating concentration of OT in mares is greater at the expected time of luteolysis (Day 15) than that before luteolysis (Days 0, 3, or 7) [12]. The role of OT during spontaneous luteolysis has not been clarified in mares, but in other farm species, OT is an intermediary in the stimulation of PGF2α secretion from the uterus (review, [16]). In previous studies on the effect of exogenous OT on PGFM in mares, the doses were apparently based on clinical recommendations (e.g., 20 IU to evacuate the uterus [17]). Treatment of nonpregnant mares with OT doses of 10 to 25 IU [18–21] or the OT response to uterine biopsy [22,23] stimulates an increase in PGFM. The OT treatments are especially effective near the expected day of luteolysis when the number of uterine OT receptors is maximum (Days 14–17) [22–24]. Despite the induced increase in PGFM, a negative effect of OT treatment on circulating P4 concentration has not been documented in mares.

Treatment with OT early in the estrous cycle in cattle is luteolytic [25], but in mares, treatment from Days 1 to 7 with a high dose (200 IU [26]) or Days 4 to 8 (150 IU [27]) did not induce luteolysis or shorten the interovulatory interval (IOI). In this regard, the number of OT receptors in the uterus is minimal on Days 4 to 8 in mares [27]. Despite the stimulation of PGFM by OT doses of 10 to 25 IU/mare [18,20], chronic administration of OT in high doses (e.g., 60 IU/day on Days 7–14) prolongs the luteal phase at least until Day 30 [28–31]. Furthermore, 60 IU of OT on Days 8 to 14 prolongs the luteal phase (P4 > 1.0 ng/mL until Day 30), reduces endometrial cyclooxygenase-2 expression, and lowers plasma concentrations of PGFM [31].

The dose and method of OT administration that could be considered physiological in mares are unknown. That is, the effect of OT treatment on the CL has been examined only on a pharmacologic basis. The objectives for the present studies in mares were to (1) determine a dose and method of administration of OT that will stimulate a PGFM pulse similar to a spontaneous pulse and (2) study the role of OT in luteolysis. The hypotheses were (1) simulation of a PGFM pulse can be done by infusion of a specific dose of OT during 2 hours but not by a single OT bolus dose, (2) OT induction of a simulated PGFM pulse stimulates a decrease in P4, and (3) OT exerts a luteolytic effect only through stimulation of PGF2α secretion.

2. Materials and methods

2.1. Mares and treatments

Mixed breeds (light, riding-type horses, and apparent pony–horse crosses) of 21 nonlactating mares aged 4 to 18 years and weighing 300 to 600 kg were used in the northern temperate zone. Abnormalities of the reproductive tract were not detected by transrectal ultrasonic scanning [32]. The mares had not been bred for at least 3 years. The mares were housed under natural light in an open shelter and outdoor paddock and were maintained by free access to primarily grass hay, trace mineralized salt, and water. All mares remained healthy and in good body condition throughout the studies. Animals were handled according to the United States Department of Agriculture Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching. Mares were examined daily throughout each of four experiments by transrectal ultrasonic imaging as described [32]. A duplex B-mode (gray scale) and color Doppler instrument equipped with a linear-array 7.5-MHz transducer was used. The day of ovulation was designated Day 0.

Mares were assigned by randomization to experimental groups, including a vehicle or control group (0 dose). Each designated treatment in each experiment was given into a jugular vein. Oxytocin (Oxytocin Injection, Bimeda, Inc., Le Sueur, MN, USA) was used in each experiment. Flunixin meglumine (FluMeglumine, Phoenix, St Joseph, MO, USA) was also used in experiments 3 and 4. The FM acts on cyclooxygenase enzymes in the endometrium [33] and thereby blocks PGF2α synthesis [34]. A single systemic treatment with FM inhibits PGF2α secretion in the mare, but the effect wanes by 8 hours [7].

2.2. Experiment 1. Effect of bolus treatment with OT on PGFM

This experiment was done to determine if a single injection of OT would induce a pulse of PGFM that would be similar to an endogenous pulse. On Day 13, mares in a vehicle group were given 2 mL of saline, and mares in five OT-treated groups were given a single bolus treatment of OT as follows: OT-1.0 (1.0 IU of OT/mare), OT-2.5, OT-5, OT-7.5, and OT-10.0 (n = 3/group). The doses of OT were diluted in saline to a final volume of 2 mL for each dose. The minute of the treatment was designated minute 0. Blood samples were collected into heparinized tubes by venipuncture of the jugular vein. The mares were docile and did not object to venipuncture with a 20-ga needle, as indicated by collection without head restraint. Samples were collected at minutes 0, 1, 2, 3, 4, 5, 10, 15, 30, 45, and 60 and hourly thereafter until hour 4. The minute-0 sample was collected immediately before treatment. Plasma samples were assayed for PGFM.

2.3. Experiment 2. Effect of 2-hour infusion of OT on PGFM pulses

This experiment was done to determine a dose of OT that would simulate an endogenous pulse of PGFM when