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Prostaglandins & other Lipid Mediators 85 (2008) 33–41

PROSTAGLANDINS & other LIPID MEDIATORS

Mechanism whereby nitric oxide (NO) infused chronically intrauterine in ewes is antiluteolytic rather than being luteolytic

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Received 8 July 2007; received in revised form 8 October 2007; accepted 17 October 2007
Available online 24 October 2007

Abstract

Nitric oxide (NO) has been reported to be luteolytic *in vitro* and *in vivo* in cows. However, an NO donor reversed $PGF_2\alpha$-induced inhibition of rat luteal progesterone secretion *in vitro* and an NO donor or endothelin-1 stimulated bovine luteal tissue secretion of prostaglandins E (PGE; PGE_1, PGE_2) *in vitro* without affecting progesterone or $PGF_2\alpha$ secretion. In addition, chronic infusion of an NO donor into the interstitial tissue of the ovarian vascular pedicle adjacent the luteal-containing ovary prevented the decline in circulating progesterone, while a nitric oxide synthase (NOS) inhibitor did not affect luteolysis. The objective of this experiment was to determine whether an NO donor or NOS inhibitor infused chronically intrauterine adjacent to the luteal-containing ovary during the ovine estrous cycle was luteolytic or antiluteolytic. Ewes were treated either with vehicle ($N=5$), diethylenetriamine (DETA-control for DETANONOate; $N=5$), (*Z*)-1-[2-(2-aminoethyl)-*N*-(2-ammonioethyl)amino]diazen-1-ium-1,2-diolate (DETANONOate-long acting NO donor; $N=6$), L-arginine ($N=5$), L-nitro-arginine methyl ester (L-NAME-NOS inhibitor; $N=6$), or NG-monomethyl-L-arginine acetate (L-NMMA; NOS inhibitor; $N=5$) every 6 h from 2400 h (0 h) on day 8 through 1800 h on day 18 of the estrous cycle. Jugular venous blood and inferior vena cava plasma via a saphenous vein cathether 5 cm anterior to the juncture of the ovarian vein and inferior vena cava were collected every 6 h for analysis for progesterone and $PGF_2\alpha$ and PGE, respectively, by RIA. Corpora lutea were collected at 1800 h on day 18 and weighed. Weights of corpora lutea were heavier ($P \leq 0.05$) in DETANONOate-treated ewes when compared to vehicle, DETA, L-arginine, L-NAME, or L-NMMA-treated ewes, L-arginine luteal weights were heavier than vehicle, DETA, L-arginine, L-NAME, or L-NMMA-treated ewes, and luteal weights of vehicle, DETA, L-NAME, or L-NMMA-treated ewes did not differ amongst each other ($P \geq 0.05$). Profiles of progesterone in jugular venous blood on days 8–18 differed ($P \leq 0.05$) in DETANONOate-treated ewes when compared to vehicle, DETA, L-arginine, L-NMMA or L-NAME-treated ewes, which did not differ ($P \geq 0.05$) amongst each other. The PGE:$PGF_2\alpha$ ratio profile in inferior vena cava plasma of DETANONOate-treated ewes was increased ($P \leq 0.05$) when compared to all other treatment groups. In a second experiment, conversion of [^{3}H PGE_2] to [^{3}H $PGF_2\alpha$] by day 15 ovine caruncular endometrium *in vitro* was determined in vehicle, DETA, or DETANONOate-treatment groups. Conversion of [^{3}H PGE_2] to [^{3}H $PGF_2\alpha$] was decreased ($P \leq 0.05$) only by DETANONOate. It is concluded that NO is not luteolytic during the ovine estrous cycle, but may instead be antiluteolytic and prevent luteolysis by altering the PGE:$PGF_2\alpha$ ratio secreted by the uterus.
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Keywords: Sheep; Progesterone; Nitric oxide; L-NAME; L-NMMA; $PGF_2\alpha$; PGE; Estrous cycle

1. Introduction

Approximately one-third of ovine, bovine, and porcine embryos are lost during the first third of pregnancy [1–11]. Additional losses of 6–8% occur after the first third of pregnancy [34]. These losses are probably due to deficiencies in progesterone secretion, since progesterone is required throughout gestation to maintain pregnancy in ewes [13–16]. Secretion of progesterone by the corpus luteum during the estrous cycle is regulated by LH stimulation of small luteal cell production of c-AMP [17–19]. Loss of progesterone secretion by the corpus luteum at the end of the estrous cycle is via uterine secretion of $PGF_2\alpha$, which is delivered locally from the uterine vein to the adjacent ovarian artery [20–22]. Uterine secretion of $PGF_2\alpha$ to initiate luteolysis begins around day 12 in ewes and day 16 in cows [13–17,20,23]. Although $PGF_2\alpha$ is a vasoconstrictor and could cause ischaemia of the corpus luteum [24], loss of luteal LH receptors [13–17] and decreases in ovarian blood flow

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1098-8823/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.prostaglandins.2007.10.003

[24,25] occur after the onset of luteolysis and after cAMP and progesterone had declined [17].

Endometrial LH receptors may play a role in luteolysis through increases in uterine $PGF_2\alpha$ secretion during the estrous cycle [26–30]. Endometrial LH receptors increase the last few days of the estrous cycle and LH increases $PGF_2\alpha$ secretion *in vitro* and *in vivo* late in the estrous cycle and thus may enhance the luteolytic process [31,32]. However, LH increased placental caruncular endometrial secretion of PGE secretion *in vitro* when an embryo was present [33]. PGE_1 and PGE_2 are vasodilators and increase luteal progesterone secretion *in vitro*, which is in contrast to the actions of $PGF_2\alpha$ [15–18].

Nitric oxide is also a vasodilator and increases throughout pregnancy [34]. Nitric oxide (NO) has been reported to play a role in luteolysis, since L-nitro-arginine methyl ester (L-NAME), a NOS inhibitor, perfused into the bovine corpus luteum *in vivo* delayed the decrease in progesterone for only a few days [35,36] and NO decreased progesterone by dissociated bovine luteal cells in culture [37,38]. However, an NO donor reversed $PGF_2\alpha$-induced inhibition of luteal progesterone secretion *in vitro* in the rat [39]. Bovine luteal tissue of the estrous cycle or pregnancy secretes PGE_2 and $PGF_2\alpha$, which increases linearly with time in culture and the PGE_2:$PGF_2\alpha$ ratio remains 1:1 [40], which does not occur with ovine luteal tissue of the estrous cycle *in vitro* [41]. Ovine luteal tissue of pregnancy secretes PGE (PGE_1/PGE_2) and PGE, but not LH is the luteotropin in ewes by day 50 [42] and by day 200 in cows [43]. NO donors and endothelin-1 do not alter bovine luteal tissue progesterone or $PGF_2\alpha$ secretion *in vitro*, but increased PGE, while NOS antagonists did not affect secretion of progesterone, $PGF_2\alpha$, or PGE [44,45]. PGE [46–51], NO [52–58], and estradiol-17β and progesterone [46–51] are obligatory for implantation in rodents and NO may regulate endometrial PGE secretion for implantation in rodents [49–53] via estrogen-induced increases in NOS III [57,59]. NOS inhibitors prevented implantation in rodents [52–55]. Estrogen increased blood flow to the uterus of livestock during implantation [24,25] and may be mediated in part by NO, which is a vasodilator [34]. NO may also be important throughout pregnancy to relax the uterine myometrium [60–66] and uterine NO decreased just prior to parturition [67–70]. Arginine is the main source for production of NO [70] and arginine increases in porcine uterine fluids during gestation [71], which may be to support increases in uterine blood flow [34]. In addition, placental NOS activity increased on days 20–40 in the ovine conceptus [72]. NO [52–56], PGE [47–51] and progesterone and estradiol-17β [47–51] are obligatory for implantation in rodents. The corpus luteum is the source of this progesterone [13–18] and PGE is the major direct antiluteolytic signal from the uterine horn to the adjacent luteal-containing ovary to prevent luteolysis during early pregnancy [13–16]. Since chronic infusion of an NO donor into the interstitial tissue of the luteal-containing ovary prevented luteolysis in ewes [73], the objective of this experiment was to determine whether an NO donor or NOS inhibitors infused chronically intrauterine adjacent to the luteal-containing ovary affected ovine luteal function and the PGE:$PGF_2\alpha$ ratio in inferior vena cava blood during the ovine estrous cycle.

2. Materials and methods

2.1. Animals

The experimental protocol was approved at the University of Hawaii IACUC. Mature Merino crossbred ewes were checked twice daily (0800 and 1700 h) for estrus with brisket-painted vasectomized rams. Estrus was designated as day 0 of the estrous cycle.

3. Experimental procedures

3.1. Experiment 1

On day 8 of the estrous cycle, ewes were fasted for 12 h before surgery and received 1 cm^3 (0.54 mg) atropine sulfate (Phoenix Pharmaceuticals, Inc., St. Joseph, MO) intramuscularly as a pre-anesthetic. Ewes were laparotomized under pentobarbital sodium anesthesia (Anthony Products Co., Arcadia, CA). A polyvinyl catheter (Cole Parmer, Chicago, IL) was installed in the jugular vein and a cathether was installed intrauterine adjacent to the ovary containing the corpus luteum via a mid-ventral incision and exteriorized through the flank to administer treatments. Treatments were vehicle ($N=5$), DETA (control for DETANONOate; $N=5$; Tocris Bioscience, Ellisville, MO), DETANONOate (long acting NO donor; $N=6$; Tocris Bioscience, Ellisville, MO), L-arginine (NO donor; $N=5$; Sigma Chemical Company, St. Louis, MO), L-NMMA (NOS inhibitor; $N=5$; Tocris Bioscience, Ellisville, MO), or L-NAME (NOS inhibitor; $N=6$; Tocris Bioscience, Ellisville, MO). Samples of jugular venous blood and inferior vena cava plasma from a saphenous vein cathether installed 5 cm anterior to the juncture of the ovarian vein and inferior vena cava blood were collected via heparinized syringes every 6 h from 2400 h on day 8 postestrus through 1800 h on day 18 for analysis for plasma progesterone in jugular venous plasma [74] and for $PGF_2\alpha$ and PGE in inferior vena cava plasma [75], respectively, by RIA. Samples of inferior vena cava blood received 0.1 ml 0.1N HCl per ml of blood collected to inhibit platelet prostanoid production prior to centrifugation to collect plasma until analysis for $PGF_2\alpha$ and PGE [21,22]. The corpus luteum was collected at 1800 h on day 18 and weighed.

3.2. Experiment 2

In a second experiment, day 15 ovine caruncles were collected from the anterior third of the uterine horn adjacent to the luteal-containing ovary for incubation with treatments to determine whether NO affected the conversion of PGE_2 to $PGF_2\alpha$. Caruncles were weighed, diced, and incubated in 3 ml of M199 containing 25 mM Hepes buffer and Earle's salts (Gibco, Grand Island, NY, USA), 0.1% bovine serum albumin (BSA-Fraction V; Sigma Chemical Co., St. Louis, MO, USA), 100 IU/ml penicillin (Sigma Chemical Co., St. Louis, MO, USA), and