Nitric Oxide as a Local Mediator of Prostaglandin $F_{2\alpha}$-Induced Regression in Bovine Corpus Luteum: An *In Vivo* Study

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To test whether nitric oxide (NO) is involved in prostaglandin (PG) $F_{2\alpha}$-induced regression of the bovine corpus luteum (CL) *in vivo*, heifers were treated as follows: Group 1, saline (3 ml/h); Group 2, dinoprost, an analogue of prostaglandin $F_{2\alpha}$ ($aPGF_{2\alpha}$; 5 mg/0.5 h); Group III, Nω-nitro-L-arginine methyl ester (L-NAME; 200 mg/4 h), an inhibitor of nitric oxide synthase; and Group IV, L-NAME (400 mg/4 h) and $aPGF_{2\alpha}$ (5 mg/0.5 h). All treatments were administered by an intraluteal microdialysis system (MDS) on day 15 of the cycle. Perfusate and jugular plasma samples were collected at half-hour intervals; additionally, jugular plasma samples were collected once daily from day 16 to day 21 of the cycle. In the perfusate samples, $aPGF_{2\alpha}$ increased P_4 ($P < 0.05$), PGE_2 ($P < 0.001$), and LTC_4 ($P < 0.05$) concentrations; L-NAME increased P_4 ($P < 0.05$) but did not change PGE_2 and LTC_4 ($P > 0.05$) concentrations as compared with the period before treatment. Simultaneous perfusion of CL with L-NAME and $aPGF_{2\alpha}$ caused a further increase of P_4 concentration ($P < 0.05$) induced by L-NAME or $aPGF_{2\alpha}$ treatment and increased PGE_2 and LTC_4 ($P < 0.001$) concentrations to the level observed after $aPGF_{2\alpha}$ treatment. Perfusion of CL with $aPGF_{2\alpha}$ caused luteal regression within 24 h, while perfusion with L-NAME prolonged the life span of CL to day 21 ($P < 0.05$). Concomitant L-NAME and $aPGF_{2\alpha}$ treatment partially counteracted ($P < 0.05$) the luteal regression caused by $aPGF_{2\alpha}$ administration. These results show that NO is involved in the process of luteolysis in the bovine CL and suggest that the luteolytic effect of $aPGF_{2\alpha}$ may be mediated by NO as an important component of an autocrine/paracrine luteolytic cascade. Exp Biol Med 228:1057–1062, 2003

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The mechanism controlling the development, secretion, and regression of the bovine corpus luteum (CL) involves many factors produced both within and without the CL (1–3). The current concept that luteolysis in cattle is brought about by prostaglandin (PG) $F_{2\alpha}$ of uterine origin secreted in response to oxytocin (OT) released by the CL is inadequate to explain many of the events that actually occur at the time of regression (2). It has been well documented that $PGF_{2\alpha}$ acts as a luteolytic agent when administered parenterally in ruminants (2, 4). However, when added to pure populations of steroidogenic luteal cells, $PGF_{2\alpha}$ does not inhibit basal P_4 secretion by the large luteal cells and actually stimulates P_4 production by the small luteal cells (5, 6) and by a mixture of large and small luteal cells (7, 8). There is very little OT present in the CL at the time of luteal regression, and several recent studies indicate that luteolysis can occur after depletion of luteal OT (9, 10) and in the absence of measurable OT release from the luteal tissue (11). Moreover, the products of the lipoxygenase pathway of the arachidonic acid cascade, particularly leukotriene C_4 (LTC_4) play a role in luteolysis (12). In microdialysis studies leukotriene B_4 and LTC_4 were found in perfusate samples from CL and rose prior to the decline in progesterone during luteolysis in untreated control heifers (11). These results suggest that the effects of parenterally administered $PGF_{2\alpha}$ may be mediated, at least in part, by vascular changes.

A number of studies indicate that substances, produced locally in the bovine CL, may mediate the luteolytic action of $PGF_{2\alpha}$. Pate (13) suggested that the immune cells and their secreted products, cytokines, are involved in the process of luteal regression. At the time of luteolysis, macrophages invade the bovine CL (14) and release cytokines,

especially tumor necrosis factor-α (TNFα) and interferon-γ (IFNγ), which can participate in the apoptotic events that finally lead to structural luteolysis (15–18). In addition to immune cells and cytokines, it has been documented that endothelial cells and their main secreted product, endothelin-1 (ET-1), may mediate the luteolytic action of $PGF_{2\alpha}$ in bovine steroidogenic luteal cells (19, 20). Yet another factor that may mediate $PGF_{2\alpha}$ action during luteolysis in cattle is nitric oxide (NO) (21, 22). We have previously shown that administration of a nitric oxide synthase (NOS) inhibitor (L-NAME) into the ovary during the late luteal phase increases P_4 secretion and prolongs the functional life span of the bovine CL (21). Moreover, NO directly inhibits P_4 secretion from bovine luteal cells *in vitro* (23, 24) and preexposing luteal cells to nitric oxide (NO) increases the effect of $PGF_{2\alpha}$. These results suggest that NO, produced locally in the bovine CL, is needed to complete luteolysis (25). It has been recently shown that local administration (infusion into the *aorta abdominals*) of L-NAME into the reproductive tract counteracts the luteolytic action of $PGF_{2\alpha}$ on the bovine CL (22). Therefore, in the present study we determined whether NO is involved as a local auto/paracrine factor in $PGF_{2\alpha}$-induced regression of the bovine CL.

Materials and Methods

Microdialysis System Implantation *In Vivo*. Normally cycling Holstein/Polish Black and White heifers (380–430 kg; $n = 15$) were injected intramuscularly (i.m.) with 25 mg of dinoprost, an analogue of $PGF_{2\alpha}$ (Dinolytic; Pharmacia & Upjohn, Belgium) during the luteal phase to induce luteolysis and estrus. On day 14 of the subsequent estrous cycle the MDS was implanted into the CL as described by Blair *et al.* (11). The animals were premedicated with xylazine at a dose of 15 to 20 mg/animal i.m. (Xylavet 2%, Scan Vet, Poland) and local anesthesia (epidural, paravertebral, and infiltration at the site of incision), was induced using 2% procaine hydrochloride (Polocainum Hydrochloricum 2%, Biowet Drwalew, Poland) prior to surgery. The MDS was threaded through the CL such that an 8-mm long dialysis tubing (Fresenius SPS 960, Frankfurt, Germany; MW cutoff = 1,000,000 Daltons; o.d. = 500 μm; i.d. = 340 μm) was localized within the CL. After surgery heifers were kept in individual stalls in a temperature-controlled room, and the CL were perfused with Ringer's solution. All perfusions were at a flow rate of 3 ml/h. The recovery rates of hormones across the MDS were measured as previously described (26) and amounted to ≈ 0.1%, 0.3%, and 1% for LTC_4, PGE_2 and P_4, respectively. All animal procedures were approved by the Local Committee of Animal Care and Use (Agreement No. 25/N).

Experimental Procedures. At day 15 of the estrous cycle 15 heifers were divided into 4 groups and treated as follows: Group 1 ($n = 4$), CL were perfused with saline (3 ml/h) for 4 hrs; Group II ($n = 4$), CL were perfused with saline for 4 hrs and beginning at the second hour of treatment, CL were perfused simultaneously for 0.5 h (at a total flow rate of 3 ml/h) with 5 mg of dinoprost ($aPGF_{2\alpha}$); Group III ($n = 3$), CL were perfused with L-NAME (Nω-Nitro-L-Arginine Methyl Ester; Sigma-Aldrich, USA), a NOS inhibitor (50 mg/h) for 4 hrs; Group IV ($n = 3$), CL were perfused with L-NAME (50 mg/h) for 4 hrs and beginning at the second hour of treatment, CL were perfused simultaneously for 0.5 h with 5 mg of $aPGF_{2\alpha}$. Each 4-hr treatment period was preceded and followed by 2- and 4-hr control periods, respectively, during which saline (3 ml/h) was perfused.

Perfusate samples were taken at half-hour intervals during the 10-hr experimental period; simultaneously, blood samples were collected through catheters implanted into the jugular vein. Additional blood samples were collected once daily by jugular venipuncture from day 16 to day 21 of the estrous cycle.

Hormone Determinations. Concentrations of P_4 in perfusate and jugular blood plasma samples were assayed using a direct enzyme immunoassay (EIA), as described by Okuda *et al.* (27). Cross-reactivities of the anti-P_4 serum (donated by Dr. S. Okrasa, University of Warmia and Mazury in Olsztyn, Poland), were determined by comparing the inhibition of binding of P_4-HRP to antiserum. Results were as follows: 100% with P_4, 38.9% with pregnenolone, 11.1% with 17α-hydroxy-progesterone, 9.8% with 17β-estradiol, 1.2% with dihydrotestosterone and testosterone, and less than 0.5% with 11-desoxycortisol, estrone, cortisol, and 4-androsten-3,17-dione. Since the bovine CL secretes very little pregnenolone, no adjustment was made in the data for its cross-reaction. The assay sensitivity was 0.3 ng/ml and intra- and interassay coefficients of variation (CVs) were 6.6% and 8.7%, respectively.

Concentrations of PGE_2 in perfusate samples were determined using a direct EIA, as previously described (23). Cross- reactivities of the anti-PGE_2 serum (donated by Dr. S. Ito, Kansai Medical University, Osaka, Japan), validated by comparing the inhibition of binding of peroxidase-labeled PGE_2 to antiserum, were as follows: PGE_2, 100%; PGE_1, 18%; PGJ_2, 14%; PGA_1, 10%; 15-keto PGE_2, 8.8%; PGB_2, 6.7%; PGA_2, 4.6%; PGD_2, 0.13%; $PGF_{2\alpha}$, 2.8%, and with dinoprost 7.9%. The assay sensitivity was 6.9 pg/ml and intra- and interassay CVs were 5.9% and 9.6%, respectively. The total concentrations of PGE_2 (without adjustment for dinoprost cross-reactivity) are presented.

Concentrations of LTC_4 in perfusate samples were determined by use of a commercially available EIA kit (Cayman Chemical Co., USA) according to instructions of the manufacturer. The assay sensitivity was 9.81 pg/ml and intra- and interassay CVs were 5.4% and 9.2%, respectively.

Statistical Analyses. The analysis of P_4, PGE_2, and LTC_4 in the perfusate and P_4 in the jugular plasma samples, collected during administration of the tested substances on day 15 of the cycle and P_4 in jugular plasma samples, collected daily from day 16 to day 21 of the estrous cycle, was performed using a repeated measure design approach with

L-NAME, $aPGF_{2\alpha}$, and time (hours or days, respectively) being fixed effects with all interactions included. Time was considered a repeated factor. The dependencies on time were modeled by an autoregressive type I covariance structure. The particular hypotheses were tested as slice effects in a corresponding mixed model. All analyses were done using SAS Version 9.0; $P < 0.05$ was considered statistically significant.

Results

P_4 Concentrations. Concentrations of P_4 in the jugular plasma after perfusion of CL with saline (Group I) and L-NAME (Group III) were higher ($P < 0.05$) compared with both Groups receiving $aPGF_{2\alpha}$ (Fig. 1). Moreover, an increase in P_4 concentrations was observed in Group III when compared with the period before treatment (Fig. 1). Perfusion of CL with $aPGF_{2\alpha}$ (Group II) and L-NAME with $aPGF_{2\alpha}$ (Group IV) caused significant decreases ($P < 0.05$) in P_4 concentrations in the jugular plasma when compared with concentrations of this hormone to the period before treatment (Fig. 1). However, the decrease in P_4 concentrations after $aPGF_{2\alpha}$ administration was preceded by a brief increase (Fig. 1).

In the perfusate samples, concentrations of P_4 were significantly increased ($P < 0.05$) by $aPGF_{2\alpha}$ as well as by administration of L-NAME when compared with the periods before treatment (Fig. 2). Moreover, P_4 concentrations after $aPGF_{2\alpha}$ treatment were significantly higher ($P < 0.05$) when compared with the concentrations of this hormone after saline administration (Fig. 2). Simultaneous perfusion of CL with L-NAME and $aPGF_{2\alpha}$ caused a further significant increase ($P < 0.05$) in P_4 concentrations compared with $aPGF_{2\alpha}$ or L-NAME treatment (Fig. 2).

For P_4 concentrations the three-way interactions were measured between place of sample collection, L-NAME,

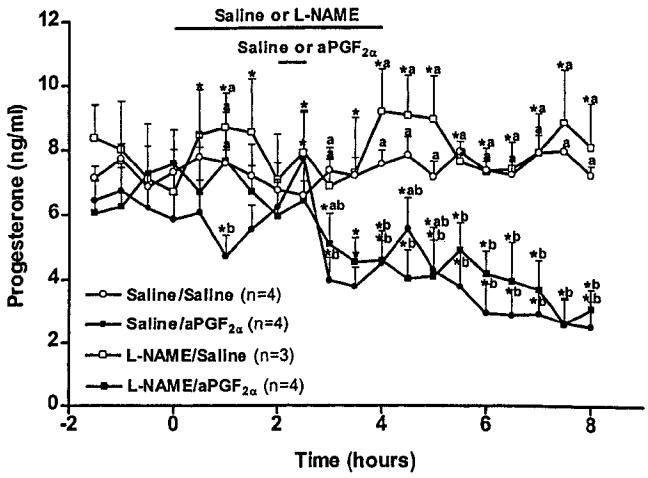

Figure 1. Concentrations (mean ± SEM) of progesterone in jugular plasma samples after simultaneous intraluteal administration of saline (4 h) or L-NAME (200 mg/4 h) and saline (0.5 h) or dinoprost (5 mg; $aPGF_{2\alpha}$) on day 15 of the estrous cycle. * $P < 0.05$ vs. period before treatment; $^{a-b}$different subscripts indicate differences ($P < 0.05$) between groups at the same time point.

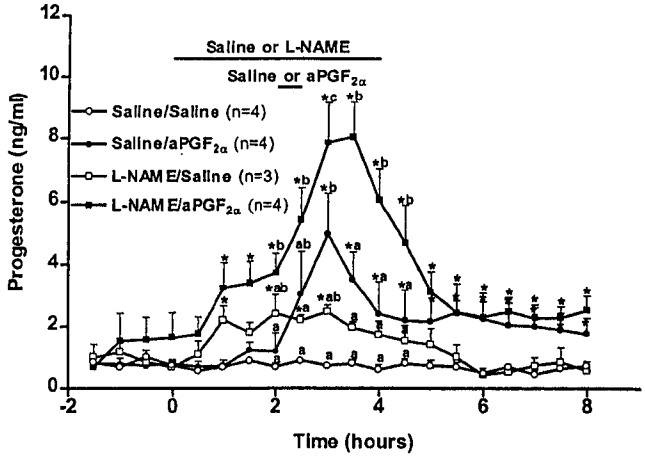

Figure 2. Concentrations (mean ± SEM) of progesterone in perfusate samples after simultaneous intraluteal administration of saline or dinoprost (5 mg), a $PGF_{2\alpha}$ analog, for 0.5 h during intraluteal perfusion (4 h) of either saline or L-NAME (200 mg), an inhibitor of nitric oxide synthase, on day 15 of the estrous cycle. *$P < 0.05$ vs. period before treatment; $^{a-c}$different subscripts indicate differences ($P < 0.05$) between groups at the same time point.

and $aPGF_{2\alpha}$ treatment ($P < 0.05$) as well as for place of sample collection, L-NAME, and time of sample collection ($P < 0.01$). Two-way interactions were observed between place of blood collection and treatment with $aPGF_{2\alpha}$ ($P < 0.01$) and between place of sample collection and time of sample collection ($P < 0.01$) as well as treatment with $aPGF_{2\alpha}$ and time of sample collection ($P < 0.01$).

There were no significant changes ($P > 0.05$) in P_4 concentrations in jugular plasma samples collected daily during days 14 to 21 of the estrous cycle in the animals treated simultaneously with L-NAME and $aPGF_{2\alpha}$ when compared with saline administration (Fig. 3). After administration of $aPGF_{2\alpha}$ the average concentration of P_4 decreased in all heifers to less than 20% of baseline (day 14) at day 16 of the cycle and remained significantly ($P < 0.05$) lower until day 17 or 18 when compared with Group IV (simultaneous L-NAME and $aPGF_{2\alpha}$ treatment) or Group I (saline), respectively (Fig. 3). The concentrations of P_4 after CL perfusion with L-NAME remained high until day 21 of the cycle and were significantly higher ($P < 0.05$) than in heifers treated with saline, $aPGF_{2\alpha}$, and L-NAME and $aPGF_{2\alpha}$ on days 18 to 21, 16 to 21, and 17 to 21, respectively (Fig. 3).

PGE_2 Concentrations. Perfusion of CL with saline or L-NAME did not affect ($P > 0.05$) PGE_2 concentrations in the perfusate samples compared with the periods before treatment (Fig. 4). Perfusion of CL with $aPGF_{2\alpha}$ or with L-NAME and $aPGF_{2\alpha}$ increased ($P < 0.001$) PGE_2 concentrations as compared with the periods before treatment as well as with PGE_2 concentrations after saline (Group I) and L-NAME (Group III) treatment (Fig. 4).

For PGE_2 concentrations, a two-way interaction was found only between treatment with $aPGF_{2\alpha}$ and time of sample collection ($P < 0.001$).

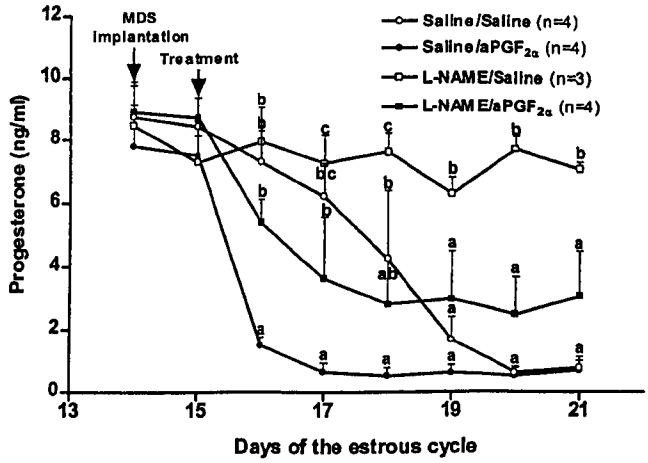

Figure 3. Concentrations (mean ± SEM) of progesterone in jugular plasma samples (from day 14–21) after intraluteal administration of saline or dinoprost (5 mg), a $PGF_{2\alpha}$ analog, for 0.5 h during intraluteal perfusion (4 h) of either saline or L-NAME (200 mg), an inhibitor of nitric oxide synthase, on day 15 of the estrous cycle. [a–c]Different subscripts indicate differences ($P < 0.05$) between groups at the same day.

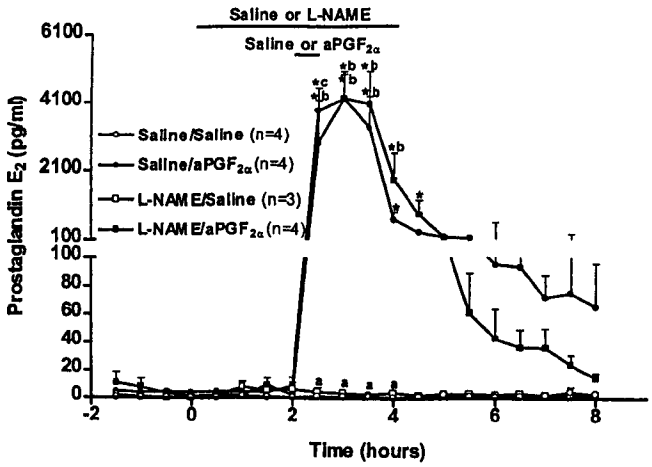

Figure 4. Concentrations (mean ± SEM) of prostaglandin E_2 in perfusate samples after intraluteal administration of saline or dinoprost (5 mg), a $PGF_{2\alpha}$ analog, for 0.5 h during intraluteal perfusion (4 h) of either saline or L-NAME (200 mg), an inhibitor of nitric oxide synthase, on day 15 of the estrous cycle. * $P < 0.05$ vs. period before treatment; [a–c]different subscripts indicate differences ($P < 0.05$) between groups at the same time point.

LTC_4 Concentrations. Perfusion of CL with saline or L-NAME had no effect ($P > 0.05$) on LTC_4 concentrations in perfusate samples as compared with the period before treatment (Fig. 5). LTC_4 concentrations were significantly ($P < 0.05$) increased by treatment with $aPGF_{2\alpha}$ and concomitant L-NAME and $aPGF_{2\alpha}$ administration as compared with the periods before treatment or with saline (Group I) and L-NAME (Group III) administration (Fig. 5).

For LTC_4 concentrations two-way interactions were measured between L-NAME treatment and time of sample collection ($P < 0.05$) and between $aPGF_{2\alpha}$ treatment and time of sample collection ($P < 0.001$). A three-way interaction was found between L-NAME and $aPGF_{2\alpha}$ treatment and time of sample collection ($P < 0.01$).

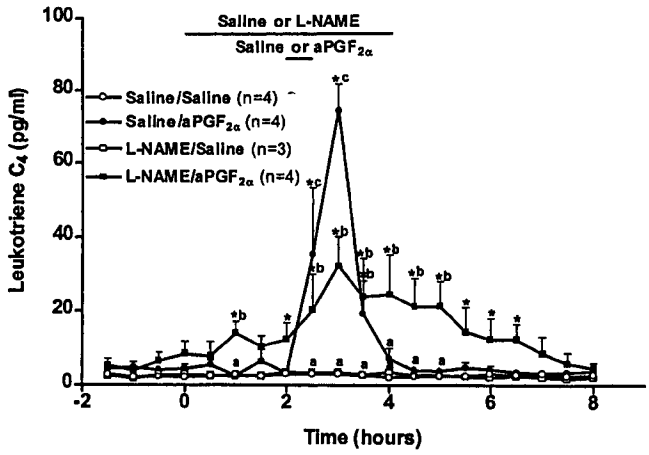

Figure 5. Concentrations (mean ± SEM) of leukotriene C_4 in perfusate samples after intraluteal administration of saline or dinoprost (5 mg), a $PGF_{2\alpha}$ analog, for 0.5 h during intraluteal perfusion (4 h) of either saline or L-NAME (200 mg), an inhibitor of nitric oxide synthase, on day 15 of the estrous cycle. * $P < 0.05$ vs. period before treatment; [a–c]different subscripts indicate differences ($P < 0.05$) between groups at the same time point.

Discussion

Our study showed that intraluteal administration of $aPGF_{2\alpha}$ caused a decrease of P_4 concentrations in peripheral blood samples to less than 20% of baseline after 24 h, a result similar to that reported after intramuscular injection (28, 29). Despite the fact that $aPGF_{2\alpha}$ administered by MDS reduced plasma P_4 concentration within 24 h, it caused a brief temporary increase in P_4 concentration. A similar brief increase in P_4 concentration after $aPGF_{2\alpha}$ treatment was also observed in microdialysed bovine CL *in vitro* (30) and in peripheral blood plasma samples after $aPGF_{2\alpha}$ administration into the aorta abdominalis (4, 22). These results, obtained from different experimental models, clearly indicate that the final decrease in P_4 concentration observed after $PGF_{2\alpha}$-induced luteolysis is preceded by a transient increase. The mechanism responsible for these changes is not fully understood, but the transient increase in P_4 secretion may result from a direct influence of $PGF_{2\alpha}$ on steroidogenic activity of luteal cells, particularly the small luteal cells. This phenomenon may explain the stimulatory effect of $PGF_{2\alpha}$ on P_4 production by bovine steroidogenic luteal cells *in vitro* reported by many authors (5, 7, 24, 31). The final decrease in P_4 secretion after intraluteal administration of a $PGF_{2\alpha}$ may be a consequence of changes in intraluteal blood flow (28) caused by disruption of the normal balance of vasodilatory (NO) and vasoconstrictive (endothelin) activities.

Perfusion of CL with L-NAME increased P_4 concentrations in the perfusate samples and maintained concentrations of this hormone in peripheral blood at a high level until day 21 of the cycle. This result is in agreement with our previous study showing that spontaneous luteolysis was inhibited by L-NAME administered by MDS on days 17 and 18 of the cycle (21). In the present study we also

showed that intraluteal administration of $aPGF_{2\alpha}$ during L-NAME perfusion intensified the L-NAME-induced P_4 secretion by the CL and that the luteolytic action of $aPGF_{2\alpha}$ was partially counteracted by L-NAME. Collectively, these results suggest that NO plays a role as an auto/paracrine factor in regulation of luteal steroidogenesis, and that it may mediate $PGF_{2\alpha}$ action during luteolysis. It has been shown that a number of NO donors negatively affect P_4 secretion, while NOS inhibitors stimulate P_4 production by dispersed bovine luteal cells (20, 24), confirming the role of NO as a regulator of steroidogenesis.

Our present study indicates that the luteolytic action of $aPGF_{2\alpha}$ was partially counteracted by L-NAME. An evident blockade of $PGF_{2\alpha}$ action was also observed when an analog of $aPGF_{2\alpha}$ (cloprostenol) and L-NAME were administered into the aorta abdominalis (22). $PGF_{2\alpha}$ is most effective as a luteolytic factor when it reaches the CL through blood vessels (32). We suggest that the discrepancy in the potency of L-NAME in inhibiting $aPGF_{2\alpha}$-induced luteolysis observed in our two different experimental procedures (local versus systemic application of L-NAME) are a consequence of the fact that the $PGF_{2\alpha}$ reached the luteal tissue by way of blood vessels after systemic administration.

Nitric oxide is a potent vasodilator and increases blood flow (33). Acosta *et al.* (20) showed that blood flow within the bovine CL initially increased at 0.5 to 2 h, decreased at 4 h to the level observed at 0 h and then decreased to a lower level beginning at 8 h after $aPGF_{2\alpha}$ treatment. In our previous study (22), plasma concentrations of nitrite/nitrate were also elevated during the first 2 hr after $aPGF_{2\alpha}$ injection; after that they slowly decreased, indicating that the factor responsible for the increase in blood flow after $aPGF2_{\alpha}$ treatment is NO. Therefore, the inhibition of luteolysis observed after L-NAME treatment that reduces NO release may also be a consequence of the changes in the blood flow within the bovine reproductive tract. Moreover, Lopez Collazo *et al.* (34) showed that $[Ca^{2+}]_i$-mobilizing agents and cytokines elicit an apoptotic response in the vascular endothelial cells through mechanisms that require NO synthesis. The expression of inducible NOS is correlated with cytotoxic/cytostatic events and results in a sustained synthesis of NO, which in turn induces apoptotic cell death (35). Recently we showed that spermine NONOate, an NO donor, strongly reduced viability of the bovine luteal cells, while L-NAME had opposite effects (Korzekwa A, Okuda K, Jaroszewski JJ, Skarzynski DJ, unpublished data). Therefore, the administration of L-NAME may also protect against apoptosis induced by $PGF_{2\alpha}$ during luteolysis.

It was recently shown that intraluteal administration of a NO donor *in vivo* strongly stimulated both LTC_4 and $PGF_{2\alpha}$ (36), two factors involved in the process of luteal regression in bovine CL (2, 12). It has been shown in our previous study that another analogue of $PGF_{2\alpha}$ (cloprostenol) strongly stimulated output of two luteolytic factors: $PGF_{2\alpha}$ (4, 22) and NO (22) in the bovine reproductive tract. Our present study showed that $aPGF_{2\alpha}$ increased secretion of another luteolytic factor—LTC_4. Thus, $PGF_{2\alpha}$-induced regression of the CL is a very complex process, which involves not only an utero-ovarian $PGF_{2\alpha}$ auto-amplification loop (4, 29), but also several paracrine mechanisms including immune (6, 11, 13, 16, 17) and vascular factors (6, 19, 20, 22, 28). Indeed, in our present study $aPGF_{2\alpha}$ also increased concentrations of luteotropic PGE_2. Both NO and PGE_2 are potent vasodilators and increase blood flow. Thus, our previous (22) and present findings can explain the recent data of Acosta *et al.* (20) showing that blood flow in the bovine CL is increased just after $PGF_{2\alpha}$ treatment, not decreased as is commonly assumed. During the first 2 hr of $PGF_{2\alpha}$ action, NO may act in concert with PGE_2 to relax vascular smooth muscle cells and maintain the luteal blood flow required for invasion of immune cells into the CL during luteolysis (14, 16, 17).

In summary, our previous data showed that that NO is produced locally in the bovine CL with the highest production occurring during the late luteal phase (22) and that NO directly inhibits P_4 secretion in cattle (23, 24). The increased effects of $PGF_{2\alpha}$ on luteal cells previously exposed to NO suggest that priming of bovine CL by NO is needed for complete luteal regression (25). This suggestion is confirmed by our previous (21) and present data showing that an inhibition of ovarian NO production by perfusion of the CL with an NOS inhibitor prolonged the functional life span of CL. Moreover, the luteolytic action of $aPGF_{2\alpha}$ is inhibited (22) or partially prevented, as shown in the present study, by administration of an NOS inhibitor. Therefore, NO appears to play an important role in both functional and structural luteolysis, acting as a component of an autocrine/paracrine luteolytic cascade induced by $PGF_{2\alpha}$.

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1. Davis JS, May JV, Keel BA. Mechanisms of hormone and growth factor action in the bovine corpus luteum. Theriogenology **45**:1351–1380, 1996.
2. Hansel W, Blair R. Bovine corpus luteum: A historic overview and implications for future research. Theriogenology **45**:1267–1294, 1996.
3. Meidan R, Aberdam E, Aflalo L. Steroidogenic enzyme content and progesterone induction by cyclic adenosine 3′,5′-monophosphate-generating agents and prostaglandin $F_{2\alpha}$ in bovine theca and granulosa cells luteinized in vitro. Biol Reprod **46**:786–792, 1992.
4. Skarzynski DJ, Bogacki M, Kotwica J. Changes in ovarian oxytocin secretion as an indicator of corpus luteum response to prostaglandin $F_{2\alpha}$ treatment in cattle. Theriogenology **48**:733–742, 1997.
5. Alila HW, Dowd JP, Corradino RA, Harris WV, Hansel W. Control of progesterone production in small and large bovine luteal cells separated by flow cytometry. J Reprod Fertil **82**:645–655, 1988.
6. Meidan R, Milvae RA, Weiss S, Levy N, Friedman A. Intraovarian regulation of luteolysis. J Reprod Fertil **54**(Suppl):217–228, 1999.
7. Okuda K, Uenoyama Y, Lee KW, Sakamoto R, Skarzynski DJ. Pro-

gesterone stimulation by prostaglandin $F_{2\alpha}$ involves the protein kinase C pathway in cultured bovine luteal cells. J Reprod Dev **44**:79–84, 1998.
8. Skarzynski DJ, Okuda K. Sensitivity of bovine corpora lutea to prostaglandin $F_{2\alpha}$ is dependent on progesterone, oxytocin and prostaglandins. Biol Reprod **60**:1292–1298, 1999.
9. Skarzynski D, Kotwica J. Influence of oxytocin removal from the corpus luteum on secretory function and duration of the oestrous cycle in cattle. J Reprod Fertil **97**:411–417, 1993.
10. Jaroszewski J, Kotwica J. Reduction of ovarian oxytocin content from early luteal phase does not affect the corpus luteum secretory function in cattle. Reprod Nutr Dev **34**:175–182, 1994.
11. Blair RM, Saatman R, Liou SS, Fortune JE, Hansel W. Roles of leukotrienes in bovine corpus luteum regression: an in vivo microdialysis study. Proc Soc Exp Biol Med **216**:72–80, 1997.
12. Milvae RA, Alila HW, Hansel W. Involvement of lipoxygenase products of arachidonic acid metabolism in bovine luteal function. Biol Reprod **35**:1210–1215, 1986.
13. Pate JL. Involvement of immune cells in regulation of ovarian function. J Reprod Fertil **49**(Suppl):365–377, 1995.
14. Bauer M, Reibiger I, Spanel-Borowski K. Leucocyte proliferation in the bovine corpus luteum. Reproduction **121**:297–305, 2001.
15. Friedman A, Weiss S, Levy N, Meidan R. Role of tumor necrosis factor-α and its type-1 receptor in luteal regression: induction of programmed cell death in bovine corpus luteum-derived endothelial cells. Biol Reprod **63**:1905–1915, 2000.
16. Pate JL, Landis Keyes P. Immune cells in the corpus luteum: friends or foes. Reproduction **122**:665–676, 2001.
17. Petroff MG, Petroff BK, Pate JL. Mechanisms of cytokine-induced death of cultured bovine luteal cells. Reproduction **121**:753–760, 2001.
18. Taniguchi H, Yokomizo Y, Okuda K. Fas-Fas ligand system mediates luteal cell death in bovine corpus luteum. Biol Reprod **66**:754–759, 2002.
19. Levy N, Kobayashi S, Roth Z, Wolfenson D, Miyamoto A, Meidan R. Administration of prostaglandin $F_{2\alpha}$ during the early bovine luteal phase does not alter the expression of ET-1 and of its type A receptor: a possible cause for corpus luteum refractoriness. Biol Reprod **63**:377–382, 2000.
20. Acosta TJ, Yoshizawa N, Ohtani M, Miyamoto A. Local changes in blood flow within the early and midcycle corpus luteum after prostaglandin $F_{2\alpha}$ injection in the cow. Biol Reprod **66**:651–658, 2002.
21. Jaroszewski JJ, Hansel W. Intraluteal administration of a nitric oxide synthase blocker stimulates progesterone and oxytocin secretion and prolongs the life span of the bovine corpus luteum. Proc Soc Exp Biol Med **224**:50–55, 2000.
22. Skarzynski DJ, Jaroszewski JJ, Bah MM, Deptula KM, Barszczewska B, Gawronska B, Hansel W. Administration of a nitric oxide synthase inhibitor counteracts prostaglandin $F_{2\alpha}$-induced luteolysis in cattle. Biol Reprod **68**:1674–1681, 2003.
23. Skarzynski DJ, Okuda K. Different actions of noradrenaline and nitric oxide on the output of prostaglandins and progesterone in cultured bovine luteal cells. Prostaglandins Other Lipid Mediat **60**:35–47, 2000.
24. Jaroszewski JJ, Bogacki M, Skarzynski DJ. Progesterone production in bovine luteal cells treated with drugs that modulate nitric oxide production. Reproduction **125**:389–395, 2003.
25. Skarzynski DJ, Kobayashi S, Okuda K. Influence of nitric oxide and noradrenaline on prostaglandin $F_{2\alpha}$-induced oxytocin secretion and intracellular calcium mobilization in cultured bovine luteal cells. Biol Reprod **63**:1000–1005, 2000.
26. Jarry H, Einspanier A, Kanngiesser L, Dietrich M, Pitzel L, Holtz W, Wuttke W. Release and effects of oxytocin on estradiol and progesterone secretion in porcine corpora lutea as measured by an in vivo microdialysis system. Endocrinology **126**:2350–2358, 1990.
27. Okuda K, Uenoyama Y, Fujita Y, Iga K, Sakamoto K, Kimura T. Functional oxytocin receptors in bovine granulosa cells. Biol Reprod **56**:625–631, 1997.
28. Ohtani M, Kobayashi S, Miyamoto A, Hayashi K, Fukui Y. Real-time relationships between intraluteal and plasma concentrations of endothelin, oxytocin, and progesterone during prostaglandin $F_{2\alpha}$-induced luteolysis in the cow. Biol Reprod **58**:103–108, 1998.
29. Tsai SJ, Wiltbank MC. Prostaglandin $F_{2\alpha}$ regulates distinct physiological changes in early and mid-cycle bovine corpora lutea. Biol Reprod **58**:346–352, 1998.
30. Miyamoto A, Kobayashi S, Arata S, Ohtani M, Fukui Y, Schams D. Prostaglandin $F_{2\alpha}$ promotes the inhibitory action of endothelin-1 on the bovine luteal function in vitro. J Endocrinol **152**:R7–R11, 1997.
31. Girsh E, Greber Y, Meidan R. Luteotrophic and luteolytic interactions between bovine small and large luteal-like cells and endothelial cells. Biol Reprod **52**:954–962, 1995.
32. Hayashi K, Miyamoto A. Angiotensin II interacts with prostaglandin $F_{2\alpha}$ and endothelin-1 as a local luteolytic factor in the bovine luteum in vitro. Biol Reprod **60**:1104–1109, 1999.
33. Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev **43**:109–142, 1991.
34. Lopez-Collazo E, Mateo J, Miras-Portugal MT, Bosca L. Requirement of nitric oxide and calcium mobilization for the induction of apoptosis in adrenal vascular endothelial cells. FEBS Lett **413**:124–128, 1997.
35. Rosselli M, Keller PJ, Dubey RK. Role of nitric oxide in the biology, physiology and pathophysiology of reproduction. Hum Reprod Update **4**:3–24, 1998.
36. Bogacki M, Jaroszewski JJ, Skarzynski DJ, Hansel W. The influence of nitric oxide on cyclooxygenase and lipoxygenase products of arachidonic acid metabolism in the bovine corpus luteum in vivo. Biol Reprod **66**(Suppl 1):325, 2002.