Effect of Relaxin on Parturition in Ruminants

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Lloyd L. Anderson, professor of animal science

Summary

The biology of relaxin differs in many respects between ruminants and nonruminants. Immunoreactive blood concentration of circulating relaxin is much less in ruminant (cattle and sheep) than in nonruminant (pigs) farm animals. The ovaries of the pig produce abundant quantities of the hormone in late pregnancy, whereas tissue sources of relaxin are not clearly defined in sheep and cattle. Relaxin facilitates parturition by cervical dilation and pelvic canal expansion in several mammalian species. Relaxin injected intramuscularly during late pregnancy can cause earlier parturition in cattle, but in sheep limited evidence indicates it does not induce earlier delivery than seen in diluent-treated controls. Intravenous infusion of increasing dosages of relaxin in beef heifers the last days of pregnancy decreased plasma progesterone concentration compared with phosphate buffer controls, but oxytocin plasma concentrations remained similar throughout the posttreatment period. Although continuous intravenous infusion of relaxin depressed blood levels of progesterone, it did not result in earlier parturition than seen in the diluent treated controls. Thus, the timing and method of relaxin administration during late pregnancy in ruminants affect remodelling of collagen and pelvic canal relaxation and can result in earlier parturition.

Introduction

Induction of parturition with prostaglandin F_2a (PGF_2a), glucocorticoids, or their analogs often results in a high incidence of difficult birth (dystocia), fetal membrane retention, and reduced fertility. Dystocia is related to insufficient dilation of the birth canal, insufficient pelvic area, and calf or lamb size at birth. Relaxin is a peptide hormone with partial structural homology to insulin. It is produced in abundant quantities in the ovaries of late pregnant pigs, and thus provides a source of relaxin for studies on reproduction in other farm animal species. Although the pig is a rich source of this hormone, only a single copy of the relaxin-like gene corresponds to part of the relaxin A-chain in sheep, and there is little evidence to support biosynthesis of the hormone in cattle. Relaxin remodels connective tissue by inducing cervical dilation, pelvic relaxation, and separation of interpubic ligaments in several mammalian species. It also inhibits smooth muscle contraction of the uterus, but in contrast, increases atrial contractions in the heart. Thus, this hormone may affect important metabolic functions in addition to its actions on the reproductive system in late pregnancy. RU 486 is a 19-norsteroid with high affinity for progesterone receptor. It has strong antiglucocorticoid activities. RU 486 is an antagonist that renders progesterone biologically inactive. Our results on the effects of relaxin alone or in combination with an antiprogesterone administered to cattle and sheep during late pregnancy on parturition are summarized.

Materials and Methods

Animals

Primiparous crossbred beef heifers at the Rhodes cattle research farm, and Holstein dairy heifers at the dairy farm, Iowa State University, approaching their first calving were bred by artificial insemination at estrus (day 0). Gestation averages 283 days in these herds. Suffolk and Hampshire breeds of sheep were bred and maintained at the Iowa State University Sheep Teaching Farm. Ewes were naturally mated at estrus (day 0). The average duration of gestation in this flock is 150 days.

Relaxin (3,000 units per milligram) for these experiments was extracted from the ovaries of pregnant pigs and purified according to procedures described by Christian Schwabe, Medical University of South Carolina, Charleston. For some experiments, the animals were fitted with an indwelling cannula in the jugular vein for repeat blood sampling. Blood samples (10 milliliters) were collected in borosilicate culture tubes and maintained on ice. Tubes were centrifuged at 2,000

x **g** for 10 minutes and plasma harvested, frozen on dry ice and stored at -20°C until required for radioimmunoassay of progesterone, relaxin, oxytocin and RU 486.

The cattle and sheep were monitored continuously for signs of imminent parturition. Duration of gestation, exact time of calving or lambing, degree of difficulty of birth (dystocia), time of placenta delivery, sex and birth weight of calves and lambs were recorded.

In cattle, relaxin was administered intramuscularly or infused intravenously continuously beginning six days before expected parturition. Phosphate buffer saline (PBS) was given by these same routes and periods of treatment in the controls. In sheep, relaxin was injected intramuscularly at noon on day 144; PBS was given to the control group at the same time. The antiprogesterone, RU 486, was administered intramuscularly (2 mg/kg body weight in cattle; 4 mg/kg body weight in sheep) alone or in combination with relaxin. Diluent treatment consisted of 3 ml ethanol injected intramuscularly.

Radioimmunoassay of Hormones

Radioimmunoassay of hormones was carried out in our laboratory with fully validated assays by our previously published procedures. Plasma volume ranged from 50 to 100 microliters for these hormone assays, and all assays were carried out in duplicate. For oxytocin, progesterone and RU 486, the plasma samples were solvent extracted before radioimmunoassay. Assay sensitivity was .25 nanogram per milliliter for progesterone, 40 picograms per milliliter for relaxin, .01 picograms per tube for oxytocin, and 10 picograms per tube for RU 486.

Statistical Analysis

Results are expressed as mean ± standard error (SE). Experimental units in the study were the individual animals randomly assigned to treatments. Hormone data were analyzed by split-plot design using both the general linear model and Student's *t*-test for comparisons between treatment groups.

Results and Discussion

The profile of immunoreactive relaxin blood concentration in nonruminants (pigs) remains low during most of pregnancy, but it increases the last days of pregnancy and reaches peak levels (i.e., 60-90 nanogram per milliliter) a few hours before parturition. In ruminants (cattle and sheep) relaxin immunoreactivity is low, usually less than .20 nanogram per milliliter (i.e., 200 picograms per milliliter) a week before parturition. In cattle, it increases to peak concentrations of >800 picograms per milliliter on the day of parturition. In sheep, relaxin plasma concentrations increase with advancing gestation, from an average of .60 nanogram per milliliter reaching a peak of 3.90 nanogram per milliliter on day 146 (four days before parturition). At parturition however, relaxin concentration averaged only .80 nanogram per milliliter. Thus, an antepartum relaxin surge occurs in late pregnant ewes, but the modest amplitude of the relaxin surge differs greatly from that seen in nonruminants, and it occurs earlier in sheep than pigs. There is no evidence of an endogenous transient prepartum relaxin peak in cattle.

Administration of porcine relaxin (RLX) intramuscularly to beef heifers five days before expected term causes a transient, but significant ($p < .05$), decrease in plasma progesterone concentration within 90 minutes compared with PBS-treated controls (Table 1). In late pregnant ewes, relaxin injected intramuscularly on days 144 and 145 resulted in significantly lower plasma concentrations of progesterone, but did not cause significantly earlier parturition than seen in the PBS-treated controls. During the pretreatment period in these ewes, plasma progesterone averaged 11 ± .6 nanogram per milliliter, but relaxin treatment reduced ($p < .05$) progesterone to 8 ± .4 nanogram per milliliter. Continuous infusion of high levels of porcine relaxin (200-700 units per hour) beginning day 277 of pregnancy in beef heifers significantly reduced circulating plasma progesterone concentration, but did not result in earlier parturition compared with PBS-infused controls.

RU 486 treatment in cattle on days 277 and 278 of pregnancy significantly decreased ($p < .05$) progesterone concentration compared with diluent-treated controls. Likewise in sheep, RU 486 injected on days 144 and 145 of pregnancy abruptly decreased ($p < .05$) circulating progesterone concentration compared with diluent-treated controls. Progesterone concentration in

peripheral plasma decreased by 30 hours after RU 486 treatment in cattle and sheep. At induced parturition progesterone concentration was similar to the diluent-treated controls (2.0 nanogram per milliliter in cattle; 4.0 nanogram per milliliter in sheep). Thus, a significant decrease in circulating progesterone concentration must be obtained in these species to result in hormone-induced or normal parturition. The second injection of RU 486 24 hours later in cattle and sheep assured a rapid and consistent decrease in circulating progesterone.

The concentration of relaxin and RU 486 was monitored in peripheral blood plasma of these hormone treated cattle and sheep during late pregnancy (Figures 1 and 2). In cattle, plasma concentrations of RU 486 increased abruptly within 15 minutes after first RU 486 injection on day 277, peaked at 7.2 nanogram per milliliter by 45 minutes, and were maintained at 5.5 nanogram per milliliter throughout the 24-hour period. On day 278, the second RU 486 treatment further increased RU 486 to 14.3 nanogram per milliliter within 30 minutes. RU 486 averaged 7.9 nanogram per milliliter on the day of induced calving (day 279), a time when progesterone was at basal concentration. Relaxin increased to a peak concentration of 4.1 nanogram per milliliter within 60 minutes after relaxin injection, and decreased to basal levels of less than .5 nanogram per milliliter by 18 hours. Relaxin treatment on the second day of RU 486 treatment in cattle did not result in a further shortening of the interval to calving compared with RU 486 treatment alone. In multiparous cows there was no incidence of dystocia at induced parturition. In primiparous heifers the incidence of dystocia was significantly reduced by RU 486 treatment compared with diluent-treated controls, but difficult calving was not completely eliminated by this hormone treatment.

The mechanism by which relaxin promotes cervical dilation is still poorly understood. Relaxin acts on collagen of the cervix and pelvis and causes relaxation by inducing the disintegration of the collagen matrix. Several studies have indicated that relaxin acts directly on cervical tissue as indicated by high affinity binding of the hormone, increased cyclic adenylyl cyclase, and decreased proline incorporation. The results of our studies were interpreted to indicate that relaxin promotes cervical dilation, probably by remodeling connective tissue collagen during late pregnancy.

Implications

Relaxin treatment of late pregnant cows and heifers causes cervical softening and dilation probably by remodeling collagen in cervical tissues. Relaxin treatments were given during the spring calving season, and there were variable results in the timing of delivery when comparing one season to the next. Limited results indicated that relaxin treatment of ewes did not affect the time of lambing. RU 486 treatment of late pregnant cattle and sheep precisely controlled the time to induced parturition, but did not eliminate dystocia in primiparous heifers.

Table 1. Effect of relaxin (RLX) on pregnancy and parturition in ruminants.

	Treatment	Number of animal	Hours from first treatment to parturition
Beef cattle[b]	PBS	16	127 ± 22
	3,000 U RLX	14	60 ± 19**
	2 x 3,000 U RLX	17	48 ± 28*
Beef heifers[d] (Spring 1992)	PBS	16	146 ± 12
	2 x 5,000 U RLX	16	80 ± 13**
Beef heifers[d] (Spring 1993)	PBS	22	75 ± 11
	2 x 5,000 U RLX	24	74 ± 13
Beef cows	Diluent	6	210 ± 57
	RU 486 (2 mg/kg BW)	6	55 ± 3**
	RU 486 + 3,000 U RLX	6	53 ± 11**
Beef heifers	Diluent	7	182 ± 38
	RU 486 (2 mg/kg BW)	7	43 ± 7**
	RU 486 + 3,000 U RLX	7	52 ± 6**
Dairy heifers[a]	PBS	7	125 ± 34
	3,000 U RLX	7	80± 19**
	2 x 3,000 U RLX	7	64 ± 17**
Sheep[c]	Diluent	8	121 ± 27
	3,000 U RLX	9	109 ± 23
	RU 486 (4 mg/kg BW)	5	31 ± 2*

[a] Injection started on day 276 of gestation.
[b] Treatment started on day 278 of gestation.
[c] Injection started on day 144 of gestation.
[d] Treatment started on day 277 of gestation.
Values are mean ± SE.
*$p < .05$.
**$p < .01$.

Figure 1. (A) Peripheral plasma concentrations of RU 486 during the pretreatment period (°; n = 18) and after intramuscular injection of RU 486 (2 mg/kg BW) at 0800 hours on day 277 and again on day 278 (n; n = 12) compared with those in diluent-treated controls (Ÿ; n = 6). (B) Peripheral plasma concentrations of relaxin during the pretreatment period (°; n = 18), and after subcutaneous injection of relaxin (3,000 units) at 0800 hours on day 278 (n; n = 6), compared with those in diluent-treated controls (Ÿ; n = 12). Blood was collected at 15-, 30-, and 60-min intervals immediately after hormone or placebo treatment and at 2000 and 2400 hours those same days. The times of hormone and diluent treatments are indicated by *open arrows*. Values are the mean ± SE.

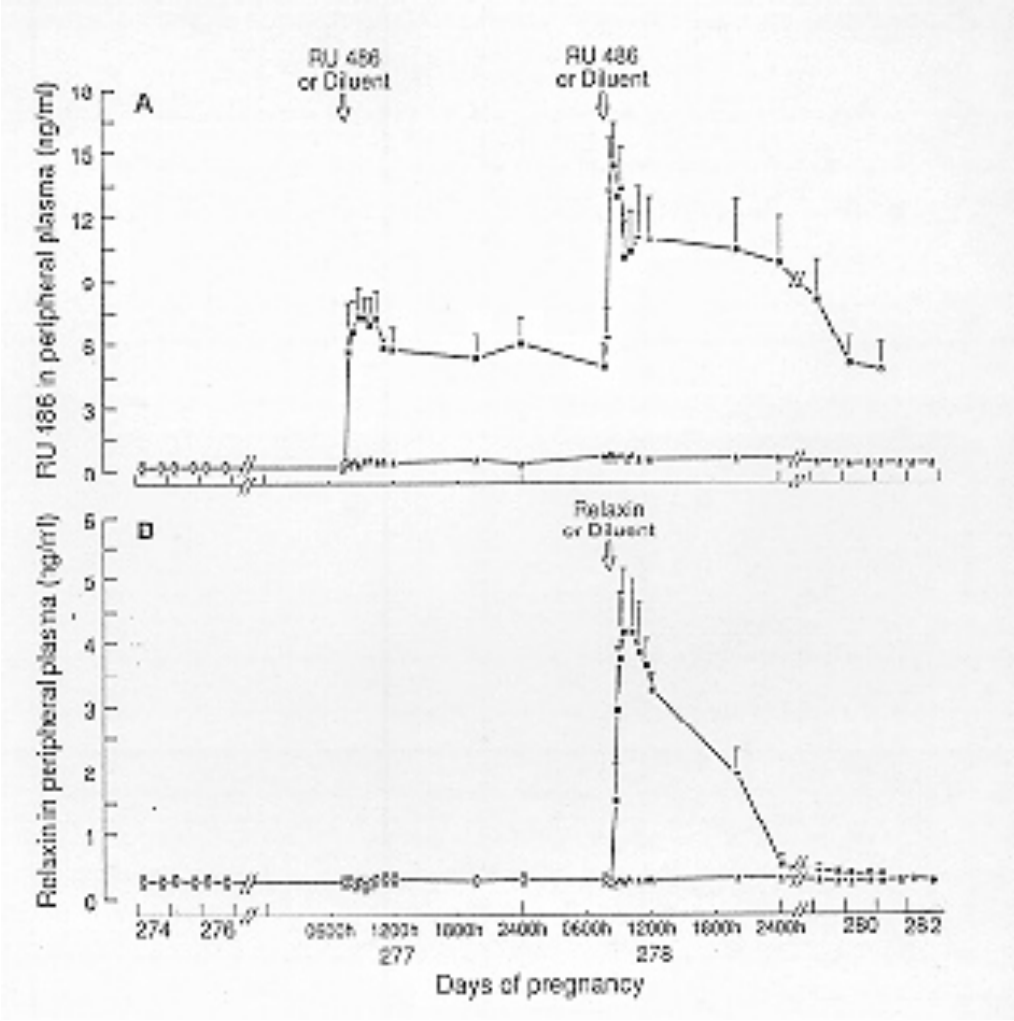

Figure 2. Comparison of (A) RU 486 and (B) relaxin in peripheral plasma during late pregnancy in sheep. On day 140, an indwelling catheter was inserted into a jugular vein for repeated blood sample collection from days 140-143 and day of lambing to two days postpartum. Blood was collected once a day at 1200 hours; on days 144 and 145 at 1200 hours, the ewes were injected with (A) RU 486 (4 mg/kg BW, intramuscular), and (B) relaxin (3,000 units, intramuscular). The controls received intramuscular injections of 3 ml diluent. Blood was collected at -60, -45, -30, -15 min before treatment and immediately after treatment at 15, 30, 45, 60 min and at 2, 3, 9, and 15 hours. (°); pretreatment (n = 22); (n); treatment with RU 486 (4 mg/kg BW; intramuscular, n = 5); (l), treatment with 3,000 U relaxin (intramuscular, n = 9); (Ÿ), diluent-treated controls (n = 8). The times of hormone and diluent treatments are indicated by *open arrows*. Values are the mean ± SE.