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LUTEOTROPIC PROPERTIES OF LUTEINIZING HORMONE AND NATURE OF OXYTOCIN INDUCED LUTEAL INHIBITION IN CATTLE [1]

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ABSTRACT

Fifty-three Holstein heifers were used to study the effects of oxytocin administered alone and in combination with various gonadotropins on the weights and progesterone contents of corpora lutea. Pituitary gonadotropin levels were compared in untreated and oxytocin-treated heifers at three stages of the estrous cycle.

Oxytocin injections on Days 2 through 6 significantly reduced the weights and total progesterone contents but not the progesterone concentrations of glands removed on Day 7. The concurrent administration of either purified bovine luteinizing hormone (LH) or human chorionic gonadotropin (HCG) significantly increased these parameters from the oxytocin treatment levels to normal or supra-normal values. Neither purified bovine prolactin nor urea-incubated HCG overcame the inhibitory effects of oxytocin. Neither oxytocin treatment on Days 2 and 3 nor oxytocin together with HCG significantly altered luteal function when the glands were taken on Day 4.

It was concluded that LH is the luteotropic hormone and that the cow requires at least two periods of luteotropic stimulation for normal corpus luteum development: one at ovulation and another after Day 4 of the cycle.

Oxytocin depleted total pituitary gonadotropin levels by about half during estrus or on Day 7, but did not alter levels at Day 4.

Heifers' pituitary gonadotropin levels and the total progesterone in their corpora lutea were negatively correlated in control (-0.75 on Days 4 and 7 pooled) and positively correlated in oxytocin- (0.78 on Days 4 and 7 pooled) treated groups ($P < 0.0001$). On Day 7 the correlation coefficient in the oxytocin-treated group was 0.96. The positive correlations in the treated animals are interpreted to mean that pituitary gonadotropin levels reflect plasma levels, and that these levels are directly controlling (and limiting) progesterone synthesis in the corpus luteum. The negative correlations reflect pituitary storage of gonadotropin.

Daily injections of oxytocin during the first third of the estrous cycle shortened the bovine cycle from a normal of about 22 days to 8-12 days (4). Simmons and Hansel (35) postulated that any hormone capable of preventing oxytocin from shortening the cycle possessed luteotropic properties. Several hormones were tested: human chorionic gonadotropin (HCG) and crude bovine pituitary extracts proved luteotropic, whereas bovine growth hormone, ovine prolactin, or equine luteinizing hormone (LH) did not. The luteotropic effects of prolactin in the rat have been clearly demonstrated (5, 11), but attempts to show that prolactin was luteotropic in guinea pigs (1, 31), rabbits (18, 30), cattle (35, 36), sheep (12, 26), goats (10), swine (32), and monkeys (8) have been unsuccessful or unconvincing. Kilpatrick et al. (18) presented evidence that LH is luteotropic in hypophysectomized rabbits.

Oxytocin, in producing precocious estrus in cattle, caused regression of the corpus luteum (4) and depletion of progesterone in luteal tissue (37). However, Mares and Casida (22) reported that oxytocin given on Days 12 and 13 of the cycle stimulated the corpus luteum so that increased levels of progesterone were measured on Day 14. Oxytocin injected early in estrus hastened ovulation in the cow by some 5 hr (17).

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Increased urinary excretions of 17-ketosteroids and urinary gonadotropins were measured in dogs and men following oxytocin injection (34). Oxytocin given over a long period to immature male rabbits caused increased testes weights by stimulation of the interstitial cells (3). Both oxytocin and vasopressin increased urinary gonadotropins in rabbits (24). Oxytocin instilled slowly into the third ventricle of the brain hastened sexual maturation in the intact or hypothalamic-lesioned immature female rat (9). These experiments suggest that oxytocin can cause release of pituitary gonadotropins in both sexes of several species.

MATERIALS AND METHODS

Fifty-three normally cycling Holstein heifers weighing 600-900 lb were used. They were subjected to three basic treatments: control, oxytocin,[3] and oxytocin plus gonadotropin. Eleven heifers were used once, 17 twice, and 25 three times. A normal estrous cycle was allowed between treatments, and with only three exceptions no heifer received two treatments with gonadotropins. The gonadotropins used were bovine LH,[4] bovine prolactin,[5] human chronic gonadotropin (HCG),[6] and HCG incubated with 6 M urea at 40 C for 24 hr to destroy its LH component (UHCG) (13, 33).

Heifers were treated on Days 2-6 (day of estrus is Day 0) and the corpus luteum removed at laparotomy or slaughter on Day 7. Twelve corpora were removed after no treatment, ten after oxytocin, ten after LH plus oxytocin, seven after HCG plus oxytocin, five after UHCG plus oxytocin, and five after prolactin plus oxytocin. A further 16 control corpora were removed at Day 4 of the cycle, 16 after oxytocin on Days 2 and 3, and ten after HCG plus oxytocin, also on Days 2 and 3 of the cycle.

The oxytocin was given subcutaneously at the rate of 0.33 USP units/kg body weight, and the HCG and UHCG at a dose level of 2,000 IU. Bovine LH was always given as the first treatment. The first five heifers on LH received the 0-71 preparation at the rate of 30 mg (16.2 units NIH-LH-SI) daily. The next five received the 0-72 preparation, two at 30 mg (0.54 units NIH-LH-SI) and three at 50 mg (0.90 units NIH-LH-SI). Bovine prolactin was always given as a first treatment at the rate of 60 mg (84 units of the N.I.H. prolactin standard) daily. Both the LH and prolactin were given intramuscularly, suspended in 5% beeswax in sesame oil.

Thirty pituitaries were collected at slaughter, ten 6-7 hr after beginning of estrus, ten at Day 4, and ten at Day 7. Five of the pituitaries on each day were collected following oxytocin treatment at the beginning of estrus, or on Days 2-4 or 2-7, respectively.

Corpora lutea were removed at laparotomy or within 15 min of slaughter. They were weighed and sampled for progesterone determinations by the method of Staples and Hansel (37).

The posterior lobe of the pituitary was dissected from the anterior lobe, which was then split mid-sagittally, and half was lyophilized and stored at −20 C. It was later homogenized in physiological saline containing 100 mg blood albumin per liter. The pituitaries were assayed for total gonadotropin by the assay described by Florsheim et al. (16). The method is based on the augmentation of radiophosphorus uptake by chick testes. NIH-LH-SI[7] was the standard hormone preparation used and the potency used (0.64 unit/mg) was the sum of its LH (0.53 unit/mg) and FSH (0.11 unit/mg) component potencies. Three assays were conducted, the ten pituitaries collected on each of the three days of the cycle (7, 4, and 0), being assayed in separate assays (1, 2, and 3, respectively). The standard solutions used were prepared to contain 13.25, 26.5, 53.0, 106.0, and 212.0 μg of gonadotropin activity in 0.2 ml of saline albumin. The homogenized pituitaries were administered in 0.2 ml at two dosage levels, one being twice the concentration of the other. Eight chicks were used at each dosage level. The statistical analyses for testing the validity of the assays were by the methods of Finney (15).

[3] Oxytocin, P.O.P., kindly supplied by M. E. Davenport of Armour Pharmaceutical Company, Kankakee, Illinois.

[4] Luteinizing Hormone Bovine L586284-0-71 and L586284-0-72 kindly supplied by N. G. Brink of Merck Sharp and Dohme, Rahway, New Jersey. The 0-71 preparation was the F-3-4 fraction of the Reichert (29) procedure for the preparation of purified bovine luteinizing hormone. The 0-72 preparation was worked up from 1.3 kg of beef pituitaries by the two-step procedure of Ellis (14). The yield was 5.5 g. Both preparations were assayed by A. F. Parlow, Emory University, Atlanta, Georgia, for LH and FSH.

[5] Prolactin Bovine Lot #216-178-8 and 216-178-9, kindly supplied by M. E. Davenport of Armour Pharmaceutical Company, Kankakee, Illinois.

[6] HCG, Follutein, kindly supplied by D. E. Rankin of E. R. Squibb and Sons, New York, control no. 2H76947.

[7] Kindly supplied by National Institute of Health, Endocrinology Study Section, Bethesda, Maryland.

RESULTS

Oxytocin treatments given on Days 2-6 significantly reduced the average weight of the corpora lutea taken on Day 7 from 4.62 to 2.68 g and the total progesterone content from 145 to 58 μg (Table 1). The reduction in progesterone content per gram of luteal tissue (31.7 to 19.6 μg) was not significant ($0.1 < P < 0.2$). Bovine LH or HCG treatments overcame oxytocin inhibition and increased corpus weight, progesterone concentration, and total progesterone to normal or higher than normal values. Urea incubation of HCG destroyed its ability to increase corpus luteum weight, progesterone concentration, and its total progesterone content above oxytocin treatment values, and bovine prolactin did not increase these parameters.

Oxytocine treatment on Days 2 and 3 did not significantly ($P > 0.1$) alter the Day 4 corpus luteum weights, total progesterone content, or progesterone concentration. Likewise, HCG plus oxytocin did not significantly alter these parameters ($P > 0.1$) (Table 2).

P^{32} Assays. A summary of the analyses testing the statistical validity of the assays is given in Table 3. The responses to unknown preparations and the standard hormones were parallel in Assays 1 and 2 but not in Assay 3. However, in Assay 3 the unknown preparations were parallel within themselves. The very large mean square for preparations in Assay 3 indicates that the pituitary preparations had lower activity than the standards.

Oxytocin administered 3-5 hr before slaughter, 6 hr after the beginning of estrus, significantly depleted pituitary gonadotropin contents from 3.0 μg/mg dried anterior pituitary tissue to 1.7 μg (Table 4). At Day 7 oxytocin depleted pituitary gonadotropins from 19.6 to 8.3 μg when administered on Days 2-7. At Day 4 oxytocin (administered on Days 2-4) did not alter pituitary gonadotropin content (9.1 and 10.5 μg).

Within treatment groups correlation coefficients were calculated between heifers' pituitary gonadotropin levels and the total progesterone in their corpora lutea (Table 5). There was a

TABLE 1

Net weight, progesterone concentration, and total progesterone content of corpora lutea removed on Day 7 of the estrous cycle

Treatment no.	Treatment	No. CL's	Net weight (g)	Progesterone: Concentration (μg/g)	Progesterone: Total (μg)
1	Control	12	$4.62^{1} \pm 0.29$ [c, d, e]	$31.7^{1} \pm 3.7$ [c]	$145^{1} \pm 15.5$ [e]
2	Oxytocin (O)	10	$2.68^{2} \pm 0.25$	$19.6^{2} \pm 5.7$	$58^{2} \pm 21.5$
3	O + Prolactin	5	$3.21^{2} \pm 0.31$	$16.9^{2} \pm 4.7$	$57^{2} \pm 18.4$
4	O + UHCG [a]	5	$2.73^{2} \pm 0.36$	$34.7^{1} \pm 14.2$	$100^{1} \pm 40.7$
5	O + HCG	7	$4.72^{1} \pm 0.78$	$61.9^{3} \pm 11.9$	$334^{3} \pm 92.6$
6	O + LH [b]	10	$4.85^{1} \pm 0.62$	$41.3^{3} \pm 5.3$	$212^{3} \pm 44.9$

[a] UHCG is human chorionic gonadotropin incubated with 6 M urea at 40 C for 24 hr.
[b] Luteinizing hormone.
[c] Standard error of the mean.
[d] Means with same superscript number do not differ significantly ($P < 0.05$); those with different numbers differ significantly.
[e] Residual mean squares for net weight, progesterone concentration, and total progesterone are 1.89, 414.2, and 17,559, respectively.

TABLE 2

Net weight, progesterone concentration, and total progesterone content of corpora lutea removed on Day 4 of the estrous cycle

Treatment	No. CL's	Net weight (g)	Progesterone/gram (μg)	Total progesterone (μg)
Control	16	1.25 ± 0.16 [b, c]	30.0 ± 2.1 [c]	38.0 ± 5.4 [c]
Oxytocin	16	1.50 ± 0.15	34.3 ± 1.1	51.2 ± 6.1
O + HCG [a]	10	1.18 ± 0.11	31.0 ± 1.8	36.0 ± 3.7

[a] HCG = human chorionic gonadotropin.
[b] Standard error of the mean.
[c] Residual mean squares for net weight, progesterone/gram, and total progesterone are 0.31, 67.5, and 469.9, respectively.

TABLE 3
Analysis of variance testing statistical validity of P^{32} assays for gonadotropins

Assay	Source	df	MS	F	Gaddum's λ	Finney's *(g)*
1	Doses	23	941	4.31***		
	Regression	1	6,365	29.19***		
	Preparations [a]	1	210	0.96		
	Parallelism [b]	1	824	3.78		
	Quadratic	1	86	0.40		
	Cubic	1	50	0.23		
	Error	138	218		0.486 ±0.031	0.134
2	Doses	23	883	5.79***		
	Regression	1	9,942	65.17***		
	Preparations	1	3,867	25.35***		
	Parallelism	1	389	2.55		
	Quadratic	1	129	0.85		
	Cubic	1	14	0.09		
	Error	129	153		0.285 ±0.021	0.060
3	Doses	23	1,990	13.02***		
	Regression	1	7,546	49.38***		
	Preparations	1	24,050	157.40***		
	Parallelism	1	1,251	8.19**		
	Parallelism [c]	1	259	1.69		
	Quadratic	1	157	1.03		
	Cubic	1	89	0.58	0.365 ±0.023	0.079
	Error	132				

** $P < 0.01$.
*** $P < 0.001$.
[a] Standard preparations vs. unknown preparations.
[b] Regression × preparations.
[c] Regression × first unknown preparation vs. other unknown preparations. A similar mean square was obtained when other combinations of unknown preparations were compared.

large negative correlation (−0.75) in control cows on Days 4 and 7 (pooled) and a large positive correlation of 0.96 in oxytocin-treated cows on Day 7 and 0.78 on Days 4 and 7 pooled. The probability that the pooled correlations are the same is less than 0.0001, and that the correlations are the same on Day 4 and 7, 0.0606 and 0.0018, respectively.

DISCUSSION

Both HCG and bovine LH were luteotropic in the system used; prolactin was not. The urea treatment of HCG destroyed the LH component (13, 33) and the preparation was no longer luteotropic. These results indicate that LH is the luteotropin in cattle. This conclusion is consistent with results of in vitro investigations where progesterone synthesis in bovine luteal slices was stimulated specifically with LH (2, 23, 25) and with the ovulation-hastening effect of oxytocin when injected into heifers at the beginning of estrus (17). At Day 4 the corpus luteum was unaffected by oxytocin or HCG treatment, whereas by Day 7 oxytocin reduced both its size and progesterone content. However, at Day 7 exogenous gonadotropin restored size and progesterone content to normal or above.

TABLE 4
Mean gonadotropic potencies of pituitaries collected from heifers on Days 0, 4, or 7 of the cycle

Day	Control ± S. E. *(μg NIH-LH-S1/mg dried tissue)*	Oxytocin ± S. E. *(μg NIH-LH-S1/mg dried tissue)*	P
0	3.0 ± 0.33	1.7 ± 0.47	<0.05 [a]
4	9.1 ± 0.62	10.5 ± 2.54	>0.25 [a]
7	19.6 ± 8.34	8.3 ± 1.41	<0.10 [a,b]

[a] Residual mean squares for Days 0, 4, and 7 are 0.8, 4.0, and 130.7, respectively, with eight degrees of freedom.
[b] $F = 5.2$ and $P = 0.05$ when $F = 5.3$.

The pituitary hormone preparations were not pure for the hormone they represented. However, the LH hormone preparations did not contain measurable quantities of FSH (< 0.018 × the NIH standard). The prolactin preparation was not luteotropic. Thus, if LH is not the bovine luteotropin, the luteotropic properties must be due to a synergism of several hormones. However, HCG was also luteotropic and it is prepared from a different source (human pregnancy urine) than the pituitary LH; thus, the possibility of any synergistic action is somewhat reduced.

The lowered Day 0 pituitary gonadotropin

TABLE 5

Correlation between pituitary gonadotropin [a] levels and the total progesterone content [b] of the corpus luteum in glands collected on Days 4 or 7 of the cycle

Day 4				Day 7			
Control		Oxytocin		Control		Oxytocin	
Pit. Gonad.	Total Prog.	Pit. Gonad.	Total Prog.	Pit. Gonad.	Total Prog.	Pit. Gonad.	Total Prog.
6.9	63	5.3	24	3.6 [c]	186	5.0	5
8.7	41	7.7	37	9.1	164	6.7	18
9.5	33	8.4	18	15.1	223	7.7	24
9.7	26	10.8	71	19.2	171	8.9	39
10.6	42	20.0	50	50.9	114	13.4	151
Correlation coefficients							
−0.76*		+0.49*		−0.75*		+0.96**	

* Ho[d] ($\rho 1 \neq 0$) $P > 0.05$.
** Ho ($\rho 1 \neq 0$) $P < 0.01$.
Ho ($\rho 1 = \rho 2$) Day 4 $P = 0.0606$.
Day 7 $P = 0.0018$.
[a] μg/mg dried anterior pituitary.
[b] μg.
[c] The hypothalamus of this heifer was accidentally stimulated with a needle during collection of cavernous sinus blood about 30 min before slaughter.
[d] Ho = null hypothesis.

content supports the conclusion that gonadotropin, probably LH, is the ovulating hormone, and provides an initial stimulus for corpus luteum development. On Days 2 and 3 the pituitary was refractory to oxytocin-induced depletion of gonadotropin and at the same time the corpus luteum was refractory to exogenous gonadotropin. The refractoriness of the corpus luteum may result from the fact that at this time the corpus luteum is already responding at a maximal rate of growth to a level of gonadotropins already in excess of requirements.

At about Days 5 and 6 oxytocin caused either the release of gonadotropins or decreased their synthesis. Several observations suggest that release rather than decreased synthesis is the primary mechanism involved. Gonadotropin stimulation of target organs following oxytocin administration has been noted by several workers (3, 9, 17, 22). The ability of oxytocin administered at the beginning of estrus to hasten ovulation in heifers (17) can scarcely be explained on any other basis. Nevertheless, the possibility that decreased synthesis occurred cannot be ruled out.

It was further concluded that the oxytocin-treated heifers were incapable of synthesizing and releasing sufficient gonadotropin to maintain corpus luteum size and progesterone production following the initial release. This was suggested by the fact that the corpora lutea were responsive to exogenous LH. In addition, it has been found (unpublished data) that regressing corpora taken on Day 7 from oxytocin-treated heifers can be stimulated in vitro to produce increased amounts of progesterone in response to LH. This result again suggests that luteal regression in oxytocin-treated heifers is due to declining plasma LH levels.

The large correlation coefficient between pituitary gonadotropins and corpus luteum progesterone in oxytocin-treated cows was interpreted as evidence that pituitary gonadotropin levels accurately reflected plasma gonadotropin levels, and that the function of the corpus luteum was being directly controlled by these plasma levels. The large negative correlation between pituitary gonadotropins and corpus luteum progesterone content in control animals was interpreted to represent storage of gonadotropin in the pituitary. Therefore, at Day 7, in untreated cows, pituitary gonadotropin content does not reflect plasma levels which still must be limiting corpus luteum function, since injection of exogenous gonadotropin (HCG or pituitary extracts) will increase corpus luteum size and progesterone content (20). Presumably, the exogenous gonadotropins increased plasma gonadotropin levels.

A direct implication of these results is that the bovine corpus luteum, like that of the rabbit (18), requires more than one, several, or continuous stimuli by the luteotropin for normal function. This is in distinction to the pig (7, 27), in which a single stimulus has been reported to be effective.

An alternative interpretation, that oxytocin in addition to reducing pituitary gonadotropin levels is releasing a hormone which is actively

luteolytic, seems doubtful in the light of the high correlation between pituitary gonadotropin and progesterone content in oxytocin-treated cows, and the facility with which exogenous gonadotropin overcomes the effects of the hypothesized luteolytic hormone. Malven and Hansel (21) failed to find evidence for a pituitary luteolytic hormone in cattle. The ability of LH-containing preparations to stimulate corpora lutea to supra-normal weights and progesterone contents also argues against this concept.

The P^{32} assays unfortunately gave a measure of total gonadotropin and not LH alone. The pituitaries in Assay 3 all fell below the standard doses and, thus, the assay was statistically not valid. However, the values were low, and as the pituitary dose responses were parallel within themselves it seemed safe to make comparisons within these pituitaries. Because their range of potencies was small, the bias due to nonparallelism with the calculated standard curve should be similar for each pituitary.

Recently, Labhsetwar et al. (19) injected oxytocin into normal heifers from Days 0-5 and produced smaller corpora lutea and reduced progesterone concentration on Day 6. They attempted to compare FSH and LH levels of pituitaries taken from oxytocin and untreated heifers (four heifers per group). The authors (19) utilized a modification of the Steelman and Pohley (38) FSH assay and Parlow's (28) LH assay. No standard hormone preparations were used and only two rats per pituitary at one dosage level for FSH and at three dosage levels for the LH assay were used. Rats ovarian weight and ovarian ascorbic acid depletion were used as the respective response parameters. No differences in LH or FSH levels were found between oxytocin-treated and untreated heifers. With this design it is unlikely that the methods used were sensitive enough to detect differences of the magnitude found in the present experiment.

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