Animal Reproduction Science, 14(1987) 95–101
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

Effect of Chronic Treatment with Bromocryptine on the Corpus Luteum Function of the Cow

M.M BEVERS and S.J. DIELEMAN

Clinic for Veterinary Obstetrics, A.I. and Reproduction, Veterinary Faculty, State University of Utrecht, Yalelaan 7, 3508 TD Utrecht (The Netherlands)

(Accepted 16 March 1978)

ABSTRACT

Bevers, M.M. and Dieleman, S.J., 1987. Effect of chronic treatment with bromocryptine on the corpus luteum function of the cow. *Anim. Reprod. Sci.*, 14: 95–101.

Six heifers received an intramuscular injection of 15 mg bromocryptine twice daily from day 1 (the day of standing oestrus was defined as day 0) until 50 h after the start of luteal regression. The overall mean level of prolactin was 0.22 ± 0.01 μg/l (SEM; $n=6$) in the bromocryptine-treated group and 10.7 ± 2.7 μg/l (SEM; $n=6$) in the control group. No significant differences in the overall mean level of progesterone and LH, the mean length of the early-luteal phase, the luteal phase and the period of luteal regression were measured between the two groups. The results provide strong evidence that prolactin has no luteotrophic properties in the cow during the oestrous cycle.

INTRODUCTION

The luteotrophic effect of prolactin has been described for rodents, sheep and ferret (for review see McNeilly et al., 1982). Investigations on the influence of prolactin on the luteal phase of the oestrous cycle in the cow are scarce and the results are contradictory.

Bartosik et al. (1967) demonstrated an increased progesterone production during perfusion of bovine ovaries in vitro with prolactin. Administration of prolactin to heifers to overcome oxytocin-induced luteal inhibition (Donaldson et al., 1965) did not increase progesterone levels to normal values. Administration of a lactogen preparation during the luteal phase of heifers had no effect on the duration of the oestrous cycle compared to the previous cycle (Smith et al., 1957). Moreover, Hoffmann et al. (1974) observed no change in the progesterone level after a marked reduction of prolactin which was caused by the administration of bromocryptine in the luteal phase of heifers.

In general, the in-vivo studies concerned experiments over a short time period.

0378-4320/87/$03.50

Recently, Dieleman et al. (1986) reported significantly higher levels of prolactin during the luteal phase of heifers compared to the follicular phase, suggesting the involvement of the hormone. This paper deals with the effect of a prolonged suppression of prolactin on the maintenance of the corpus luteum of the cow and may therefore contribute to the elucidation of the role of prolactin in this animal.

MATERIALS AND METHODS

Animals and experimental design

Experiments were performed from August 1984 until February 1985 with 12 heifers weighing between 300 and 350 kg. Experiments were started after at least one cycle indoors with normal luteal function and oestrus. At day 5 of the oestrous cycle during which experiments were carried out, the heifers were provided with a catheter in the vena circumflexa, under general anaesthesia. The catheter was fixed by three mersilene sutures. The tip of the catheter ended in the vena cava cranially to the bifurcation with the vena ovarica, and correct placing was assessed according to Walters et al. (1984) by estimating the progesterone level with a rapid radioimmunoassay (Dieleman et al., 1983a).

From day 1 (the day of standing oestrus was defined as day 0) until 50 h after the start of luteal regression, six randomly chosen heifers were injected intramuscularly with 15 mg (1.5 ml) bromocryptine twice daily. Bromocryptine (Parlodel; a generous gift of Sandoz, Basel, Switzerland) was dissolved in an aqueous vehicle containing 15% (*v/v*) propylene-glycol and 4% (*v/v*) ethanol. The other six heifers served as a control group.

The onset of luteal regression was assessed by estimating the progesterone level in the peripheral blood with a rapid radiommunoassay twice a day at 07.00 and 19.00 h (Dieleman et al., 1983a). From day 1 jugular venous blood samples were taken by venipuncture each day at 01.00, 07.00, 13.00 and 19.00 h until day 14 and thereafter at 03.00, 07.00, 11.00, 15.00, 19.00 and 23.00 h. At day 10 of the oestrous cycle, blood samples were taken via the catheter in the vena circumflexa at 10-min intervals for 8 h, starting at about 08.00 h.

Blood was collected in heparinized tubes, cooled immediately and centrifuged, and plasma was stored at $-25\,^{\circ}C$ until analysis.

Radioimmunoassays

Concentrations of progesterone were estimated by a direct solid phase ^{125}I RIA method (Coat-A-Count TKPG; Diagnostic Products Corporation, Los Angeles, CA, U.S.A.) in 100-μl samples in duplicate according to the manufacturer. The main cross-reactivities were 2.4, 2.0, 1.7 and 1.3% for deoxycortisol, 20α-hydroxypregn-4-ene-3-one, deoxycorticosterone and 5β-pregnane-3,20-

dione, respectively, and $<1\%$ for other steroids tested, according to the manufacturer. The sensitivity was 0.15 nmol/l and the interassay coefficient of variation was 11% ($n=16$). Prolactin and LH levels were estimated by previously validated radioimmunoassays (Dieleman et al., 1983b). The sensitivity was 0.10 μg/l NIH-P-B3 and 0.12 μg/l bLH7981 for the assay of prolactin and LH respectively.

Statistical analysis of the data

Mean hormone concentrations during the luteal phase were calculated for each individual animal from the levels measured in the blood samples collected from the jugular vein. The overall mean for a particular hormone within a group was calculated as the average of the means per animal. Similarly, mean hormone concentrations were calculated for the frequent sampling period on day 10 from the levels measured in the vena cava, and within a group an overall mean was determined.

A hormone pulse is defined as an increase of at least 50% from the adjacent baseline value with at least two 10-min sample points on the downwards limb, similar to the definition used by Peters et al. (1981).

Differences between the bromocryptine-treated and control group were tested for significance by non-paired Student's t-test (two-tailed). Data were judged as being significant at $P<0.05$.

RESULTS

Intramuscular administration of 15 mg of bromocryptine twice daily to heifers suppressed prolactin levels in the peripheral blood effectively (Table 1). The dose of bromocryptine (approximately 50 μg per kg body weight) is comparable with doses used in other mammalian species (sow, bitch, woman). There was no indication that the chronic treatment with the drug exerted negative effects on the health of the animals.

The treatment with bromocryptine caused no change in the levels of progesterone and LH in the peripheral blood as is seen from the overall mean levels of both hormones during the luteal phase in Table 1. The luteal phase is defined as the period during which the progesterone level is above 14 nmol/l (Dieleman et al., 1986). The maximum progesterone level was 36.6 ± 3.2 nmol/l (SEM; $n=6$) in the bromocryptine group and 34.3 ± 1.9 nmol/l (SEM; $n=6$) in the control group.

Insertion of the catheter on day 5 of the oestrous cycle had no apparent influence on the levels of progesterone, LH and prolactin in the twelve animals. The early luteal phase, the luteal phase and the period of luteal regression did not change upon treatment with bromocryptine (Table 2).

Table 3 shows the mean progesterone levels for each individual animal and

TABLE 1

Hormone levels in the peripheral blood during the luteal phase of bromocryptine-treated and control heifers

Treatment	Overall mean level ± SEM		
	Prolactin (μg/l)	LH (μg/l)	Progesterone (nmol/l)
Bromocryptine ($n=6$)	0.22 ± 0.01	0.64 ± 0.10	22.6 ± 1.9
Control ($n=6$)	10.7 ± 2.7	0.66 ± 0.06	22.6 ± 1.6

The length of the luteal phase is defined as the period during which the progesterone concentration is higher than 14 nmol/l.

TABLE 2

Effect of bromocryptine treatment on the length of the early-luteal phase, the luteal phase and the period of luteal regression

Treatment	Length of the early luteal phase (hour ± SEM)	Length of the luteal phase (hour ± SEM)	Length of the period of luteal regression (hour ± SEM)
Bromocryptine ($n=6$)	69 ± 7	266 ± 18	23 ± 2
Control ($n=6$)	60 ± 4	250 ± 10	23 ± 3

The early luteal phase is defined as the period during which the progesterone level increases from 3.2 to 14 nmol/l.
The luteal phase is defined as the period during which the progesterone concentration is higher than 14 nmol/l.
The period of luteal regression is defined as the interval between the time after which the progesterone level decreased in at least three successive samples and the time after which it fell below 3.2 nmol/l.

the overall mean for both groups during an 8-h sampling period during which a blood sample was collected every 10 min. This was performed in the luteal phase at day 10. The values represent the progesterone concentrations in the vena cava in the cranial direction close to the bifurcation with the vena ovarica. For comparison the mean progesterone levels in the vena jugularis at day 10 are also presented. One heifer of the bromocryptine-treated group had a mean progesterone level in the vena cava of 27.2 ± 1.5 nmol/l (SEM;$a=49$) and of 23.3 ± 2.8 nmol/l (SEM; $a=4$) in the jugular vein which indicates that the levels in the vena cava samples did not reflect the progesterone level close to the corpus luteum, probably because of a wrong placing of the catheter. Therefore the data for this cow are not presented in Table 3 and not included in the statistical analysis of the data in this table.

The values in parentheses represent the number of hormone pulses during

TABLE 3

The mean progesterone concentration during an 8-h frequent sampling period on day 10 of the luteal phase in bromocryptine-treated and control heifers

Bromocryptine-treated group			Control group		
Heifer	Mean progesterone (nmol/l ± SEM)		Heifers	Mean progesterone (nmol/l ± SEM)	
	vena cava $a=49$	vena jugularis $a=4$		vena cava $a=49$	vena jugularis $a=4$
15	53.64 ± 1.91 (3)	16.98 ± 1.69	5	77.01 ± 4.36 (2)	26.07 ± 2.04
22	161.24 ± 11.80 (9)	21.15 ± 1.91	16	56.28 ± 3.94 (2)	15.80 ± 1.30
46	64.10 ± 2.42 (5)	20.64 ± 0.16	24	110.62 ± 3.69 (5)	27.19 ± 1.24
63	49.34 ± 1.97 (4)	30.62 ± 2.19	28	67.31 ± 2.93 (3)	25.31 ± 4.96
73	45.85 ± 2.58 (5)	14.79 ± 0.54	29	54.47 ± 1.97 (7)	12.43 ± 0.54
			31	73.74 ± 9.16 (6)	21.62 ± 1.65
Overall mean $n=5$	74.72 ± 21.75 (5.2 ± 2.3)		Overall mean $n=6$	73.23 ± 8.36 (4.2 ± 0.9)	

a = number of blood samples per animal; n = number of heifers.
Overall mean = the average of the mean per animal for the 8-h sampling period of vena cava blood samples.
Values in parentheses represent the number of hormone pulses during the 8-h sampling period. A hormone pulse is defined as an increase of at least 50% from the adjacent baseline value with at least two 10-min sample points on the downwards limb.

the 8-h sampling period. The data in Table 3 demonstrate that chronic treatment with bromocryptine had no effect on the level and the pulse frequency of the progesterone output of the corpus luteum. Mean LH levels during the frequent sampling period were of the same magnitude as the levels measured in the jugular vein, as could be expected.

There was no difference in the overall mean level and the mean frequency of LH pulses between the two groups: bromocryptine-treated group 0.49 ± 0.05 μg LH/l and 2.0 ± 0.4 pulses (SEM; $n=5$); control group 0.67 ± 0.21 μg LH/l and 2.0 ± 0.6 pulses (SEM; $n=6$).

DISCUSSION

Hoffmann et al. (1974) have convincingly shown that LH is luteotrophic in the cyclic cow. However, no change in the progesterone concentration in the peripheral blood was noticed after a single daily injection of bromocryptine on days 11 and 12 of the oestrous cycle. Since the injection caused a temporary

reduction of the prolactin levels by 80–90%, the authors concluded that prolactin has little or no luteotrophic activity.

Donaldson et al. (1965) and Smith et al. (1957) concluded that prolactin has no luteotrophic properties in the cow on the results of experiments in which the endogenous concentration of prolactin was increased by the injection of prolactin preparations. It appeared that the increased level of prolactin did not affect the concentration of progesterone in the peripheral blood or the duration of the oestrous cycle.

The results of chronic suppression of prolactin release by bromocryptine treatment throughout the oestrous cycle, as described in this report, provide strong evidence that prolactin plays no role in the maintenance of the corpus luteum of the cow as far as the oestrous cycle is concerned. Prolactin suppression not only had no effect on the progesterone concentration in the peripheral blood; it also had no effect on the progesterone concentration and its pulsatile secretion pattern near the ovary. This excludes the possibility of overlooking a relatively minor action of prolactin on the corpus luteum, which may not be reflected in the peripheral blood.

The observation that suppressed prolactin levels caused no elevated LH levels excludes the possibility that a positive influence of the hormone is taken over by LH. On the basis of experiments with hypophysectomized sheep, Denamur et al. (1973) reported that prolactin is necessary for normal luteal function in sheep. Chronic treatment of sheep with bromocryptine during the oestrous cycle suppressed prolactin to levels lower than 0.5 ng/ml but had no effect on the length of the luteal period and the levels of progesterone and LH (Niswender, 1974). This may suggest that extremely low levels of prolactin are sufficient to ensure normal luteal function. However, comparable low levels of prolactin established in dogs by chronic treatment with bromocryptine during the oestrous cycle significantly shortened the luteal phase (Okkens et al., 1985), indicating the need for normal prolactin levels for the maintenance of the cyclic corpus luteum in this species.

ACKNOWLEDGEMENTS

The authors wish to thank Dr.P. Fontijne and Dr. G.C. van der Weyden and assistants for surgery. The technical assistance of Mrs. D.M. Blankenstein, Mrs. H.T.M. van Tol and Mr. A.V.P. van de Poll is greatly appreciated. Thanks are also due to Mr. S.H.J. Mook and co-workers for tending the animals.

REFERENCES

Bartosik, D., Romanoff, E.B., Watson, D.J. and Scricco, E., 1967. Luteotrophic effects of prolactin in the bovine ovary. Endocrinology, 81: 186–194.

Denamur, R., Martinet, J. and Short, R.V., 1973. Pituitary control of the ovine corpus luteum. J. Reprod. Fertil., 32: 207-216.

Dieleman, S.J., Kruip, Th.A.M., Fontijne, P., De Jong, W.H.R. and Van der Weyden, G.C., 1983a. Changes in oestradiol, progesterone and testosterone concentrations in follicular fluid and in the micromorphology of preovulatory bovine follicles relative to the peak of luteinizing hormone. J. Endocrinol., 97: 31-42.

Dieleman, S.J., Bevers, M.M., Poortman, J. and Van Tol, H.T.M., 1983b. Steroid and pituitary hormone concentrations in the fluid of preovulatory bovine follicles relative to the peak of LH in the peripheral blood. J. Reprod. Fertil., 69: 641-649.

Dieleman, S.J., Bevers, M.M., Van Tol, H.T.M. and Willemse, A.H., 1986. Peripheral plasma concentrations of oestradiol, progesterone, cortisol, LH and prolactin during the oestrous cycle in the cow, with emphasis on the peri-oestrous period. Anim. Reprod. Sci., 10: 275-292.

Donaldson, L.E., Hansel, W. and Van Vleck, L.D., 1965. Luteotrophic properties of luteinizing hormone and nature of oxytocin induced luteal inhibition in cattle. J. Dairy Sci., 48: 331-337.

Hoffmann, B., Schams, D., Bopp, R., Ender, M.L., Gimenez, T. and Karg, H., 1974. Luteotrophic factors in the cow: evidence for LH rather than prolactin. J. Reprod. Fertil., 40: 77-85.

McNeilly, A.S., Glasier, A., Jonassen, J. and Howie, P.W., 1982. Evidence for direct inhibition of ovarian function by prolactin. J. Reprod. Fertil., 65: 559-569.

Niswender, G.D., 1974. Influence of 2-Br-a-ergocryptine on serum levels of prolactin and the estrous cycle in sheep. Endocrinology, 94: 612-615.

Okkens, A.C., Bevers, M.M., Dieleman, S.J. and Willemse, A.H., 1985. Shortening of the inter-oestrous interval and the lifespan of the corpus luteum of the cyclic dog by bromocryptine treatment. Vet. Q., 7: 169-173.

Peters, A.R., Lamming, G.E. and Fisher, G., 1981. A comparison of plasma LH concentrations in milked and suckling post-partum cows. J. Reprod. Fertil., 62: 567-573.

Smith, V.R., McShan, W.H. and Casida, L.E., 1957. On maintenance of the corpora lutea of the bovine lactogen. J. Dairy Sci., 40: 443.

Walters, D.L., Schams, D. and Schallenberger, E. 1984. Pulsatile secretion of gonadotrophins, ovarian steroids and ovarian oxytocin during the luteal phase of the oestrous cycle in the cow. J. Reprod. Fertil., 71: 479-491.