Suppression of luteal function in dogs by luteinizing hormone antiserum and by bromocriptine

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Summary. Beagle bitches were treated with equine anti-LH serum (ALHS) or the dopamine agonist bromocriptine at selected times during the 2-month luteal phase of the ovarian cycle or pregnancy. After a single injection of ALHS (10 ml, i.m.) at Day 42 of pregnancy (N = 2) or the ovarian cycle (N = 3), progesterone was reduced (P < 0.05) to 7–24% of preinjection values within 1–2 days, and by 4–8 days returned to levels not different from those in control bitches treated with normal horse serum. Injections of bromocriptine (0.1 mg/kg, i.m.) daily for 6 days caused abrupt declines in progesterone which lasted 6–8 days in bitches treated at Day 8 or 22 of pregnancy (N = 5). In bitches treated at Day 42 of pregnancy (N = 3) or in non-pregnant cycles (N = 4) the bromocriptine treatment caused declines (P < 0.05) in progesterone which were permanent, extensive (<2 ng/ml), and therefore abortive. The declines in progesterone in response to immunoneutralization of LH and to prolactin-lowering doses of a dopamine agonist demonstrate that normal luteal function in dogs requires both LH and prolactin.

Introduction

The nature and extent of pituitary hormone support required for luteal progesterone secretion varies considerably amongst species (Rothchild, 1981). In the dog there is an absolute requirement for pituitary hormone secretion and LH can be luteotrophic. Hypophysectomy terminates luteal function and administration of exogenous LH during the luteal phase causes transient elevations in serum progesterone (Concannon, 1980). However, there are no detailed reports of studies demonstrating whether basal secretion of LH or any other pituitary hormone is specifically required for maintenance of normal luteal-phase progesterone secretion in the dog. Relevant studies in other carnivores are limited. In mink, prolactin appears to be a required luteotrophin. Luteal function in mink, as evidenced by elevated progesterone and normally occurring near the vernal equinox, was advanced by prolactin and inhibited by the dopamine agonist bromocriptine (Papke et al., 1980), and exogenous prolactin initiated progesterone secretion in hypophysectomized mink (Murphy et al., 1981). A luteotrophic requirement for LH in mink has not been demonstrated. In ferrets, LH as well as prolactin may be luteotrophic (Donovan, 1967; Murphy, 1979; Agu et al., 1986). The present studies were conducted to determine whether normal luteal function in dogs requires the continuous secretion of LH or prolactin. The experiments involved inhibition of endogenous LH activity by administration of anti-LH serum and suppression of endogenous prolactin secretion by administration of the dopamine agonist bromocriptine, at known times during the 2-month luteal phase (Concannon, 1986) in pregnant and non-pregnant bitches.

Materials and Methods

Animals. The beagle bitches were 2–4 years of age, maintained indoors in individual cages under a lighting schedule of 12 h light:12 h dark (lights on 07:00 h), and monitored for reproductive function and sex behaviour as
previously described (Concannon et al., 1977a, 1978, 1983). In brief, during the 2–5 months of anoestrus, animals were observed 3 times a week for pro-oestrous signs of vulval swelling and vaginal discharge of serosanguinous fluid. Daily during the 4–14 days of pro-oestrus and the 1–2 weeks of oestrus, bitches were observed with males for receptive behaviour, and, in some instances, bled for later assay of serum LH concentrations and/or examined for the presence of a 95–100% incidence of cornified superficial cells typically observed in vaginal smears during late pro-oestrus and the fertile days of oestrus. For the 33 cycles studied, Day 0 of the cycle was defined as the day of the preovulatory LH peak (N = 5), 8 days before the end of vaginal cytology characteristic of oestrus (N = 8) or the first day of behavioural oestrus (N = 20). Other studies have demonstrated the close relation of the last two times to the time of the preovulatory LH peak (Concannon et al., 1977a; Concannon & Di Gregorio, 1986). Blood samples were collected by jugular venepuncture and allowed to clot for 6–20 h at 5°C before centrifugation and preparation of serum. Serum samples were stored frozen until assayed.

**Anti-LH serum treatments.** Beginning at Day 42 of the cycle each of 5 bitches (2 pregnant and 3 non-pregnant) received a single intramuscular (i.m.) injection of 10 ml of a horse anti-bovine LH serum. Six control bitches (3 pregnant, 3 non-pregnant) received an injection of normal horse serum (NHS). Serum samples were obtained at 12–24-h intervals from 6 days before until 4 days after injection, and at 6, 8 and 10 days after injection. All samples were assayed for progesterone content. The preparation, characteristics, cross-reactivity and biopotencies of the anti-LH serum have been described (Snook, 1968; Morishige & Rothchild, 1974). The antiserum used (KAE0669) has been demonstrated to block ovulation or terminate pregnancy in rats at a dose of 0.5 ml/rat (Morishige & Rothchild, 1974).

**Bromocriptine treatments.** Bromocriptine (CB-154, Batch 76002, Sandoz Pharmaceuticals, E. Hanover, NJ, U.S.A.), dissolved at a concentration of 2 mg/ml in a solution of 20% ethanol in saline (9 g NaCl/l), was administered i.m. at a dose of 0.1 mg/kg daily for 6 days to each of 11 bitches during the luteal phase. Solutions were freshly prepared every 2–3 days and stored away from light at 5°C. Treatment was begun at known days of the cycle including Day 8 of pregnancy (N = 2), Day 22 of pregnancy (N = 2), Day 42 of pregnancy (N = 3) and Day 42 in non-pregnant bitches (N = 4). Control vehicle (20% ethanol in saline) was administered i.m. (1.0 ml/day) for 6 days to each of 11 control bitches, at times of cycles equivalent to those of bromocriptine-treated animals. Serum samples were obtained daily from 9 days before to 10 or 12 days after start of treatment and assayed for progesterone content.

**Progesterone assays.** Serum progesterone concentrations were determined by radioimmunoassay in duplicate for each of duplicate petroleum ether extracts of each sample, as previously described (Concannon et al., 1977b; Concannon, 1980). The assay was sensitive to 50 pg/tube (0.5 ng/ml) and had a mean within-assay coefficient of variation of 10% based on all samples assayed. All samples for a treated dog were included within a single assay along with those of a control dog. The mean coefficient of variation between assays was 14% for serum pools of 1, 4 and 15 ng/ml included in each assay. Values reported were not corrected for extraction efficiency or for the 100–400 pg/ml assayed in serum of ovariectomized bitches. Values are reported as mean ± s.e.m. Differences in mean progesterone concentrations between groups were evaluated by analysis of variance and levels of significance between mean concentrations were determined by Fisher’s protected lsd tests (Steel & Torrie, 1980).

**Results**

In each bitch treated with anti-LH serum progesterone values were rapidly reduced from pre-injection levels of 10–14 ng/ml to concentrations that were 9–32% of preinjection levels by 24 h after injection, and 7–24% by 36 h. Nadir values of 0.8–3.4 ng/ml were 6–24% of preinjection levels, occurred at 24–48 h after injection and were followed by elevations to 55–77% of pre-injection levels by 4–8 days after injection. Results in pregnant and non-pregnant bitches were similar (Fig. 1). Comparable declines in progesterone were not observed in any control bitches treated with NHS. Mean progesterone in antiserum-treated bitches was lower (P < 0.05) than in control bitches for the first 3 days after treatment (Fig. 2).

In each bitch treated with bromocriptine, serum progesterone declined abruptly during treatment. In bitches treated beginning at Day 22 after the LH peak, or later, progesterone concentrations declined to nadir values which were 10% or less of pretreatment levels (9–32 ng/ml) by 2–4 days of treatment. Abrupt declines in progesterone were also observed in bitches treated at Day 8 but were only to levels approximately 50% of pretreatment values (25–34 ng/ml). Comparable declines were not observed in control bitches (Fig. 3).

Bitches treated beginning at Day 8 or 22 of pregnancy remained pregnant and gave birth to normal litters. Progesterone concentrations increased at or shortly after the end of treatment and within 2–6 days returned to values within the normal range for that stage of pregnancy. Bitches treated beginning at Day 42 of pregnancy aborted all fetuses at 2-5 to 4 days of treatment and after
Fig. 1. Serum progesterone concentration, as a percentage of pretreatment values, in a pregnant and a non-pregnant bitch treated with 10 ml horse anti-bovine luteinizing hormone serum (ALHS) on Day 42 after the onset of oestrus and when concentrations were 12.8 and 10.3 ng/ml, respectively.

Fig. 2. Mean (± s.e.m.) concentrations of progesterone in serum of bitches treated with 10 ml horse anti-bovine LH serum (ALHS) or normal horse serum (NHS) at 42 days after the onset of oestrus. Each group included 3 pregnant bitches which subsequently whelped normal litters.
Fig. 3. Scrum progesterone concentrations in individual pregnant and non-pregnant bitches treated with bromocriptine (BrC) at a dose of 0.1 mg/kg/day, or control vehicle (20% ethanol in saline), i.m. for 6 days starting at 8, 22 or 42 days after the preovulatory LH peak.

a decline in progesterone from pretreatment levels of 8–19 ng/ml to < 2 ng/ml. Progesterone concentrations remained low after abortion except for 1–2-day transient increases of 2–3 ng/ml at 2–5 days after abortion in 2 bitches (Fig. 3). In 4 non-pregnant bitches treated with bromocriptine at Day 42 of the luteal phase, progesterone concentrations were initially 8–19 ng/ml, declined to ≤ 1 ng/ml between 2 and 5 days of treatment, increased to 2–7 ng/ml for 3–4 days after the end of treatment, and again declined to low levels. Mean progesterone concentrations in pregnant and non-pregnant bitches treated from Day 42 of the luteal phase are shown in Fig. 4. Progesterone was lower (P < 0.05) in bromocriptine-treated than in control bitches from 2 to 6 days of treatment.

Side effects observed throughout administration of bromocriptine, and most prominent at 1–3 h after injection, included vomiting, listlessness, inappetence, inanition, and increased drinking.
Fig. 4. Mean (±s.e.m.) progesterone concentrations in serum of pregnant (N = 3) and non-pregnant (N = 4) bitches treated with bromocriptine injections of 0·1 mg/kg/day, or control vehicle, for 6 days starting at 42 days after the preovulatory LH peak.

Discussion

The rapid decline in progesterone caused by anti-LH serum demonstrates the LH-dependence of normal luteal function in dogs during the second half of the luteal phase. The extent of LH-dependence during the early luteal phase remains to be determined. The ability of anti-bovine LH serum to neutralize canine LH was not unexpected since it is effective in other non-ruminant species (Morishige & Rothchild, 1974; Greenwald & Terranova, 1981) and anti-ruminant LH serum has been used to assay endogenous canine LH (Nett et al., 1975; Concannon et al., 1980, 1983). The 2- or 3-day decline in progesterone is assumed to represent neutralization of a significant portion of endogenous LH and a corresponding decreased availability of endogenous LH for binding to luteal cell LH receptors. The rapid recovery of progesterone levels may represent a failure of a limited amount of antiserum to neutralize continued secretion of normal amounts of LH or a compensatory increase in LH secretion. Presumably higher doses of anti-LH serum or repeated administration would be needed to obtain protracted declines in progesterone below 2 ng/ml and termination of pregnancy as obtained with bromocriptine treatment.

The decrease in progesterone consistently caused by bromocriptine suggests that normal luteal function in dogs is dependent upon normal endogenous levels of prolactin throughout the luteal phase. The daily dosage of bromocriptine used in the present study (0·1 mg/kg/dog) was similar to the dose (1 mg/dog) previously demonstrated to reduce serum prolactin concentrations in dogs to 25% of preinjection levels after a single injection (Reimers et al., 1978). The effects of bromocriptine could have involved changes in secretion of hormones other than prolactin, including decreased LH secretion (Lachelin et al., 1977; Shaban & Terranova, 1986). However, the results of ongoing experiments suggest that the luteolytic effect primarily involves reduction in prolactin secretion and can be prevented by concomitant administration of prolactin (P. W. Concannon & D. Frank, unpublished data). Our findings are in agreement with a report that chronic oral administration of lower doses of bromocriptine resulted in slightly shortened luteal phases and reduced interoestrous intervals in bitches (Oikkens et al., 1985). The abortions in bromocriptine-treated bitches after declines in progesterone to < 2 ng/ml correspond to abortions under similar conditions during induction of luteolysis by administration of prostaglandin F-2α (Concannon &
Hansel, 1977). Maintenance of pregnancy in dogs requires luteal progesterone secretion throughout gestation and the ovaries are the only significant source of progesterone in dogs (Concannon et al., 1977b). The abortions suggest that bromocriptine administration could be used for elective termination of pregnancy in dogs. However, concerns about side effects, repeatability and dosage would need to be addressed.

In summary, the present studies indicate that both LH and prolactin are part of the normal luteotropic requirement in the dog and that prolactin suppression by bromocriptine can be abortifacient in dogs.

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