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Effect of Arginine Vasotocin on Luteinizing Hormone and Prolactin Concentrations in Serum of Cattle[1] (41167)

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Abstract. Arginine vasotocin (AVT) was given to cattle to determine its effects on concentrations of luteinizing hormone (LH) and prolactin (PRL) in serum. Doses of AVT ranging from 0.3 to 300 μg/kg BW, when given as single injection, did not alter the magnitude or frequency of normal pulsatile secretion of LH or concentrations of PRL in steers. Furthermore, AVT (0.7 mg/injection) given every 2 hr from −2 to 20 hr after 1 mg estradiol did not block the LH surge in ovariectomized cows. However, the 54% increase in concentrations of PRL which occurred 11 to 20 hr after injecting estradiol was blocked by repetitive injections of AVT. We conclude that AVT may alter PRL secretion in cattle. However, there is no indication that AVT affected LH release.

Arginine vasotocin (AVT), a nonapeptide secreted by the pineal gland (1), prevents or delays the preovulatory surge of luteinizing hormone (LH; 2, 3) and prolactin (PRL; 4) in rats. In addition, AVT is capable of increasing secretion of LH (5) and PRL (5, 6) *in vitro* or decreasing LH secretion *in vivo* (7, 8) in rats.

Concentrations of PRL in serum are directly related to duration of photoperiod in cattle (9, 10). Recently, we showed that AVT caused PRL release from bovine pituitary cells (11). Since the pineal gland and its secretions are often associated with photoperiodic effects, it is possible that AVT may play a role in mediating the effects of photoperiod on PRL secretion. The objective of the experiments presented was to determine the effects of AVT on LH and PRL secretion in cattle during basal conditions and after an estradiol stimulus.

Methods and Materials. *Effect of AVT on concentrations of LH and PRL in serum of steers.* Our objective was to determine if AVT would affect pulsatile LH release which characterizes LH secretion in steers, or affect prolactin concentration in serum. Four Holstein bulls averaging 151 kg body weight (BW), were castrated at least 5 days prior to use in this experiment. Steers were used to avoid confounding effects of AVT on the testis. Treatments were arranged as for a latin square design. Each of four steers were injected iv via jugular cannulae with 0.3, 3, 30 and 300 μg AVT[2]/kg BW, a single dose on each of 4 consecutive days. The range of doses is centered about the doses which affect LH and PRL secretion in rats (2, 4). Blood was collected via jugular cannulae. LH was measured in blood at 15-min intervals from −90 to 180 min after giving AVT, plus one additional sample at 6 min. PRL was measured in blood collected at −45, −30, −15, 0, 2, 4, 6, 8, 10, 15, 20, 30, 45, 60, 90, 120, and 180 min.

Effect of AVT on LH and PRL after giving estradiol. Eight nonlactating dairy cows (Jersey and Guernsey) averaging 410 kg BW, were ovariectomized (ovx) at least 30 days prior to use in this experiment. Cows were randomly assigned to one of two treatments. All cows were given 1 mg estradiol injections im at time zero and either 0.7 mg AVT dissolved in 5.8 ml saline or saline alone. This dose of AVT or saline was injected into a carotid artery via cannulae every 2 hr from −2 to 20 hr. Choice of

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[2] AVT purchased from Bachem Fine Chemicals, Torrance, Calif.; Lot No. R1615 used for first experiment, Lot No. R1750 used for second experiment; biological activity of both lots equalled 225 units/mg.

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this dose of AVT was based on the per kilogram of BW dose that blocks the preovulatory LH and PRL surge in rats (2, 4). One milligram estradiol was chosen because of its ability to induce a preovulatory-like LH surge in ovariectomized cows (12). Relative to giving estradiol, blood was collected hourly via jugular cannulae from −2 to 30 hr plus every 15 min during the intervals −2 to 0 hr, 12 to 14 hr, and 16 to 18 hr. Samples were also collected 5 and 10 min after AVT was first injected. The rationale for this sampling scheme was to monitor changes in the chronic, as well as the pulsatile or episodic secretion profiles of LH and PRL. All serum samples were assayed for LH and PRL.

Body temperature, respiration rate, and heart rate were monitored in both experiments. AVT did not alter these parameters nor behavior.

Housing environment. The first and second experiments were conducted on November 14 through 17, 1978 and February 25 through 26, 1979; respectively, at East Lansing, Michigan. Ranges in ambient temperature on these days were −3 to 17° and −12 to −5°, respectively. Animals were kept in open front sheds with natural lighting until 3 days before the experiments. Thereafter, they were housed in enclosed, unheated buildings with continuous lighting.

Assaying and statistical procedures. LH and PRL were assayed in serum using radioimmunoassays previously described (13, 14). Data were transformed logarithmically [transformed value = log(value + 1)] to correct for heterogeneous variance and then analyzed using split-plot analysis of variance for repeat measures (15). Specific, nonorthogonal comparisons were performed using Bonferroni's *t* test (15).

Results. *Effect of AVT on concentrations of LH and PRL in serum of steers.* Neither magnitude nor frequency of pulsatile release of LH, which is characteristic of steers, was affected ($P > 0.05$) by a single injection of AVT at doses ranging from 0.3 to 300 μg/kg BW (Fig. 1). Similarly PRL averaged 9.3 ng/ml in serum collected before AVT was injected and concentration was not altered ($P > 0.05$) by any of the doses of AVT tested (Table I). The insignificant increase in mean PRL concentration in steers given 300 μg AVT/kg BW was due to the response of one animal.

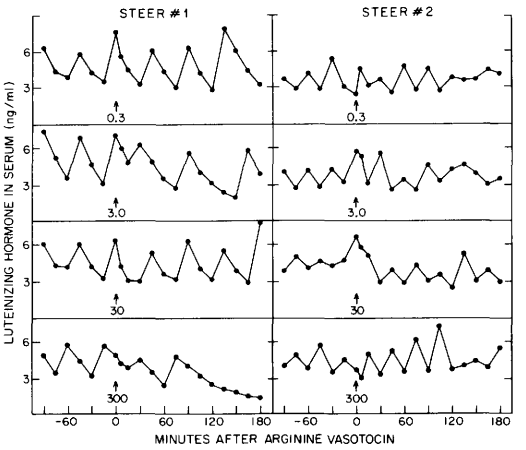

FIG. 1. Serum LH concentrations for two of the four Holstein steers given 0.3, 3, 30, and 300 μg AVT/kg BW. AVT was equally ineffective in the other two steers. Standard error was calculated from the mean square error of logarithmically transformed data and equals 0.1485.

Effect of AVT on LH and PRL release after giving estradiol. LH surges similar to those occurring prior to ovulation, were induced in ovx cows given 1 mg estradiol and pulses of saline every 2 hr (Fig. 2A). These LH surges were not blocked by injecting 0.7 mg AVT every 2 hr from −2 to 20 hr relative to giving estradiol (Fig. 2B). The mean interval ± SE from injection of

TABLE I. MEAN CONCENTRATIONS OF PRL IN SERUM OF HOLSTEIN STEERS BEFORE AND AFTER GIVING VARIOUS DOSES OF AVT[a]

Time after AVT (min)	AVT dose (μg/kg body wt)			
	0.3	3.0	30.0	300.0
−45 to 0	2.31 (10.1)	2.26 (9.0)	2.05 (6.8)	2.37 (10.4)
2 to 150	2.14 (8.2)	2.14 (8.3)	2.08 (7.2)	2.52 (14.2)

[a] Means represent logarithmically transformed values (untransformed values are parenthesized) from 4 or 13 samples per steer collected at various times before and after AVT, respectively (see Methods and Materials for sampling schedule). Four steers were given each dose as for a latin square design. Standard error was calculated from the mean square error and equals 0.7514.

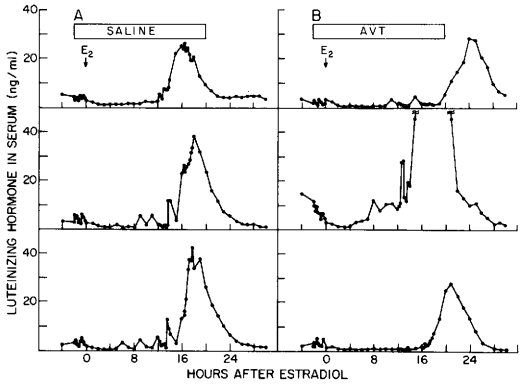

FIG. 2. Serum LH concentrations for individual ovx cows given 1 mg estradiol at time zero and either (A) saline or (B) 0.7 mg AVT every 2 hr from −2 to 20 hr. LH surges were not detected for one cow in each treatment group and therefore LH profiles for these cows are not depicted.

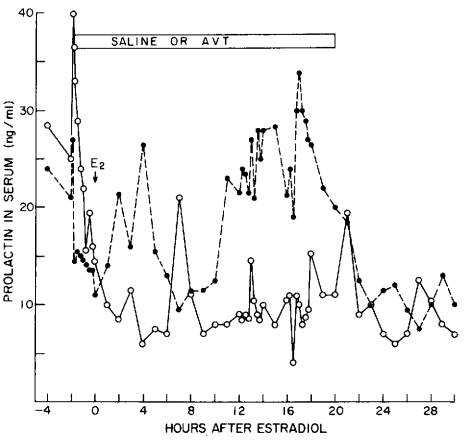

FIG. 3. Mean serum PRL concentrations for ovx cows given 1 mg estradiol at time zero and either saline (●) or 0.7 mg AVT/injection (○) every 2 hr from −2 to 20 hr; four cows per treatment. Standard error was calculated from the mean square error of logarithmically transformed data and equals 0.1714.

estradiol until LH surge peak was 17.7 ± 0.3 and 21.0 ± 1.4 for cows given saline and AVT, respectively ($P \simeq 0.1$). LH surges were not induced by 30 hr in one cow from each treatment group and thus data from these cows were omitted from analysis.

PRL averaged 16.4 ng/ml in serum collected before 1 mg estradiol was given to ovx cows and increased ($P < 0.005$) to 25.2 ng/ml between 11 and 20 hr after estradiol (Fig. 3). Injections of AVT every 2 hr completely blocked ($P < 0.005$) this increase in serum PRL, as the corresponding mean equalled 10.3 ng/ml. The injection of AVT given before estradiol had no effect ($P > 0.05$) on concentrations of PRL in serum of ovx cows.

Discussion. Under conditions of the present studies in cattle, AVT had no detectable effects on LH secretion. This was true despite administering AVT over a wide range of doses, as a single injection or repetitive injections, and in the presence and absence of estradiol. AVT may have slightly delayed the estradiol-induced LH surges, but the limited number of cows prevented a reasonable assessment of this possibility. Our results sharply contrast with the effects of AVT on LH secretion reported to occur in rats. Thus, in rats AVT has been shown to block or delay naturally occurring or estradiol-induced LH surges (2, 3), attenuate the postcastrational increase in LH concentrations in serum (7), augment the LHRH-induced release of LH in male rats (16), and increase basal LH release *in vitro* from pituitaries of intact male rats or LHRH-induced LH release from pituitaries of estrogen−progesterone-primed ovx female rats (5). Interestingly, photoperiod, like AVT, also affects LH secretion in rats (17), but not in cattle (18, 19).

Although we previously reported that AVT was a potent secretagogue of PRL when added to bovine cell cultures (11), a single injection of AVT had no effect on basal concentrations of PRL in serum of steers. In rats, on the other hand, AVT increased PRL release in estrogen−progesterone-treated males (6) or from cultured pituitaries from males or ovx females primed with estrogen and progesterone (5).

PRL concentrations in serum increased between 11 and 20 hr after giving estradiol to cows. Since sham controls for estradiol were not examined, we cannot determine whether estradiol caused the increased PRL release, i.e., effects of estradiol are confounded with time. In fact, it is not clear whether estradiol acts as a physiological regulator of PRL in cattle (18−25). Nevertheless, AVT did reduce serum PRL concentrations in these estradiol-treated ovx cows. This observation is consistent with

the possibility that AVT may influence the ability of estradiol to alter PRL release in cattle.

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