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Effects of 8-Arginine Vasotocin on Plasma Prolactin and Follicle-Stimulating Hormone Surges in the Proestrous Rat (39938)

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We recently reported that 8-arginine vasotocin (AVT) suppressed the preovulatory surge of luteinizing hormone (LH) and inhibited subsequent ovulation in proestrous rats when given in picogram amounts intraventricularly or in microgram amounts iv (1, 2). In diestrous rats, AVT did not affect tonic blood levels of LH, nor did it alter the elevated blood LH patterns in rats used 2, 14, or 30 days after ovariectomy. We undertook the present study to determine if AVT is capable of suppressing the endogenous increases in levels of plasma prolactin (3-6) and follicle-stimulating hormone (FSH) (4-8) that occur on the afternoon of proestrous.

Materials and methods. Animals. Female Sprague-Dawley rats (200-280 g) were maintained under controlled lighting conditions (illumination from 0500 to 1900 hr). On the basis of daily vaginal smears, only those animals with at least two consecutive 4-day estrous cycles were used. These were anesthetized with ether on the morning of proestrus, and a polyethylene (PE-50) cannula for blood collection and compound injection was inserted via the jugular vein to the superior vena cava, adjacent to the heart; all surgery was completed by 1030 hr. The rats were then housed in individual cages and kept unanesthetized and unrestrained. Prior to blood collection, 100 U of heparin was administered via the cannula.

Peptides. Antidiuretic hormone (ADH) and oxytocin (OT) were used as control compounds in addition to the saline vehicle, because they are chemically similar to AVT and cause some similar biological responses (9). The AVT (Lot 5468 for the prolactin studies and Lot 8893 for the FSH studies), ADH (Lot 8022), and OT (Lot 5693) were all obtained from Bachem, Inc., Los Angeles. These materials were stored lyophilized at 4° until the day of an experiment, when they were diluted with saline solution and administered iv in 10-μl injections.

Experiments. The endogenous increase of plasma prolactin commences between 1300 and 1600 hr on the day of proestrus (3-6), and, accordingly, the 250-μl samples of blood were drawn hourly from 1200 through 1800 hr. The 93 rats in the prolactin experiments included 29 saline controls; the remainder were divided into groups for each compound, with 1-μg injections being administered at various times between 1300 and 1600 hr (see Table I).

The increase in plasma FSH occurring on proestrus is initiated at approximately 1400-1600 hr and remains elevated until late afternoon the following day (5-8). Blood samples of 600 μl were obtained every 3 hr either from 0900 to 2100 hr or from 1500 to 2400 hr on the day of proestrus and at 0500 and 1200 hr the following day. Of the 61 rats used, 24 received saline solution alone and the remaining 37 were divided into groups receiving AVT, ADH, or OT. The compounds were administered at 1 μg/hr from 0900 to 1600 hr. Additional experiments were conducted using AVT (1 μg/hr) from 0700 to 1600 hr and from 1100 to 1800 hr, and 2 μg of AVT/hr from 0900 to 1600 hr.

Radioimmunoassay. Prolactin concentrations in the 100-μl plasma samples were determined with the double-antibody method of Niswender *et al.* (10), except that phosphate-buffered saline with 0.1% gelatin was used as the diluent. Rat prolactin antiserum No. 10, used at a 1:20,000 dilution (11), was kindly provided by J. D. Neill; the rat prolactin fraction LER-1382-3 used for iodination was a gift from L. E. Reichert, Jr. The reference preparation,

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TABLE I. EFFECTS OF NONAPEPTIDES ON THE ENDOGENOUS SURGE OF PLASMA PROLACTIN IN PROESTROUS RATS.

Treatment[a]	Total dose per rat (μg)	Incidence of prolactin surge[b]	
		(n/n')[c]	(%)
Saline	0	27/29	93
AVT			
1 μg/30 min from 1300 to 1600 hr	7	1/23	4
1 μg/60 min from 1300 to 1600 hr	4	1/7	14
1 μg at 1300 and 1500 hr	2	0/6	0
1 μg at 1300 hr	1	4/5	80
ADH			
1 μg/30 min from 1300 to 1600 hr	7	4/5	80
1 μg at 1300 and 1500 hr	2	4/7	57
OT			
1 μg/30 min from 1300 to 1600 hr	7	4/5	80
1 μg at 1300 and 1500 hr	2	5/6	84

[a] Compound administered iv in 10 μl of saline solution.

[b] The prolactin surge was considered suppressed when values were less than 30 ng/ml from 1500 through 1800 hr; it was considered present when values were >100 ng/ml on at least two consecutive determinations.

[c] n = Number of animals evidencing the surge; n' = total number of animals.

NIAMDD-Rat Prolactin-RP-1, was generously provided by the Rat Pituitary Hormone Program and had a biological potency of approximately 30 IU/mg in the intradermal pigeon crop-sac assay. The sensitivity of the assay was 2 ng/ml plasma and the coefficient of variation was 5% within an assay and 12% between assays.

Values of FSH in 300-μl plasma samples were determined with the radioimmunoassay kit provided by the NIAMDD. The rat FSH preparation (NIAMDD-Rat FSH-RP-1) had a biological potency of approximately 2.1 × NIH-FSH-S1 (HCG-Augmentation Assay). The sensitivity of this assay was 0.8 ng/ml and the coefficient of variation was 5% within an assay and 13% between assays. Differences between treatment groups were analyzed by the unpaired t test.

Results and discussion. The dramatic inhibitory effect of AVT on the surge of prolactin is shown in Fig. 1. At all sampling times after 1300 hr, the values of those animals given AVT were significantly lower ($P < 0.001$) than those of the saline controls, which exhibited the prolactin surge at times similar to earlier reports (3–6). Neither ADH nor OT had this suppressive effect, except for an inhibited response at 1500 hr with OT ($P < 0.05$ when compared with the saline group), for which the reason was not apparent.

As can be seen in Table I, when the 1-μg dose of AVT was administered every 30 min from 1300 to 1600 hr, 96% of the animals showed suppression of the prolactin surge; when given every hour, 84% showed suppression; when given bihourly (at 1300 and 1500 hr), 100% suppression was observed. However, when the 1-μg dose was given at 1300 hr only, the incidence of surge was comparable with that of saline controls and animals receiving ADH and OT.

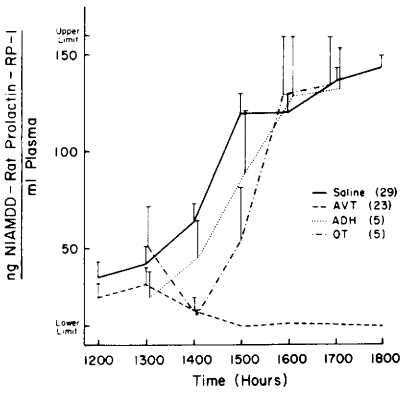

FIG. 1. Effects of AVT on the surge of prolactin in proestrous rats. AVT and the control compounds ADH and OT were administered at 1 μg every 30 min from 1300 through 1600 hr. The number of animals in each treatment group is in parentheses; vertical bars represent the SEM. The prolactin values of AVT-treated animals were suppressed ($P < 0.001$) when compared with the ADH-, OT-, and saline-treated controls at 1500, 1600, 1700, and 1800 hr.

None of the three compounds affected the increase in plasma FSH (Fig. 2), for which the timing, duration, and magnitude in our studies were similar to those previously reported (5–8). Neither lengthening the administration time of AVT nor increasing the dosage (up to eight times the amount that inhibited the prolactin surge) caused suppression of the endogenously elevated levels. There was no significant difference between the elevated plasma FSH levels of the 12 saline control animals and the group of 6 rats receiving 1 μg of AVT/hr from either 0700 through 1600 hr or 1100 through 1800 hr, or a 2-μg/hr dose from 0900 through 1600 hr.

Both the failure of AVT to affect FSH levels and the finding that the prolactin surge was not inhibited by ADH and OT militate against the suggestion that experimental stress depressed the prolactin levels (12–14). Likewise, it is improbable that AVT's inhibitory effect can be attributed to a vasoconstrictor mechanism indirectly altering pituitary secretion, because the pressor activity of AVT is less than one-half that of ADH (9).

An apparent conflict exists between our finding that AVT blocks the proestrous surge of prolactin and a report showing that this peptide increases plasma prolactin titers in estrogen/progesterone-primed male rats (15). However, experimental differences—in particular the fact that our animals were female rats that had not been presensitized with gonadal steroids (16)—could account for this discrepancy. Also, the inhibitory effect of AVT may be intimately linked to the mechanisms regulating cyclicity in the female and may not have the same effects in the tonically regulated male rat (17).

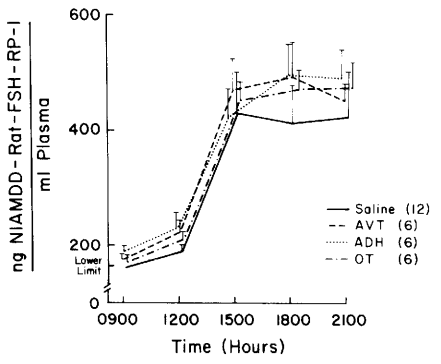

FIG. 2. Effects of AVT on the surge of FSH in proestrous rats. AVT and the control compounds ADH and OT were administered at 1 μg every hr from 0900 through 1600 hr. The number of animals in each treatment group is in parentheses; vertical bars represent the SEM.

Recent reports question the ability of FSH to induce ovulation in the cycling rat (18, 19). This inference is supported by the present finding that AVT does not suppress the proestrous surge of FSH and the previous report in which AVT was shown to inhibit both the proestrous LH surge and subsequent ovulation (1, 2).

The preovulatory surge of LH, in addition to inducing ovulation, participates in progesterone biosynthesis in the rat luteal cells (20). Prolactin has also been implicated in this synthesis (21). A possible physiological role of the blocking of the prolactin surge by AVT is the interference with this synthesis of progesterone by the corpora lutea. Currently, experiments are being conducted to investigate the effects of AVT on development of the corpus lutea and on progesterone production.

The presence of AVT in the rat pineal has been demonstrated using bioassay (22) and radioimmunoassay (23), and it is tempting to speculate that pineal secretion of AVT may be involved in the regulation of fertility.

Summary. Previous findings in this laboratory that AVT inhibited the preovulatory surge of LH and subsequent ovulation prompted the present study demonstrating that AVT effects a marked suppression of the prolactin surge during proestrus, but does not affect the concurrent surge of FSH. Microgram amounts of AVT (administered from 1300 to 1600 hr on the afternoon of proestrus) suppressed the prolactin surge significantly, relative to saline-treated controls and comparable doses of antidiuretic hormone and oxytocin ($P < 0.001$); and prolonged administration with increased amounts of AVT (up to 16 μg over the period from 0900 through 1600 hr) had no effect on the FSH surge. These results support earlier suggestions that different mechanisms are involved in the cyclic release of

LH and FSH and that the surge of FSH is minimally involved in ovulation in the cycling rat.

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