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Effects of Arginine Vasotocin on Levels of Plasma Gonadotropins and Prolactin in Ovariectomized Conscious Rats (41624)

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Abstract. Arginine vasotocin was injected into the third ventricle or intravenously in conscious, ovariectomized rats and its effect on gonadotropin and prolactin release evaluated. The peptide lowered plasma levels of both LH and prolactin in doses of 40 or 100 ng given intraventricularly. The higher dose was slightly more effective than the lower dose. Intravenous injection of a 1-μg dose of vasotocin failed to alter plasma LH in the ovariectomized animals; however, a 5-μg dose induced a slight depression apparent at only 60 min following injection. Intravenous injection of 1 μg produced a significant lowering of plasma prolactin, whereas a dramatic lowering followed the injection of the higher dose. Plasma FSH was unaffected in these experiments. Incubation of dispersed anterior pituitary cells from ovariectomized rats with various doses of vasotocin revealed no effect of the peptide on the release of FSH, LH, or prolactin. It also did not alter the response to LHRH, but it partially blocked the action of dopamine to inhibit prolactin release. The data indicate that quite low doses of arginine vasotocin act within the brain to inhibit LH and prolactin secretion in ovariectomized, conscious animals.

Arginine vasotocin (AVT), a nonapeptide with antigonadotropic properties, has been detected in the pineal gland of a number of mammalian species including the human fetus (1–5). Peripheral (micrograms) or intraventricular (picograms) injection of AVT suppressed the preovulatory surge of LH and inhibited subsequent ovulation in proestrous rats (6, 7). AVT also induced a marked suppression of the prolactin surge during proestrus (8). The present study was undertaken to determine if AVT is active in the conscious, ovariectomized rat to alter plasma prolactin and the elevated gonadotropin levels found after removal of gonadal steroid feedback.

Material and Methods. Virgin, female Spraque–Dawley rats (Simonsen Laboratories, Gilroy, Calif.) weighing 200–220 g were housed under controlled conditions of light and temperature (lights on 0500–1900 hr) with free access to Purina rat chow and water. They were ovariectomized (OVX) while lightly anesthetized with ether and used for the experiments 3–4 weeks later.

In vivo studies. Four to 6 days prior to experimentation, a 23-gauge stainless-steel cannula (17 mm length) was implanted into the third ventricle and indwelling Silastic catheters fitted into the external jugular vein as described earlier (9). On the day of the experiment, the animals were transferred in their individual cages to a quiet laboratory and a polyethylene extension tube (PE 50, 12 in. long) filled with a solution of heparin in 0.9% NaCl was attached to the distal end of the jugular cannula. A preinjection blood sample was withdrawn 30–60 min later and intraventricular injections performed. Postinjection samples were drawn at different intervals while the animal was freely moving in the cage. The volume of each sample was replaced after each bleeding by an equal volume of 0.9% NaCl.

Arginine vasotocin (A-Grade, Lot No. 500308 and 602238, Calbiochem, San Diego, Calif.) was freshly dissolved in 0.9% NaCl and injected into the third ventricle in a volume of 2 μl at a dose of 40 or 100 ng. Controls received an equal volume of saline. To determine whether the effects of intraventricular AVT on gonadotropin and Prl release were mediated centrally, AVT at doses of 1 and 5 μg in 0.1 ml 0.9% NaCl was injected systemically by iv, pulse injection. The first injection was given immediately after the removal of a preinjection blood sample. Similarly, the second iv injection was given after the 60-min

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sampling. In all cases the injection time was about 60 sec and all experiments were performed in the morning between 0900 and 1100 hr.

Heparinized blood samples (0.9 ml) were collected from the external jugular vein cannula at varying intervals. Plasma was separated by centrifugation at 4°C and stored frozen until the day of assay. Plasma LH concentrations were measured by the RIA procedure of Niswender *et al.* (10) using RP-1 rat pituitary LH reference standard and LH titers were expressed in terms of the NIH-LH-S1 reference preparation. Plasma FSH and prolactin (PRL) were measured by the respective RIA kits supplied by NIAMDD for each hormone and the results were expressed in terms of the RP reference standard provided with the kit. To eliminate interassay variation, all samples from each experiment were run in one assay at two different dilutions, each in duplicate.

Statistical significance of the results was evaluated by the paired *t* test for sequential changes within the same group and by Student's *t* test for differences between two groups for a particular time.

In vitro studies. Anterior pituitaries were removed after decapitation and dispersed in the presence of trypsin (Difco) as previously described (11). Cells were incubated overnight (37°C) in medium 199 containing 20 m*M* *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES buffer, Gibco). Cells were then pelleted on the day of experimentation and preincubation media removed. The cells were subsequently resuspended and incubated for 2 hr (37°C) in either 1 ml medium 199 (0.1% bovine serum albumin, 1% penicillin–streptomycin, 20 m*M* HEPES buffer, 2 $\times 10^{-5}$ *M* bacitracin) or 1 ml of the same medium containing AVT (Bachem, R1750) in log doses ranging from 10^{-5} to 10^{-8} *M* or in the presence of 10^{-6} *M* thyrotropin-releasing hormone (TRH) or 10^{-7} *M* luteinizing hormone-releasing hormone (LHRH) as peptide controls (Peninsula). In two additional experiments the possible interaction effects of dopamine (Sigma) and AVT and LHRH and AVT were similarly tested. Incubations were terminated by centrifugation (25°C, 10 min, 600*g*) and media stored frozen until measurement of hormone content. Results were expressed in terms of nanograms hormone released per 10^5 cells and data were analyzed by means of the Student's *t* test.

Results. *Behavioral changes after intraventricular AVT.* Intraventricular injection of 40- or 100-ng doses of AVT induced "barrel rolling" within 2 min of injection, in which the animals revolved longitudinally usually to the left side for about 2–3 min. In some cases the rat would rotate first to the right side and then to the left. Systemic injection of the peptide in doses of 1 or 5 μg produced no such behavioral effects.

Initial plasma hormone levels in OVX rats. As expecteld, plasma LH and FSH titers were significantly elevated compared to basal levels in normal female rats in this laboratory. On the other hand, plasma PRL was relatively low in OVX animals compared to values in normal females. Plasma hormone levels were not altered by third ventricular injections of 2 μl of 0.9% NaCl or by iv injections of 0.1 ml saline (Figs. 1–4).

Intraventricular injection of 100 ng AVT produced a significant suppression in plasma LH within 15 min which persisted for the 120-min duration of the experiment. The lower dose of 40 ng also suppressed LH, but this effect was significant and maximal at 30 min and had disappeared by 120 min (Fig. 1).

Third ventricular injection of AVT (40 or 100 ng) produced a dramatic decline in plasma

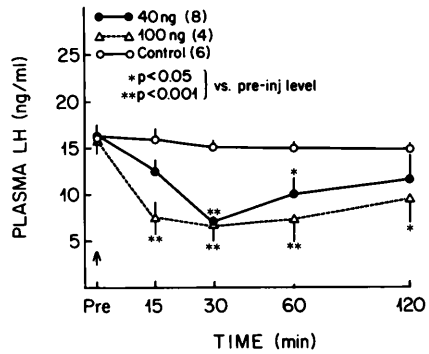

FIG. 1. Plasma LH levels in conscious OVX rats after third ventricular injection of 0.9% NaCl and/or 8-Arginine vasotocin. In this and subsequent figures arrows indicate the time of injection, vertical lines above or below the mean represent the SEM, and numbers in parentheses indicate number of animals in each group. Preinjection values for all groups were pooled and represented as mean ± SEM.

PRL 15 min after injection. Prolactin remained low for the duration of the experiment (Fig. 2). There was no significant difference between the decrease produced by either dose except that the effect with the 100-ng dose was still highly significant at 120 min after injection ($P < 0.001$ for 100 ng and $P < 0.05$ for 40 ng).

Effects of intravenous AVT. A single iv injection of 1 μg or a subsequent infusion of an additional 1 μg at 60 min after the first injection produced no significant effect on plasma LH, while a dose of 5 μg caused significant lowering of LH ($P < 0.05$) at 60 min (Fig. 3). Plasma PRL levels, on the other hand, were significantly reduced at 30 min ($P < 0.05$) by both doses at iv AVT. Prolactin levels declined further ($P < 0.001$ vs 30 min or control) to very low values at 60 and 120 min after the 5-μg dose. Prolactin levels began to rise after 30 min in rats injected with 1 μg. However, a second infusion of the 1-μg dose at 60 min again decreased PRL to levels comparable to those obtained with the 5-μg dose by 120 min (Fig. 4).

No significant changes in plasma FSH levels were observed after any of the third ventricular or systemic doses of AVT employed in the present study (data not shown).

In vitro studies. Arginine vasotocin failed to alter significantly the *in vitro* release of LH, FSH, or PRL in two separate experiments (Table I); however, these cell preparations did respond to the peptide controls. In both experiments (Table I), 10^{-6} *M* TRH significantly ($P < 0.001$) stimulated PRL release. In Experiment 2, significant stimulations of LH and FSH release were seen in the presence of LHRH.

Two additional dispersed-cell preparations were tested and again no direct action of AVT (10^{-5} and 10^{-6} *M*) to alter PRL (Table II) or LH and FSH (Table III) releases was seen. However, AVT (10^{-5} *M*) was capable of significantly ameliorating, but not totally reversing, the potent PRL-inhibitory effect of 10^{-6} *M* DA in both experiments (Table II).

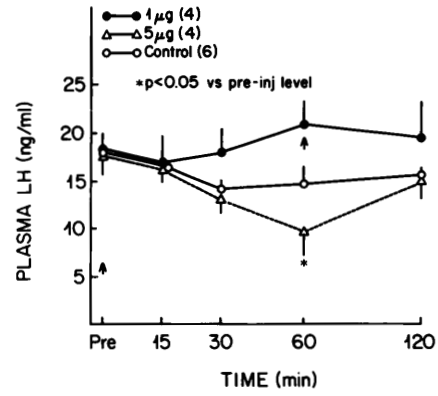

FIG. 3. Plasma LH levels in conscious OVX rats after iv pulse injection of saline and/or arginine vasotocin. Arrow at 60 min in this figure and in Fig. 4 indicates a second injection of a 1-μg dose.

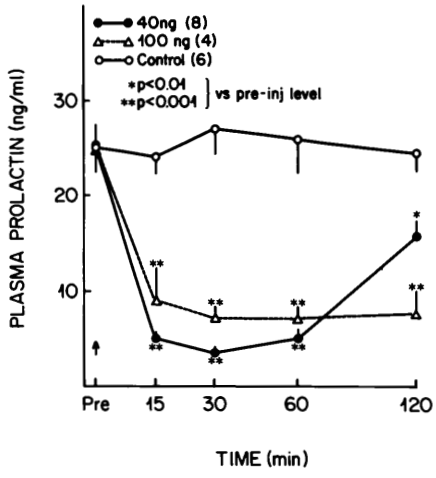

FIG. 2. Effects of intraventricular injection of saline or arginine vasotocin on plasma PRL levels.

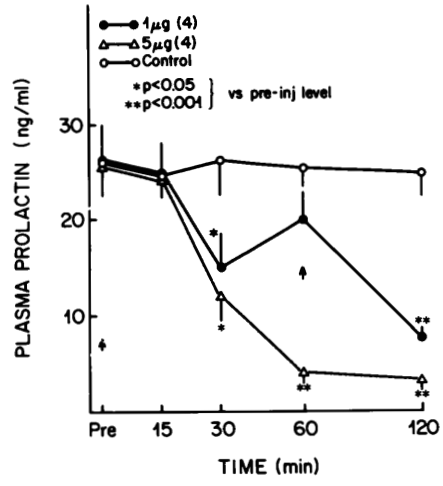

FIG. 4. Plasma PRL levels in conscious OVX rats after iv injection of saline and/or arginine vasotocin.

TABLE I. EFFECT OF AVT ON *in Vitro* HORMONE RELEASE (ng HORMONE/10^5 CELLS, $X \pm$ SEM) FROM CELLS DISPERSED FROM ANTERIOR PITUITARIES OF OVARIECTOMIZED FEMALE RATS

Treatment	(*n*)	LH	FSH	PRL
Experiment 1				
Control	14	5.95 ± 0.43	52.8 ± 5.6	97.8 ± 9.0
10^{-5} *M* AVT	14	5.79 ± 0.39	63.7 ± 7.1	102.2 ± 2.2
10^{-6} *M* AVT	14	6.26 ± 0.29	57.1 ± 3.9	103.2 ± 2.6
10^{-7} *M* AVT	14	5.62 ± 0.33	59.4 ± 2.7	101.4 ± 2.4
10^{-8} *M* AVT	14	6.54 ± 0.40	52.5 ± 5.3	107.0 ± 4.5
10^{-6} *M* TRH	9	5.73 ± 0.21	56.2 ± 4.5	120.6 ± 4.9***
Experiment 2				
Control	14	3.47 ± 0.47	92.9 ± 3.5	149.8 ± 9.0
10^{-5} *M* AVT	10	3.49 ± 0.27	86.4 ± 6.8	132.9 ± 19.6
10^{-6} *M* AVT	10	4.45 ± 0.84	90.8 ± 6.8	162.2 ± 22.6
10^{-7} *M* AVT	10	2.88 ± 0.20	77.5 ± 10.1	140.4 ± 11.8
10^{-8} *M* AVT	10	3.37 ± 0.25	78.0 ± 7.0	151.6 ± 21.0
10^{-6} *M* TRH	10	3.31 ± 0.27	88.9 ± 9.0	269.9 ± 33.0***
10^{-7} *M* LHRH	10	18.63 ± 0.89***	173.4 ± 33.1**	181.9 ± 22.8

** $P < 0.01$ versus control, *t* test.
*** $P < 0.001$ versus control, *t* test.

The less potent inhibitory action of 10^{-7} *M* DA was not altered significantly by coincubation with 10^{-5} *M* AVT.

Additionally, coincubation of 10^{-5} *M* AVT with both 10^{-8} and 10^{-9} *M* LHRH did not alter the potent LH-releasing activity of LHRH in either experiment (Table III). Similarly, AVT did not alter the LHRH stimulation of FSH release except for a slight, but significant ($P < 0.05$), diminution of the 10^{-8} *M* LHRH-induced FSH release in the first experiment alone (Table III).

Discussion. Arginine vasotocin-induced barrel rotation has been reported earlier in mice (12) but not rats. This type of behavioral change has been observed previously, after injection of somatostatin into the third ventricle (13) or lateral ventricle (14) of unanesthetized rats. It is interesting that the rats usually rotated to the left hand side. This is probably an indication of "handedness" in the rat. The rat may have a dominant hemisphere just as higher animals.

Control microinjections of saline into the ventricle and removal of blood samples from the conscious animals had no effect on hormonal levels, which indicates that the procedure was relatively innocuous. The present

TABLE II. EFFECT OF AVT ON PRL RELEASE IN THE PRESENCE AND ABSENCE OF DOPAMINE (DA): NANOGRAMS PRL RELEASED/10^5 CELLS $X \pm$ SEM

Treatment	(*n*)	Experiment 1	Experiment 2
Control	10	85.75 ± 4.62	100.52 ± 4.40
10^{-5} *M* AVT	7	76.74 ± 7.63	104.48 ± 3.20
10^{-6} *M* AVT	7	82.10 ± 5.49	103.60 ± 2.96
10^{-6} *M* DA	7	24.26 ± 0.49***	72.72 ± 1.92
10^{-6} *M* DA + 10^{-5} *M* AVT	7	33.54 ± 1.33***††	79.92 ± 1.80**†
10^{-7} *M* DA	7	40.87 ± 2.58***	82.24 ± 5.08*
10^{-7} *M* DA + 10^{-5} *M* AVT	7	46.19 ± 3.56***	80.96 ± 5.20*

* $P < 0.02$ compared to control.
** $P < 0.002$ compared to control.
*** $P < 0.001$ compared to control.
† $P < 0.02$ compared to same doses of DA without AVT.
†† $P < 0.001$ compared to same doses of DA without AVT.

TABLE III. EFFECT OF AVT ON LH AND FSH AND ON LHRH STIMULATION OF LH AND FSH RELEASE IN CELLS DISPERSED FROM OVARIECTOMIZED FEMALE RATS: NANOGRAMS HORMONE/10^5 CELLS, $X \pm$ SEM

		LH		FSH	
Treatment	(n)	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Control	10	4.70 ± 0.23	3.66 ± 0.14	52.72 ± 3.93	51.71 ± 4.92
10^{-5} M AVT	7	4.71 ± 0.10	4.13 ± 0.31	49.79 ± 2.36	37.41 ± 5.04
10^{-6} M AVT	7	4.20 ± 0.12	3.69 ± 0.26	48.83 ± 4.71	49.38 ± 5.27
10^{-8} M LRH	7	11.93 ± 1.04**	14.29 ± 0.56**	119.91 ± 4.01**	143.20 ± 14.93**
10^{-8} M LRH + 10^{-5} M AVT	7	10.80 ± 0.29**	13.31 ± 0.37**	107.99 ± 2.08**†	139.91 ± 3.28**
10^{-9} M LRH	7	8.64 ± 0.15**	8.76 ± 0.22**	94.68 ± 1.66**	89.30 ± 12.43*
10^{-9} M LRH + 10^{-5} M AVT	7	8.94 ± 0.25**	8.14 ± 0.20**	85.49 ± 5.58**	108.48 ± 5.29**

* $P < 0.005$ compared to control.
** $P < 0.001$ compared to control.
† $P < 0.05$ compared to 10^{-8} M LRH alone.

results indicate that intraventricular injections of AVT can inhibit pituitary LH and PRL release in conscious OVX rats, probably by influencing the discharge of hypothalamic releasing and/or inhibiting hormones. It has been shown previously that intravenous injection of microgram quantities of AVT or intraventricular infusion of nanogram amounts every hour from 1300 to 1600 hr on the afternoon of proestrus (6, 7) can inhibit the preovulatory LH surge and accompanying ovulation in proestrous rats. The preovulatory surge of prolactin was also blocked in the proestrous animal but the FSH surge was unaffected. Furthermore, AVT prevented the LH surge which occurs in the OVX, estradiol treated animal without altering FSH levels (15). The present results indicate that AVT has similar actions following its intraventricular injection in the OVX animal and can suppress the elevated LH release in the castrate. The action is presumably mediated by a suppression of LHRH release since there was little effect of much higher doses of AVT given iv. Previous studies have shown that AVT does not alter the sensitivity to exogenous LHRH in either OVX or OVX steroid-primed rats (6). As was the case for the preovulatory release (6, 7), AVT failed to block FSH release in the castrate which suggests that FSH may be controlled by a separate releasing hormone and that AVT can suppress release of LHRH but not FSH-RH. Other evidence indicates the probable existence of FSH-RH (16). A previous study (6) indicated that AVT was unable to lower the elevated levels of LH in OVX rats. Arginine vasotocin was administered iv rather than injected into the ventricle in the earlier work (6). Our data indicate that AVT can suppress LH release when injected into the third ventricle in the castrate.

The suppression of prolactin release seen in castrates is in agreement with the previous findings that AVT could block the preovulatory release of prolactin. In this case considerable effect was seen with systemic administration of the peptide. Therefore it is possible that AVT may not only suppress prolactin via hypothalamic action but possibly by a site of action at the pituitary.

The failure of AVT, in four separate cell dispersals (Tables I–III) to alter either LH or PRL release, however, argues for a CNS site of action of the peptide to inhibit the release of these hormones. Additionally, the failure of AVT to antagonize the stimulatory action of LHRH on LH release, or to potentiate the inhibitory effect of DA on PRL release *in vitro* provides even stronger evidence for a hypothalamic site of action of AVT. It is possible that AVT injected iv exerts its effect at the level of the median eminence where there is no blood–brain barrier. Furthermore, the ability of AVT (10^{-5} M) to lessen the inhibitory action of DA (10^{-6} M) on PRL release *in vitro* suggests an opposite action of AVT at the pituitary level. Several reports have appeared in the literature claiming a direct pituitary site of action of AVT to alter LH or PRL release (17–20); however, all of these

studies were extremely preliminary in nature (no replication of experiments) and involved the use of animal donors and culture systems that varied greatly from the one employed in this report. The reproducibility of our findings, however, has been documented herein.

If indeed AVT is present in the rat pineal, it could have a physiological role in suppression of gonadotropin and prolactin release. Its presence has been claimed by both bio- (21) and radioimmunoassay (22); however, recent studies suggest that there may be no AVT in the gland in the rat (23). If this is the case, then the physiological significance of AVT in the rat is diminished.

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