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In Vitro Analysis of the Participation of Oxytocin and Vasopressin in the Gonadotropin Releasing Hormone-Induced Release of LH[1, 2] (40367)

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Both anatomical (1) and physiological (2) evidence suggest that the neurohypophyseal hormones may be involved in the control of the anterior pituitary gland. Cells of the anterior pituitary are exposed to concentrations of vasopressin (AVP) and oxytocin (OT) that are hundreds of times greater than those found in the peripheral circulation (3, 4). One aspect of the question of anterior–posterior pituitary interactions concerns the influence of neurohypophyseal hormones on the secretion of gonadotropins. In fact, pituitary stores of the neurohypophyseal hormones have been found to vary according to the estrous cycle of the rat, the highest concentrations being measured at estrus and proestrus followed by a marked depletion during diestrus (5). In humans, peripheral levels of AVP exhibit a rhythmic pattern during the menstrual cycle (6). An analysis of the menstrual cycle of the monkey revealed a peak of estrogen-stimulated neurophysin, the carrier protein thought to be associated with OT, which coincided with the peaks for estrogen and LH prior to ovulation (4). The hypothesis that there may be some relationship between AVP and gonadotropin releasing hormone (GnRH) was first tested in 1964 by Sakiz and Guillemin (7) who used a bioassay for LH, the ovarian ascorbic acid depletion (OAAD) assay. A dose of synthetic AVP with no activity in the OAAD assay did not further enhance the test response when used in combination with LH. However, when the same dose of AVP was administered concomitantly with hypothalamic extracts, OAAD activity was increased over controls. More recent *in vivo* studies in the rat also suggest an interaction between AVP and GnRH. When a tripeptide identical to the terminal amino acid sequence of AVP was injected into chlorpromazine-blocked proestrus rats, there was an increased number of ova shed 18 hours later in response to GnRH or LH (8). In another experiment (9), lysine vasopressin increased the ovulatory response in immature rats primed with pregnant mare serum gonadotropin. There is some indication, then, that the neurohypophyseal hormones may affect gonadotropin secretion via an interaction with GnRH. The purpose of the present experiments was to clarify the relationship between the neurohypophyseal hormones and GnRH using an *in vitro* approach.

Materials and methods. Anterior pituitary glands from male Wistar rats (170–350 g) were used for all perifusion experiments. Rats were decapitated immediately before each experiment and the pituitary gland was removed. The posterior pituitary was discarded and the anterior pituitary was hemisected. The halved anterior pituitaries (hemipituitaries) were kept at room temperature in fresh Krebs-Hensleit buffer containing 0.2% (w/v) glucose and oxygenated with 95% O_2–5% CO_2 until all the tissue had been collected. Each hemipituitary was then placed in a Tygon tubing chamber (length, 10 mm; inner diameter, 3 mm) which was narrowed at either end by the attachment of glass micropipet tips. Glass wool was inserted into the pipet tip leading to the collection tubes in order to prevent exit of cellular material from the chamber. All hemipituitaries were perifused with control buffer for two hours in a shaking water bath at 34° to obtain a steady baseline secretion of LH. The tissue was continuously perifused at a rate of 0.5 ml/min with Krebs-Hensleit buffer (plus 0.1% gelatin) that was oxygenated and warmed to 34° before com-

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ing in contact with the tissue. Effluent was collected at four minute intervals, kept ice-cold throughout the experiment, and stored at −40° until assayed. After the effluent had been collected for 20 minutes, the pituitary halves were perifused with hormone-containing media. Administration of the hormone was accomplished by quickly transferring the tubing carrying the perifusion media to the test solution without altering the speed of the infusion pump. Hormone treatments were: 0.2 ng/ml GnRH; 0.2 ng/ml GnRH plus AVP (0.02, 0.2, 2, 20 or 200 mU/ml); 0.2 ng/ml GnRH plus OT (0.002, 0.02, 0.2, 2 or 20 mU/ml); AVP alone (0.02, 0.2, 2, 20 or 200 mU/ml); or OT alone (0.02, 0.2, 2 or 20 mU/ml). In some experiments, 2 and 20 mU/ml AVP or 0.02 and 0.2 mU/ml OT were added to the media in the initial 20-min collection period as well as during GnRH treatment. The dose of GnRH (0.2 ng/ml) used here was shown in our previous experiments to cause submaximal release of LH and would, therefore, allow for any possible potentiating effects by AVP or OT to be readily detected. A fresh solution of GnRH was prepared on the day of each experiment. AVP and OT were stored at −40° in a stock solution of 400 U/ml dissolved in 0.3% acetic acid.

Hormones. Synthetic GnRH was obtained from the Hormone Distribution Officer, NIAMDD as Abbott lot 26-306AL. Synthetic AVP (biological activity, 385 U/mg) and OT (no. 5693; biological activity, 425 U/mg) were obtained from Bachem, Inc. Pooled samples from experiments in which hemipituitaries were perifused with different doses of AVP were assayed for ACTH in the laboratory of Dr. J. W. Kendall to confirm biological activity of the hormone preparation. 200 mU AVP (pooled samples from 3 hemipituitaries) caused a 100% increase in baseline ACTH values for the entire 20-min of exposure to the hormone.

Radioimmunoassay. All LH radioimmunoassays were performed according to the method of Niswender *et al.* (10). Each four-minute effluent was assayed in 500 μl duplicates. The sensitivity of the assay was 29.0 pg/tube (n=25) using NIH-LHS18 as the LH standard. Coefficients of variation were 13.4% (n=25) for interassay and 8.2% (n=9) for intra-assay variation.

Analysis of data. In order to eliminate interassay and inter-animal variation, data collected from each hemipituitary were recorded as percent of baseline over time. The baseline was calculated as the mean value of the four samples taken in the 20 min before the hormone-administration period. For statistical analysis, a cumulative value of the percent of baseline data was determined for each hemipituitary in an experimental group. This value was essentially a measure of the area under a hypothetical curve which could be plotted with the values from each individual hemipituitary. The resulting cumulative value was then expressed in terms of percent of baseline attained by each gland per sample collection period. The mean of these final values was used as the standard of comparison between groups. Dunnett's test (11) was used to determine significant differences.

Results. Experiment I. In this experiment (Table I), the responsiveness of the luteotrop to AVP and OT was examined. LH was measured in effluent from rat hemipituitaries perifused with different concentrations of AVP or OT. All hemipituitaries were perifused with control buffer for the first 2 hr of each experiment resulting in a mean steady baseline of 1.68±0.12 (SE)ng LH/ml (n=114). Responses of the hemipituitaries were then followed for 44 min. For the first 20 min, the hemipituitaries in experimental groups were treated with AVP or OT-containing media. For the remaining 24 min all

TABLE I. EFFECT OF DIFFERENT CONCENTRATIONS OF AVP AND OT ON LH RELEASE IN PERIFUSED RAT HEMIPITUITARIES.[a]

Treatments	Neurohypophyseal hormone concentrations (mU/ml)					
	0[b]	0.02	0.2	2.0	20	200
AVP	(4)[c]	(4)	(4)	(3)	(3)	(3)
	91[d]	127*	99	95	87	78
	±7[e]	±17	±9	±13	±6	±16
OT	(4)	(4)	(4)	(3)	(3)	
	91	114*	119*	103	122*	
	±7	±7	±13	±5	±9	

[a] Rat hemipituitaries were perifused first with buffer for a control period to determine baseline then with AVP or OT for 20 min followed by a 24-min washout period.
[b] Control, no hormone added.
[c] Number of hemipituitaries perifused.
[d] Percent of baseline per sample collection period.
[e] Mean ± SE.
* $P < 0.05$.

groups were perifused with hormone-free perifusion media. After most concentrations of AVP, treated hemipituitaries did not differ from controls. However, after 0.02 mU/ml AVP, release of LH was significantly elevated ($P < 0.05$) over the control value (Fig. 1). All concentrations of OT tested, except the 2 mU/ml concentration, were able to induce LH release (Fig. 1).

Experiment II. The purpose of this experiment (Table II) was to investigate the combined effects of neurohypophyseal hormones and GnRH on the release of LH. Tissue was perifused with either GnRH or GnRH plus different concentrations of AVP or OT for 20 min following the two hour perifusion with control buffer. Hemipituitaries stimulated with 0.2 ng/ml GnRH increased baseline LH release ($P < 0.01$) over controls. Doses of AVP ranging in concentration from 0.02 to 200 mU/ml did not alter tissue response to GnRH. Only one concentration of OT, 0.02 mU/ml, potentiated ($P < 0.05$) the LH response to GnRH while concentrations that were tenfold greater or smaller had no effect. A graph of the data (Fig. 2) shows that at this dose OT increased the magnitude and prolonged the release of LH from the hemipituitaries in response to 0.2 ng/ml GnRH.

Experiment III. This experiment was performed to determine if a greater release of LH could be induced by a longer exposure time to the neurohypophyseal hormones. Hemipituitaries were perifused with the hormones according to the schedule described for experiments I and II, but in addition were treated with either AVP or OT in the 20-min period prior to the usual 20 min of GnRH stimulation. This modification altered the response seen after acute exposure to the hormones. The addition of either 2 or 20 mU/ml AVP prior to as well as during GnRH treatment resulted in a mean cumulative percent of baseline per collection period of 233±40 SE (n=4) for the lower and 294±26 SE (n=4) for the higher dose. These values represent a significant ($P < 0.05$ and $P < 0.01$, respectively) and dose-related increase over GnRH control data as seen in Table II. In the same experiment, pre-exposure of the tissue to 0.02 mU/ml OT increased LH production in response to GnRH to 283±39 SE (n=4; $P < 0.01$). Hemipituitaries pretreated with 0.2 mU/ml OT responded to GnRH with a reading of 168±29 SE (n=4) percent of baseline, a value similar to that for tissue perifused with GnRH alone.

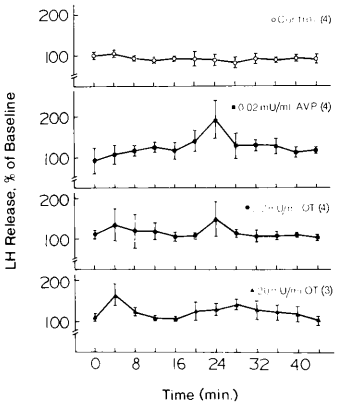

FIG. 1. LH release from perifused rat hemipituitaries which were exposed to buffer only (control) or to different concentrations of AVP alone (0.02 mU/ml) or OT alone (0.2 mU/ml, 20 mU/ml) from 0 to 20 min.

Discussion. Results from *in vitro* studies in which release of anterior pituitary hormones is stimulated by various agents imply that multiple functional receptors may be present on the tropic hormone-producing cells. Our experiments demonstrate that neurohypophyseal hormones can influence LH secretion *in vitro*. Our finding that most concentrations of AVP, when used alone, are ineffective in releasing LH confirms studies by other investigators using *in vitro* pituitary incubation systems (12, 13). The LH releasing ability of 0.02 mU/ml AVP, however, is contradictory to previous reports in which only high concentrations of Pitressin released LH after long incubation periods (14). In an investigation of TSH release by neurohypophyseal hormones in incubated pituitaries, Krass *et al.* (15) also obtained a spike-like dose response curve for AVP when only one of four concentrations of AVP (8×10^{-10} *M* or 0.3 mU/ml) caused significant release after 30 minutes. The failure of our single LH-releasing concentration of AVP to release LH in the presence of GnRH may have been due to the fact that the stimulatory effect of AVP was masked during the GnRH stimulation despite use of submaximal concentrations of the releasing hormone. Preexposure of the tissue to two previously ineffective higher concentra-

TABLE II. EFFECT OF DIFFERENT CONCENTRATIONS OF AVP AND OT ON GnRH-INDUCED RELEASE OF LH FROM PERIFUSED RAT HEMIPITUITARIES.[a]

	Neurohypophyseal hormone concentrations (mU/ml)						
Treatments	0[c]	0.002	0.02	0.2	2.0	20	200
GnRH[b] + AVP	(7)[d]		(6)	(5)	(8)	(8)	(4)
	166**[e] ± 7[f]		188 ± 23	193 ± 19	143 ± 15	180 ± 23	137 ± 13
GnRH[b] + OT	(7)	(5)	(7)	(7)	(6)	(5)	
	166** ± 7	157 ± 7	243* ± 23	165 ± 11	151 ± 11	155 ± 18	

[a] Rat hemipituitaries were perifused first with buffer for a control period to determine baseline, then for 20 min with GnRH alone or GnRH plus either AVP or OT followed by a 24-min washout period.
[b] 0.2 ng/ml.
[c] Treated with GnRH alone, 0.2 ng/ml.
[d] Number of hemipituitaries perifused.
[e] Percent of baseline per sample collection period.
[f] Mean ± SE $*P < 0.05$. $**P < 0.01$ as compared to control in Table I.

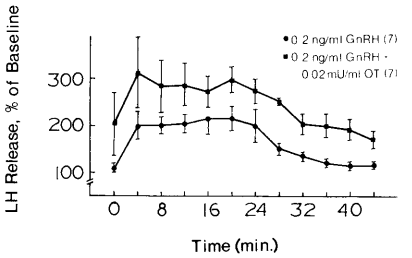

FIG. 2. LH release from perifused rat hemipituitaries which were exposed to 0.2 ng/ml GnRH from 0 to 20 min or to 0.02 mU/ml OT plus the same dose of GnRH from 0 to 20 min.

tions of AVP resulted in a significant enhancement of the subsequent GnRH-induced release of LH. This suggests that AVP may be responsible for the activation of some component of the stimulus-secretion coupling mechanism required for the release of LH from the luteotrop in response to GnRH.

In our *in vitro* system, OT alone was able to raise baseline LH production at most of the concentrations tested. The one ineffective dose, 2 mU/ml, is a concentration which may be borderline between a physiological and a pharmacological concentration. Possibly, the LH response curve for OT is a plateau which drops after 0.2 mU/ml. Krass *et al.* (15) reported a similar pattern for OT stimulation of TSH release when three different concentrations of OT elicited equal responses while lower or higher doses were ineffective. The stimulation of LH release by 20 mU/ml OT may represent a nonspecific response. In a previous investigation, Wilks and Hansel (13), testing a wide range of concentrations, concluded that OT had no LH-releasing ability. However, in their system, degradation of LH would have prevented the detection of the slight increase in LH seen under the conditions of our experiment. Only one concentration of OT in combination with GnRH was effective in releasing LH above control GnRH-stimulated values. When the tissue was preexposed to OT, this same concentration was still effective while a concentration tenfold higher was not, indicating that a precise ratio between GnRH and OT must be attained for a potentiating effect to take place. Although the GnRH-potentiating effect of OT is highly concentration dependent it is less so, in our pre-exposure experiments, for AVP. Preexposure of the anterior pituitary tissue appears to be necessary for the enhancement of the GnRH-induced release of LH by AVP whereas this pretreatment is not necessary in the case of OT. OT acts in concert with GnRH while AVP must act prior to GnRH, preparing the luteotrop for the stimulating effects of the releasing hormone.

It has been proposed that the modification of enzymatic degradation of peptide hormones at receptor sites could be one possible mechanism for the regulation of their actions (16). The fact that OT and GnRH are inactivated by the same peptidase has led to the hypothesis that elevations of OT in hypothalamic and portal blood during proestrus may enable GnRH stores to increase sufficiently to initiate the LH surge (17). This may be one of the mechanisms whereby OT enhances and/or prolongs the effect of GnRH on LH release in our pituitary perifusion system.

It is evident from the present experiments

that AVP and OT augment the GnRH-induced release of LH by the pituitary *in vitro*. The purpose of these and other peptide–peptide interactions might be the attainment of finer levels of control of secretion which would not be possible through only one hormone. The ability of AVP and OT to act alone leaves open the possibility of a completely independent mechanism although this might reflect an interaction with endogenously produced GnRH already occupying the receptor.

The results of the present studies show that AVP and OT can affect the release of LH and the responsiveness of the luteotrop to GnRH. Although evidence for participation of AVP and OT in the reproductive cycle is still not conclusive, the relationship between the neurohypophyseal hormones and GnRH warrants further investigation.

Summary. The pituitary perfusion technique was used to investigate the possible interaction between the neurohypophyseal hormones and gonadotropin releasing hormone (GnRH). The perifusion of rat anterior pituitaries with 0.2 mU/ml arginine vasopressin (AVP) resulted in a significant ($P < 0.05$) increase in LH release over baseline, while higher doses had no effect. When combined with GnRH, this and higher concentrations of AVP did not alter the GnRH-induced release of LH. Three concentrations of oxytocin (OT): 0.02, 0.2 and 20 mU/ml, increased baseline secretion of LH ($P < 0.05$) while 2 mU/ml OT did not. When added to GnRH-containing perifusion media, 0.02 mU/ml OT caused significant ($P < 0.05$) enhancement and prolongation of the LH response to GnRH. All higher concentrations of OT and one concentration that was tenfold lower, did not exhibit potentiating effects. When the pituitary tissue was pretreated with AVP or OT prior to stimulation with GnRH, only the same concentration of OT (0.02 mU/ml) was effective ($P < 0.01$) while two concentrations of AVP (2 and 20 mU/ml) which had been ineffective previously, then enhanced the LH release due to GnRH ($P < 0.05$ and $P < 0.01$, respectively). It is proposed that the data from these experiments support the hypothesis that AVP and OT may have a role in reproduction via an interaction with GnRH at the level of the anterior pituitary.

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