INFLUENCE OF THE TRIPEPTIDE, PRO-ARG-GLY-(NH₂), ON OVULATION INDUCED WITH LH OR SYNTHETIC LH-FSH RELEASING HORMONE IN CHLORPROMAZINE-TREATED PRO-OESTROUS RATS

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Vasopressin increases the ovulatory response to PMSG in immature rats (de la Lastra & Forcelledo, 1970). This effect of vasopressin on ovulation, as well as the similarity between the three terminal amino acids of arginine-vasopressin (AVP) and LH-FSH releasing hormone (LH-FSH-RH), induced us to study the influence of the peptide, Pro-Arg-Gly-NH₂ (PAGA), which is the terminal fragment of AVP, on the ovulation induced in adult rats with LH or LH-FSH-RH.

Both LH-FSH-RH and PAGA were synthesized by the solid-phase method, using standard techniques (Stewart & Young, 1969). The normal chloromethylated 1% cross-linked polystyrene was used as support, and α -amino groups were blocked with the tert-butyloxycarbonyl group. Toluenesulphonyl blocking groups were used for histidine- and arginine-side chains, and benzyl groups for serine and tyrosine. Peptides were removed from the resin by ammonolysis and remaining blocking groups were removed with liquid hydrofluoric acid. The products showed the expected electrophoretic mobility and amino acid analysis after purification.

Sprague–Dawley rats were kept under light conditions from 07.00 to 21.00 hours and injected with chlorpromazine (1 mg/100 g body weight) at 10.00 hours on the day of pro-oestrus to inhibit spontaneous ovulation. Bovine LH (NIH-LH) or synthetic LH-FSH-RH was injected intravenously the same day between 13.00 and 14.00 hours, which is the critical period in our colony of rats. Injections of PAGA were given at various times before or after LH or LH-FSH-RH. The rats were killed with ether 18 hr later and the ova in the oviducts were counted according to the technique of Rowlands (1942). The ovaries and uteri were carefully dissected clean of surrounding tissues and weighed on a torsion balance to the nearest 0.1 mg. Uteri were dried by blotting on filter-paper before weighing. The number of ova found in the different experimental groups was compared by the Wilcoxon test for two unpaired samples (Wilcoxon, 1945, 1947). Ovarian and uterine weights were compared by Student's *t* test.

Table 1. Ovulation induced with synthetic LHF-SH-RH in chlorpromazine-treated pro-oestrous rats

Treatment (per 100 g body wt)	Ovarian wt (mg) $\pm$ S.E.	Uterine wt (mg) $\pm$ S.E.	No. of ovulating rats/no. of treated rats	Mean no. of ova/rat	P
LH-FSH-RH, 0.1 μ g	69.4 $\pm$ 3.2	335.9 $\pm$ 22.0	10/18	4.5	0.023
LH-FSH-RH, 0.2 μ g	62.0 $\pm$ 3.6	341.1 $\pm$ 19.2	9/10	9.6	
LH-FSH-RH, 0.4 μ g	67.9 $\pm$ 4.1	378.9 $\pm$ 40.1	7/7	12.1	0.111
LH, 0.5 μ g	77.7 $\pm$ 6.7	342.8 $\pm$ 33.2	9/10	5.9	
LH, 1.0 μ g	61.4 $\pm$ 3.2	329.4 $\pm$ 13.8	20/23	9.2	0.031

The number of ova were compared by the Wilcoxon test for two samples.

Ovulation was induced with 0.1, 0.2 or 0.4 μ g LH-FSH-RH (Table 1). The number of ovulations induced with 0.2 μ g LH-FSH-RH and with 1 μ g LH/100 g was practically the same as that found in spontaneous ovulation (10.7 ± 0.5 ova/rat).

When PAGA was injected 5 or 10 min after 0.1 μ g LH-FSH-RH, the ovulatory response was increased. This effect was not observed when PAGA was

Table 2. Influence of Pro-Arg-Gly-NH₂ on ovulation induced with synthetic LH-FSH-RH in chlorpromazine-treated pro-oestrous rats

LH-FSH-RH (μ g/100 g)	Treatment Addition†	Ovarian wt (mg) $\pm$ S.E.	Uterine wt (mg) $\pm$ S.E.	No. of ovulating rats/no. of treated rats	Mean no. of ova/rat	P
0.1	None	69.4 $\pm$ 3.2	335.9 $\pm$ 22.0	10/18	4.5	0.045 0.034
0.1	+ PAGA (-1 min)	65.0 $\pm$ 3.6	338.1 $\pm$ 31.0	5/7	4.7	
0.1	+ PAGA (+5 min)	68.0 $\pm$ 3.8	310.3 $\pm$ 34.9	8/9	8.6	
0.1	+ PAGA (+10 min)	70.1 $\pm$ 5.3	309.2 $\pm$ 40.8	7/8	10.0	
0.1	+ PAGA (+15 min)	80.5 $\pm$ 5.9*	347.7 $\pm$ 34.4	4/8	4.0	
0.1	+ PAGA (+120 min)	63.7 $\pm$ 3.4	317.4 $\pm$ 42.7	3/4	4.5	
0.2	None	62.0 $\pm$ 3.6	341.1 $\pm$ 19.2	9/10	9.6	10.2 10.2 10.2
0.2	+ PAGA (-1 min)	79.8 $\pm$ 8.3**	378.2 $\pm$ 35.8	5/5	10.2	
0.2	+ PAGA (+15 min)	83.1 $\pm$ 5.3***	311.3 $\pm$ 20.2	7/7	10.2	
0.2	+ PAGA (+120 min)	72.1 $\pm$ 4.8	305.0 $\pm$ 36.3	5/5	10.2	

PAGA = Pro-Arg-Gly-NH₂, 1 μ g/100 g. The numbers of ova were compared by the Wilcoxon test for two samples; ovarian and uterine weights were compared by Student's *t* test. Comparisons were made between the rats injected with LH-FSH-RH and the rats which received LH-FSH-RH+PAGA.

* $P < 0.05$. ** $P < 0.02$. *** $P < 0.01$.

† Times in parentheses are before or after injection of LH-FSH-RH.

injected 15 or 120 min after LH-FSH-RH. No increase in the number of ovulations was observed when the tripeptide was injected 1 min before, or 15 min after, the injection of 0.2 μ g LH-FSH-RH (Table 2).

Rats injected with PAGA 15 min after 0.1 and 0.2 μ g LH-FSH-RH showed a significant increase in their ovarian weights, an effect which was not observed when 120 min had elapsed between the injection of the two substances.

In other rats, ovulation was induced with 1 μ g bovine LH (approximately 0.3 μ g/100 g). The tripeptide was injected 1 or 15 min after, or 15 min before, LH (Table 3). When the tripeptide was injected 1 min after LH, the ovulatory

response increased significantly, reaching the number of ova found in spontaneous ovulation; the increase was not significant when the tripeptide was injected 15 min after LH.

The ovulatory response was also increased when the tripeptide was injected 15 min before LH.

No change in ovarian weight was observed in the rats treated with LH and PAGA.

Ovulation was induced with LH-FSH-RH or LH in normal pro-oestrous rats treated with chlorpromazine. The ovulatory response to 0.1 µg LH-FSH-RH increased when the tripeptide was injected 5 or 10 min later. This effect was not observed when the tripeptide was injected 1 min before or later than 10 min after LH-FSH-RH. This time relationship suggests that the tripeptide potentiates the activity of the LH already released rather than the release of ovulating hormone.

The short half-life of LH offers an explanation for the lack of influence on

Table 3. Influence of Pro-Arg-Gly-NH₂ on LH-induced ovulation in chlorpromazine-treated pro-oestrous rats

<i>Treatment*</i>	<i>Ovarian wt (mg) ± S.E.</i>	<i>Uterine wt (mg) ± S.E.</i>	<i>No. of ovulating rats/no. of treated rats</i>	<i>Mean no. of ova/rat</i>	<i>P</i>
LH	67.1 ± 2.6	397.2 ± 22.0	9/14	3.5	
PAGA	74.3 ± 5.3	418.4 ± 35.2	2/6	2.2	
LH + PAGA (−15 min)	73.9 ± 7.9	320.4 ± 20.4	6/6	12.2	0.003
LH + PAGA (+1 min)	76.0 ± 3.7	371.5 ± 37.2	7/7	12.9	0.001
LH + PAGA (+15 min)	75.9 ± 7.1	369.9 ± 39.7	4/4	7.8	0.217

LH, 1 µg/rat. PAGA = Pro-Arg-Gly-NH₂, 1 µg/rat. *P* values were calculated by the Wilcoxon test for unpaired experiments, comparing the number of ovulations in rats injected with LH and in rats injected with LH + PAGA.

* Times in parentheses are before or after injection of LH.

LH-FSH-RH-induced ovulation when the tripeptide is injected 1 min before or 15 min after the LH-FSH-RH. We may assume that at these times, the level of LH present in the circulation is very low. In keeping with this idea is the increase in LH-induced ovulation produced when the tripeptide was injected immediately after LH, but not 15 min after it (Table 2 and 3).

The tripeptide did not increase the ovulatory response to 0.2 µg LH-FSH-RH. We may explain this fact because the ovulations induced with that amount are near the maximum possible, since the number of ova shed corresponds to the number of mature follicles ready to ovulate at pro-oestrus (10.7 ± 0.5 ova/rat in our colony). Nevertheless, an increase in ovarian weight was observed in the rats receiving the tripeptide 15 min after the injection of 0.1 µg LH-FSH-RH and 1 to 15 min after 0.2 µg LH-FSH-RH. This effect on ovarian weight was not shown in the rats receiving LH and the tripeptide. Ovarian growth suggests FSH activity. This effect of the tripeptide may be explained either by an increased release or by potentiation of the FSH already released by LH-FSH-RH. The time relations of these phenomena suggest that the second possibility is more likely.

The facts reported here suggest that the terminal tripeptide of arginine-vasopressin is able to potentiate the activity of both FSH and LH on rat ovaries.

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