SUPPRESSION OF GONADOTROPIN SECRETION AND PREVENTION OF OVULATION IN THE RAT BY ANTISERUM TO SYNTHETIC GONADOTROPIN-RELEASING HORMONE

Y. Koch, Prakong Chobsieng, U. Zor, M. Fridkin* & H. R. Lindner

Departments of Biodynamics and Chemistry*, the Weizmann Institute of Science

Rehovot, Israel.

Received September 20, 1973

SUMMARY

Administration of an antiserum (0.10-0.25 ml/rat) to the synthetic decapeptide "luteinizing hormone releasing hormone" (LH-RH) suppressed the cyclic surge of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in proestrous rats and prevented ovulation; exogenous LH reversed the block of ovulation. Serum prolactin levels remained unaffected. In ovariectomized rats, the antiserum suppressed the elevated serum levels of both gonadotropins. These findings are compatible with the view that the synthetic decapeptide is identical with the natural hypothalamic hormone that regulates the secretion of both LH and FSH.

INTRODUCTION

The release of gonadotropins from the pituitary gland is believed to be controlled by a neurohumoral secretion which reaches the pituitary by way of the hypothalamo-pituitary portal system. A decapeptide has been isolated from the hypothalamus and subsequently synthetized, that stimulates the release of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (1, 2). This substance has been designated LH-releasing factor (LH-RF) or LH-releasing hormone (LH-RH). Some workers, however, propose that two distinct hypothalamic factors may be responsible for the release of LH and FSH (3).

If LH-RF is indeed a naturally secreted hormone, it should be possible to neutralize its biological effects, e.g. the release of one or both gonadotropins and the induction of ovulation in suitable experimental animals, by administration of an antiserum directed against the synthetic decapeptide.

MATERIALS AND METHODS

Antiserum. The synthesis of an antigenic bovine-serum-albumin-LH-RH conjugate and the properties of antisera generated by this antigen in rabbits have been described (4). The titre of the antiserum used (No. 4) was 1:200,000 against iodinated LH-RH (about 50-100 pg/assay tube). Binding of LH-RH was not inhibited by any of the known hypothalamic or pituitary peptide hormones (4).

Passive immunization experiments. Wistar-derived rats of the departmental colony were housed in air-conditioned quarters illuminated between 5.00 a.m. and 19.00 p.m. Pelleted food (Ralston Purina Co.) and water were offered without restriction. Vaginal smears were examined daily. Proestrous rats (three-month-old, 180 - 245 g body wt.) were used after completion of two normal 4-day-cycles. Ovariectomized rats (one-year-old, 280-325 g body wt.) were used ten months after the operation.

Antiserum to LH-RH or normal rabbit serum (NRS) were administered by intraperitoneal (i.p.) injection as specified in Tables 1, 2 and 3. Ovine LH (NIH-LH-S18; 10 μg/rat) was administered by subcutaneous (s.c.) injection at 14.30. On the morning of estrus the rats were killed by cervical dislocation. The oviducts were excised, scrutinized under a stereoscopic microscope (x20) and the ova, if present, were counted.

Radioimmunoassay. Blood samples were collected by cardiac puncture under light ether anesthesia. Serum levels of LH, FSH and prolactin were determined by radioimmunoassay as described by Daane & Parlow (5), using reagents supplied by the National Institute of Arthritis and Metabolic Diseases (NIAMD) through the courtesy of Dr. A.F. Parlow. The results are expressed in terms of the reference preparations NIAMD-Rat-LH-RP-1; NIAMD-Rat-FSH-RP-1, and NIAMD-Rat-prolactin-RP-1, respectively.

RESULTS

Prevention of Ovulation by anti LH-RH Serum. Anti LH-RH serum was injected at

Table 1. Effect of Anti LH-RH on Ovulation

Treatment		Response	
Anti LH-RH (ml)	Exogenous gonadotropin	Rats ovulating /rats treated	Number of ova /ovulating rat (mean)
-	-	25/28	11.6
1.0	-	0/6	-
0.5	-	0/5	-
0.25	-	0/14	-
0.15	-	0/7	-
0.10	-	1/5	10
0.05	-	4/5	11.7
0.025	-	5/5	13.5
0.25	10 μg LH	6/6	12.8

Rats were injected i.p. at 12.00 on the day of proestrus with either NRS (0.25-1.0 ml) or antiserum against synthetic LH-RH. Ovine LH was injected s.c. at 14.30 where indicated. The rats were sacrificed on the morning of estrus and the number of shed ova in the oviduct was determined.

12.00 on the day of proestrus. A single injection of 0.15-1.0 ml/rat blocked ovulation completely and 0.10 ml of antiserum blocked ovulation in 4 out of 5 rats; lower dose rates were ineffective. This blockade of ovulation could be overcome by injection of LH (Table 1).

Suppression by anti LH-RH serum of cyclic surge of LH and FSH. Rats were injected i.p. with the antiserum to LH-RH at 12.00 on the day of proestrus; control rats received an injection of normal rabbit serum. The animals were bled by cardiac puncture on the same day between 18.00 and 18.30, the expected time of the pre-

Table 2. Effect of anti LH-RH on LH, FSH and prolactin levels in proestrous rats

Treatment		Serum level (ng/ml ± SEM)		
Anti LH-RH (ml/rat)	No. of Rats	LH	FSH	Prolactin
-	10	642.5±143.5	406.2 ±44.06	236.1±27.9
0.25	4	<40*	not measured	230.0±23.8
0.15	7	<40	<100*	246.1±20.9
0.10	5	<40 (4); 460 (1)	<100 (4); 260 (1)	274.0±49.9
0.050	5	471.7 ±186.7	274.8±53.5	Not measured
0.025	5	958.0±285.0	309.4±25.2	Not measured

* Below sensitivity of the assay

Rats were injected i.p. at 12.00 on the day of proestrus with either NRS (0.25 ml: controls) or antiserum against synthetic LH-RH. Blood samples were taken by heart puncture at 18.00 - 18.30 and serum levels of LH, FSH and prolactin were determined by radioimmunoassay. For details see Materials and Methods.

ovulatory gonadotropin surge in our colony. The sera were assayed for LH, FSH and prolactin. Administration of anti LH-RH serum at dose levels of 0.10 ml/rat or higher suppressed the afternoon serum levels of LH and FSH to values below the sensitivity of the radioimmunoassay, whereas rats injected with NRS showed the normal proestrous peak (Table 2). The anti LH-RH serum had no effect on serum prolactin levels.

Suppression of serum LH and FSH levels in ovariectomized rats by antiserum to LH-RH . The serum levels of LH, and particularly of FSH, were strikingly elevated ten months after ovariectomy (Table 3). Following intraperitoneal administration of anti LH-RH serum (0.10 to 0.25 ml/rat), there was a highly significant reduction in the level of both gonadotropins (Table 3): at the higher dose level, serum LH fell below the level of sensitivity of the assay within 7 h and remained so for at least 24 h;

Table 3. Effect of anti LH-RH on LH and FSH levels in Ovariectomized Rats

Treatment			Serum Gonadotropins	
Anti LH-RH (ml/rat)	Rats (No)	Time after treatment (h)	LH (ng/ml±SEM)	FSH (ng/ml±SEM)
0	8	7	520.2 ±32.36	1070.8 ±52.9
0.10	9	7	122.7 ±8.13	467.6±103.8
0.25	7	7	<40*	576.7±59.8
	10	24	<40	134.2±9.1

* Below sensitivity of the assay

Ovariectomized rats were injected i.p. with either NRS (0.25 ml; controls) or antiserum against synthetic LH-RH. Blood samples were taken by heart puncture after the time interval indicated and serum levels of LH and FSH were determined by radioimmunoassay.

the FSH level was halved at 7 h and continued to decline to one-eighth of the control level 24 h after the injection.

DISCUSSION

A number of substances other than LH-RH, some clearly pharmacological (e.g. clomiphene) but others naturally occurring in the hypothalamus (e.g. dopamine and prostaglandin E_2), are able to elicit LH release (6, 7, 8). The identity of the synthetic decapeptide with natural LH-RH present in hypophysial stalk blood has not been rigorously established (9). The present experiments were therefore designed to answer two questions: (i) whether the decapeptide LH-RH synthetized by the groups of Schally and of Guillemin can be regarded as a true hypothalamic hormone engaged in the physiological regulation of gonadotropin secretion by the pituitary; and (ii) whether it serves to control the release of FSH as well as LH. The latter point in particular is still the subject of much controversy (9, 10, 11, 12).

The results obtained suggest that the two questions posed can both be answered in the affirmative. Administration of an antiserum raised against synthetic LH-RH to proestrous rats prevented ovulation (Table 1). This finding is in accord with a brief report (13) which appeared while this manuscript was in preparation. The ovulation-blocking action of the antiserum could be overcome by exogenous LH, indicating that this effect of the antiserum is due to prevention of LH-release. At dose-levels that prevented ovulation, the anti LH-RH serum also abolished the rise in serum LH and FSH levels normally occurring on the afternoon of proestrus. At the same time, the elevated serum prolactin levels remained unaffected (Table 2). Pituitary gonadotropin secretion is greatly augmented following removal of the negative feedback exerted by the gonadal steroids. When the antiserum to LH-RH was administered to ovariectomized rats, this effect was clearly reversed: the characteristically high post-castration serum levels of both gonadotropins were significantly reduced within 7 h by the antiserum. When the higher dose level was used (0.25 ml antiserum per rat), LH became undetectable at this time ($<8\%$ of initial level) and FSH declined to 13% of its initial value 24 h after injection (Table 3). The differential rate of decline in the serum levels of the two gonadotropins following administration of the antiserum reflects the known difference in their metabolic clearance rates (14). The efficiency of the antiserum in neutralizing the physiological action of LH-RH seems remarkable when considering that the antibody has to intercept the hormone during its brief passage from the median eminence to the pituitary gland, unless the hormone is also exposed to antibody on the surface of its target cell or its cell of origin.

The plausible conclusion from these experiments that the synthetic decapeptide LH-RH is identical with the hypothalamic hormone regulating both LH and FSH secretion must be tempered by the qualification that the antiserum used was not absolutely specific for this decapeptide. While there was no cross-reaction with prolactin-inhibiting or releasing hormone (Table 2), nor with TSH-releasing hormone,

melanocyte-inhibiting hormone, vasopressin, oxytocin and some analogues of the decapeptide, it did show partial cross-reaction with a few synthetic oligopeptides (4). Nevertheless, until contrary evidence is available, the designation "gonadotropin-releasing hormone" (Gn-RH) for the decapeptide seems justified.

Acknowledgements

We are grateful to Mr. Shalom Yosef for skilful management of the animal colony, to the Endocrinology Study Section, NIAMD, for a gift of ovine LH, and to the Ford Foundation and the Population Council Inc., N.Y., for generous financial support. H.R.L. is the Adlai E. Stevenson III Professor of Endocrinology and Reproductive Biology at the Weizmann Institute.

REFERENCES

1. Schally, A.V., Arimura, A., Kastin, A.J., Matsuo, H., Baba, Y., Redding, T.W., Nair, R.M.G., Debeljuk, L. and White, W.F., Science, 173, 1036 (1971).

2. Burgus, R., Butcher, M., Ling, N., Monahan, M., Rivier, J., Fellows, R., Amoss, M., Blackwell, R., Vale, W. and Guillemin, R., C.R. Acad. Sci., Paris, 273, 1611 (1971).

3. Bowers, C.Y., Currie, B.L., Johansson, K.N.G. and Folkers, K., Biochem. Biophys. Res. Commun., 50, 20 (1973).

4. Koch, Y., Wilchek, M., Fridkin, M., Prakong Chobsieng, Zor, U. and Lindner, H.R. Biochem. Biophys. Res. Commun.,

5. Daane, T.A. and Parlow, A.F., Endocrinology, 88, 653 (1971).

6. Keller, P.J., Naville, A.H. and Wyss, H.I., Fert. Steril., 19, 892 (1968).

7. Schneider, H.P.G. and McCann, S.M., Endocrinology, 86, 1127 (1970).

8. Tsafriri, A., Koch, Y. and Lindner, H.R., Prostaglandins, 3, 461 (1973).

9. Ondo, J.G., Eskay, R.L., Mical, R.S. and Porter, J.C., Endocrinology, 93, 205 (1973).

10. Johansson, K.N.G., Currie, B.L., Folkers, K. and Bowers, C.Y., Biochem. Biophys. Res. Commun., 50, 8 (1973).

11. Currie, B.L., Johansson, K.N.G., Folkers, K. and Bowers, C.Y., Biochem. Biophys. Res. Commun., 50, 14 (1973).

12. Sundberg, D.K., Kurlich, L., Fawcett, C.P., Illner, P. and McCann, S.M., Proc. Soc. Exper. Biol. Med., 142, 1097 (1973).

13. Fraser, H.M. and Gunn, A., Nature, 244, 160 (1973).

14. Bogdanove, E.M. and Gay, V.L., Endocrinology, 84, 1118 (1969).