Identity of Prolactin Inhibiting Factor (PIF) and Luteinizing Hormone-Releasing Factor (LRF).* (29549)

ANDREW V. SCHALLY, JOSEPH MEITES, CYRIL Y. BOWERS AND ALBERT RATNER
Endocrine and Polypeptide Laboratories, Veterans Administration Hospital, New Orleans, Department of Medicine, Tulane University School of Medicine, New Orleans, La., and Department of Physiology, Michigan State University, East Lansing

In contrast to all other anterior pituitary tropic hormones, the secretion of prolactin is inhibited by the hypothalamus(1,2). Hypothalamic lesions, pituitary stalk section or transplantation of the pituitary, result in increased synthesis and release of prolactin, as indicated by mammary growth and lactation (1). Meites *et al*(1) demonstrated that cultures of isolated anterior pituitary *in vitro* result in release of prolactin many times in excess of that originally present in fresh anterior pituitary.

When rat anterior pituitaries are incubated *in vitro* by the method of Saffran and Schally (3), addition of a homogenate or acid extract of rat hypothalamus results in a significant reduction in prolactin release into the medium(4). This indicates that the hypothalamus contains a factor which inhibits synthesis and release of prolactin, and which has been designated provisionally as "prolactin inhibiting factor"(4,5).

Several workers have demonstrated that hypothalamic extracts can induce release of luteinizing hormone (LH) from the anterior pituitary(6-9). This factor, designated as luteinizing hormone releasing factor (LRF), has been partially purified from beef and sheep hypothalamus, and has been shown to be active *in vitro* as well as *in vivo*(10). It has been suggested that the same hypothalamic area controls the discharge of both LH and prolactin(11,12), and that the same factor which stimulates LH release also inhibits release of prolactin. To test this hypothesis, partially purified preparations of LRF were characterized on the basis of *in vivo* and *in vitro* tests and were assayed for PIF activity.

Materials and methods. Purification of LRF from 2 N acetic acid extract of bovine and ovine stalk-median eminence tissue was performed by gel filtration on Sephadex G-25, as described previously(13,14,10). LH releasing activity *in vivo* was detected by ovarian ascorbic acid depletion (OAAD) in immature Sprague-Dawley rats pretreated with gonadotropin(15) and by elevation of plasma LH in chronically ovariectomized rats treated with estrogen and progesterone(8). Both the one ovary and two ovary methods were used. In the former ovarian ascorbic acid concentration was compared against controls, and in the latter the results were expressed as percentage depletion (decrease) in ovarian ascorbic acid of the second ovary as compared with the first ovary. For detection of LH releasing activity *in vitro,* anterior pituitary lobes from chronically ovariectomized estrogen progesterone-treated rats were incubated according to the methods of Saffran and Schally(3), and LH release into the medium was assayed by the OAAD method (15). In all tests involving the OAAD method, 5 or more rats were used per point. Other details of incubation and measurement of release of LH *in vitro* are described elsewhere (10).

The release of prolactin *in vitro* was similarly studied using the isolated rat anterior pituitary, except that the incubations were carried out in medium 199(4). Half of each LRF preparation was incubated together with 6 anterior pituitary halves from 3-month-old female rats in one flask, and an equal number of anterior pituitary halves from the same 6 rats were incubated without LRF in another flask. The other half of each LRF preparation was similarly treated. At the end of the 2-hour incubation period, the medium containing the LRF was assayed for prolactin against the medium not containing LRF in the same 4 pigeons, by a paired assay technique(4). A total of 8 pigeons was used to assay each LRF preparation.

* Supported in part by NIH grants AM-07467-01 and AM-04784-04.

TABLE I. Effect of Purified LRF Preparations on *in vivo* Release of LH and *in vitro* Release of Prolactin.

	Ovarian ascorbic acid depletion (OAAD)			Plasma LH activity			Prolactin release by incubated rat pituitary				
								Prolactin, I.U./100 mg pit.*			
Preparation No.	Dose LRF, μg	Ovarian ascorbic acid, mg/100 g*	P *vs* saline	Dose LRF, μg	Ovarian ascorbic acid depletion, mg/100 g*	P *vs* control	Dose LRF, μg	Control medium	Exp medium	% Stimulation or inhibition	P C *vs* E
CY 2-B-6	Saline	99.1 ± 2.2		Control plasma	— 6.6 ± 2.6						
Ovine	125	85.4 ± 3.4	.01	220	—16.4 ± 2.2	.05	2300	1.93 ± .63	1.76 ± .52	— 9.0	.5
AVS-32-19 #67-72	Saline	93.2 ± 4.3		Control plasma	— 6.6 ± 2.6						
Ovine	1300	71.0 ± 3.7	.01	500	—14.8 ± 2.2	.05	12000	1.06 ± .18	1.68 ± .43	+ 68.0	.25
	Saline	88.6 ± 4.4		Control plasma	+ 2.4 ± 1.3						
	760	75.5 ± 2.3	.05	500	— 7.8 ± 1.3	.001	6000	1.93 ± .67	3.42 ±1.2	+ 78.0	.3
AVS-32-19 #75	Saline	104 ± 2.9		Control plasma	90.9 ± 2.8†						
Bovine	750	85.6 ± 1.6	.001	700	70.0 ± 4.5†	.01	4800	1.12 ± .52	2.87 ±1.62	+158.0	.3
AVS-32-49 #70-76	Saline	88.6 ± 4.4		Control plasma	— 6.6 ± 2.6						
Bovine	1100	68.3 ± 5.3	.05	1100	—14.8 ± 3.0	.05	1500	1.31 ± .30	1.55 ± .34	+ 18.0	.5
	Saline	90.9 ± 3.5		Control plasma	+ 2.4 ± 1.3						
	800	73.4 ± 6.5	.05	1100	— 8.6 ± 1.0	.001					

C = Control. E = Experimental. * Values are mean ± S.E. † Tested by one ovary method for direct comparison of ovarian ascorbic acid concentration.

Results and discussion. The results in Table I indicate that the preparations of LRF of bovine and ovine origin were active in depleting ovarian ascorbic acid in immature rats pretreated with gonadotropin, and in elevating plasma LH in chronically ovariectomized estrogen progesterone-treated rats. These two tests show that LRF preparations were clearly effective in releasing LH *in vivo*, at doses of 125-1300 μg. The same preparations were also significantly active *in vitro* in releasing LH from the isolated rat anterior pituitary tissue. Doses of 600-800 μg of purified LRF increased LH release *in vitro* 2- or 3-fold, which is in agreement with results reported previously (10). Thus a dose of 800 μg of CY-2-B-6 stimulated LH release by 204% (95% fiducial limits of the assay 140-290%) and the variance ratio F between stimulated and unstimulated samples was highly significant at 13.3.

Addition of these active LRF preparations to incubation media did not inhibit prolactin secretion by the isolated rat pituitary. In some cases the doses tested for inhibition of prolactin secretion were 10 times larger than doses found active in the LRF tests.

The results suggested that the LRF and PIF activity are not shared by the same molecule. Other data supporting this concept comes from physiological experiments and observations(1).

Summary. Preparations of hypothalamic luteinizing hormone releasing factor (LRF) of bovine and ovine origin, found active on the basis of *in vivo* as well as *in vitro* tests, did not have an inhibitory effect on pituitary prolactin release *in vitro*. The results indicate that LRF and prolactin inhibiting factor (PIF) are not the same.

1. Meites, J., Nicoll, C. J., Talwalker, P. K., Chap. 8 in *Advances in Neuroendocrinology,* A. V. Nalbandov, Ed., Univ. of Ill. Press, Urbana, 1963.

2. Schally, A. V., Bowers, C. Y., Locke, W., *Am. J. Med. Sci.,* 1964, v248, 79.

3. Saffran, M., Schally, A. V., *Can. J. Biochem. Physiol.,* 1955, v33, 408.

4. Talwalker, P. K., Ratner, A., Meites, J., *Am. J. Physiol.,* 1963, v205, 213.

5. Schally, A. V., Meites, J., Bowers, C. Y., Ratner, A., abst. 46th Meeting, Endocrine Soc., San Francisco 1964.

6. McCann, S. M., Taleisnik, S., Friedman, H. M., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 432.

7. McCann, S. M., *Am. J. Physiol.,* 1962, v202, 395.

8. Ramirez, V. D., McCann, S. M., *Endocrinology,* 1963 v73, 193.

9. Guillemin, R., Jutisz, M., Sakiz, E., *C. R. Acad. Sci.* (Paris) 1963, v256, 504.

10. Schally, A. V., Bowers, C. Y., *Endocrinology,* 1964, v75, 312.

11. Haun, C. K., Sawyer, C. H., *ibid.,* 1960, v67, 270.

12. Everett, J. W., *ibid.,* 1956, v58, 786.

13. Guillemin, R., Schally, A. V., *Texas Rep. Biol. Med.,* 1963, v21, 541.

14. Porath, J., Schally, A. V., *Endocrinology,* 1962, v70, 738.

15. Parlow, A. F., *Human Pituitary Gonadotropins,* A. Albert, Ed., Charles C Thomas, Springfield, 1961, p300.

Received May 25, 1964. P.S.E.B.M., 1964, v117.

Phenol 3, 6 Dibromphthalein Disulfonate, A New Compound for the Study of Liver Disease. (29550)

NORMAN B. JAVITT* (Introduced by Saul J. Farber)
Department of Medicine, New York University School of Medicine

Conjugation of BSP (phenoltetrabromphthalein disulfonate, sulfobromophthalein) with glutathione(1,2) represents the major pathway in the transfer of BSP from plasma to bile. However, the role of conjugation in affecting the rate of removal of BSP from plasma or excretion from the liver cell into bile has not been determined. As a result of studies concerning the conjugation and excre-

* Recipient of Career Scientist Award of Health Research Council of City of New York under contracts 1-228 and U-1224.