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Luteinizing Hormone–Releasing Factor Potentiates Lordosis Behavior in Hypophysectomized Ovariectomized Female Rats

Abstract. *Subcutaneous injection of luteinizing hormone–releasing factor (LRF) in estrogen-primed hypophysectomized, ovariectomized female rats facilitates the appearance of the lordosis response. The LRF effect on lordosis was seen 90, 180, and 360 minutes after injection. This effect could help to synchronize the female's mating behavior with the ovulatory discharge of luteinizing hormone.*

Luteinizing hormone–releasing factor (LRF) recently has been isolated from the hypothalamus, chemically identified, and synthesized (*1*). Injected systemically, it can cause luteinizing hormone discharge from the female rat pituitary within minutes (*2*). During the estrous cycle of the female rat the ovulatory discharge of luteinizing hormone from the pituitary normally is synchronized with behavioral receptivity, indicated by lordosis responses to mounts by the male (*3*). Therefore, we conducted the following experiments to see if LRF could facilitate lordosis in the female rat.

Twenty Sprague-Dawley female rats, weighing 230 to 250 g, were obtained hypophysectomized and ovariectomized from Hormone Assay Laboratories. Upon receipt in our laboratory they received a subcutaneous injection of 10 μg of estradiol benzoate, which tends to facilitate later behavioral responses to estrogen, and then remained undisturbed for 2 weeks. They were maintained on a reversed light cycle (lights off from 10 a.m. to 10 p.m.), were fed normal lab chow and water, and also had available for drinking a bottle of physiological saline with terramycin and 2 percent sucrose.

Each testing protocol was begun by subcutaneous injection of 2 μg of estradiol benzoate in sesame oil. Sixty-five hours later, at the beginning of the dark phase of the daily light cycle, each rat was given a subcutaneous injection of either the physiological saline vehicle control or commercially available synthetic luteinizing hormone–releasing factor (LRF, Beckman Instruments, Spinco Division). The LRF was given in either of two doses, 0.4 μg or 4 μg, dissolved in 0.3 ml of physiological saline. In different weeks of testing, each rat received the control injection and both LRF doses, counterbalanced for order across the 20 rats. For additional confirmation, tests of some rats were repeated at the same dose, and no marked order effects were observed. After a vehicle control or LRF injection, rats were tested for lordosis with a vigorous stud male 10, 30, 90, 180, and 360 minutes after the injection. Lordosis was scored when the back was arched and the head raised during a mount by the male. Differences in the lordosis quotient [(number of lordoses by female/number of mounts by male) × 100] were evaluated statistically by the sign test (*4*), while differences in the percent of tests positive for lordosis were evaluated by the nonparametric McNemar test (*4*). Upon autopsy, complete ovariectomy was confirmed. Completeness of hypophysectomy was confirmed by observing the lack of body weight increase over the weeks (due to lack of pituitary growth hormone), and at the end of the experiment by microscopic examination of the sella turcica and of histological sections through the median eminence.

Under the conditions of this experiment, estrogen alone, followed only by the vehicle control injection, did not lead to high levels of lordosis behavior (Table 1). The lower dose of LRF gave small increases in lordosis, of borderline statistical significance. However, the higher dose of LRF caused significant increases in the occurrence of lordosis (Table 1). These were reflected in both the number of tests positive for lordosis and in the lordosis quotient, and appeared in the tests 90, 180, and 360 minutes after LRF injection. Additional tests, using manual stimulation of lordosis by the experimenter (scratching the female's flanks and exerting pressure on the base of the tail and on the perineal regions), also showed that the higher dose of LRF facilitates lordosis (*5*).

Thus, LRF in sufficient doses can facilitate lordosis in the estrogen-primed female rat. After submission of this report, Moss and McCann (*6*) reported independently that LRF can trigger lordosis in ovariectomized rats primed with estrone and that thyrotropin-releasing factor is ineffective. Since in our experiments the subjects were also hypophysectomized, the effect of LRF could not have been due to

Table 1. Facilitation of lordosis behavior by luteinizing hormone-releasing factor (LRF) in estrogen-primed hypophysectomized, ovariectomized female rats. Some rats were tested more than once at the same dose, so the number of tests is greater than the number of rats. The mean score was taken for each animal at each time point and dose; S.E., standard error.

Treatment	Rats (No.)	Tests positive for lordosis (%)					Lordosis quotient* (mean ± S.E.)				
		Minutes postinjection					Minutes postinjection				
		10	30	90	180	360	10	30	90	180	360
Saline control	20	5	0	8	7	0	6 ± 4	0	5 ± 3	4 ± 3	0
LRF											
0.4 μg	20	0	0	13	20	15	0	0	10 ± 5	18 ± 7	11 ± 6
4.0 μg	20	0	7	46†	57†	50†	0	4 ± 3	32 ± 6†	42 ± 7†	34 ± 7†

* (Number of lordoses by female/number of mounts by male) × 100; calculated from results for all tests, including those where the quotients equal zero. † Significantly different from saline controls ($P < .01$).

luteinizing hormone or follicle-stimulating hormone release, and in other work also these have not proved necessary for steroid-induced receptivity (*7*). In work in our laboratory preliminary to the experiments reported here, the LRF effect was most easily detected when the amount of estrogen given was just below that necessary for increased receptivity with estrogen alone. It should also be noted that LRF-facilitated lordosis in our hypophysectomized rats was not accompanied by all the signs of maximum behavioral receptivity; our females did not hop and dart, they sometimes kicked the male even during tests where other mounts resulted in lordosis, and during prolonged tests the incidence of lordosis tended to decrease.

Luteinizing hormone–releasing factor both triggers the ovulatory discharge of luteinizing hormone and facilitates lordosis. This combination of facts may help to explain the close temporal relations between endocrine and behavioral manifestations of estrus during the normal female rat cycle. In turn, behavioral events associated with copulation are known to influence subsequent endocrine processes in the female associated with impregnation (*8*). The present findings also raise the possibility that some LRF-producing neurons project, either directly or via axon collaterals, to other neurons concerned with lordosis, as well as to the median eminence. In this regard, electrophysiological effects of LRF would be of considerable interest. Finally, these results contribute to the search for biochemical effects, linked to binding of steroid sex hormones by neurons (*9*) and to the facilitation of lordosis by steroid hormones (*10*). Steroid hormone effects on the production or release of LRF may be of relevance for mating behavior mechanisms as well as for neuroendocrine events leading to ovulation.

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Von Willebrand Factor: Dissociation from Antihemophilic Factor Procoagulant Activity

Abstract. *Factor VIII corrects both the clotting defect in hemophilia A and an abnormality of platelet aggregation in von Willebrand's disease. These two activities of factor VIII (antihemophilic factor and von Willebrand factor) are both detected in the void volume when human plasma or cryoprecipitate is chromatographed on Bio-Gel 5M under conditions of isotonic salt concentration. In contrast, antihemophilic factor procoagulant activity is detected with proteins of lower molecular weight when the chromatography is performed with a buffer containing 0.8M NaCl. In this way, the two activities of factor VIII can be dissociated. It remains to be determined whether these components are separate molecules associated as a complex of high molecular weight in plasma or whether they are subunits of a complex macromolecule.*

Factor VIII is a glycoprotein (*1, 2*) that is eluted in the void volume when normal plasma or cryoprecipitate is chromatographed on agarose gels with exclusion limits greater than 2×10^6 to 5×10^6 daltons (*1, 3, 4*).Two biological properties have been ascribed to this protein. It corrects the clotting defect in the sex-linked bleeding disorder known as hemophilia A, a property termed the antihemophilic factor (AHF). Factor VIII also corrects abnormalities of platelet function which are recognized in the autosomally inherited disorder known as von Willebrand's disease; these abnormalities are the reduced retention of platelets in glass bead filters (*5, 6*) and the defective aggregation of platelets by the antibiotic ristocetin (*7*). This property of factor VIII may be called the von Willebrand factor (VWF), and a deficiency of this factor may account for the prolonged bleeding time observed in patients with von Willebrand's disease. The finding that VWF activity is normal in hemophilia (*7*) suggests that the procoagulant (AHF) and platelet (VWF) factor activities of factor VIII are located on different subunits of the molecule, or possibly on different molecules that are associated as a complex of high molecular weight in plasma.

We examined the relation between these factors by determining the elution pattern of the VWF activity when plasma is chromatographed on Bio-Gel 5M with buffers with high salt concentration. In previous studies, we and others have shown that, under these conditions, AHF procoagulant activity is detected with proteins with a much lower molecular weight than plasma