organization of the connective tissue during the following 6 months. The presence of nerve signals after 3 weeks indicates that the motoneurons have not completely degenerated. A histological investigation to verify this point will be reported elsewhere.

After an implantation time of 3 weeks the connective tissue growth begins to provide a conductive pathway for the myoelectric signal. The Silastic cover can no longer effectively isolate the myoelectric signal. However, it is still possible to effectively reduce the myoelectric signal by subtracting the appropriately weighted signals from the internal and external electrode contacts. A larger myoelectric signal will be recorded from the external than from the internal contacts, most of the nerve signal will remain while the myoelectric signal is substantially reduced. Alternatively, it should be possible to remove most of the myoelectric signal recorded with the proposed electrode configuration by filtering the signal with a band-pass of 800 hertz to 5.5 khz.

This electrode unit was designed with the ultimate objective of implanting it in humans during an amputation. The proposed configuration allows several electrode pairs to be located around the perimeter of the tube so that two or more functionally distinct nerve signals can be recorded from one implanted electrode unit. This procedure has been successful in short-term experiments (*2*) with antidromic electrically stimulated nerves. The development of electrodes of this type provides exciting possibilities for neural control of prostheses with many degrees of freedom.

CARLO J. DE LUCA

Department of Orthopaedic Surgery, Harvard Medical School, Children's Hospital Medical Center, Boston, Massachusetts 02115, and *Liberty Mutual Insurance Company Research Center, Hopkinton, Massachusetts 01748*

L. DONALD GILMORE

Liberty Mutual Insurance Company Research Center

References and Notes

1. D. R. McNeal and J. Haughey, "Annual report of progress, December 1973 to November 1974," Rehabilitation Engineering Center, Rancho Los Amigos Hospital, Downey, California (1975), p. 24.
2. B. K. Lichtenberg and C. J. De Luca, *Proc. 3rd Annu. N. Engl. Bioeng. Conf.* (May 1975), p. 2.
3. R. W. Wirta and D. R. Taylor, Jr., *Advances in External Control of Human Extremities* (Jugoslav Committee for Electronics and Automation, Belgrade, 1970), p. 245.
4. P. Herberts, C. Almstrom, R. Kadefors, P. D. Lawrence, *Acta Orthop. Scand.* **44**, 389 (1973).
5. S. J. Jacobsen, thesis, Massachusetts Institute of Technology (1973).
6. C. J. De Luca, *Advances in External Control of Human Extremities* (Jugoslav Committee for Electronics and Automation, Belgrade, 1975), p. 101.
7. R. Alter, thesis, Massachusetts Institute of Technology (1965).
8. A. F. Marks, *Anat. Rec.* **163**, 226 (1969).
9. A. Mannard, R. B. Stein, D. Charles, *Science* **183**, 547 (1974).
10. C. J. De Luca, L. D. Gilmore, S. J. Thomson, R. T. Nichols, *Proc. 2nd Annu. N. Engl. Bioeng. Conf.* (March 1975), p. 458.
11. C. J. De Luca, L. D. Gilmore, R. Hassinger, *Proc. 3rd Annu. N. Engl. Bioeng. Conf.* (May 1975), p. 297.
12. J. A. Hoffer, W. B. Marks, W. Z. Rymer, *Soc. Neurosci. Abstr. 4* (1974).
13. J. A. Hoffer and W. B. Marks, *Soc. Neurosci. Abstr. 5* (1975).
14. R. B. Stein, D. Charles, L. Davis, J. Jhamandas, A. Mannard, R. T. Nichols, *Can. J. Neurol. Soc.* **2**, 235 (1975).

15. We acknowledge the assistance of S. Burke and C. de Roos in designing and constructing the electrode units, S. J. Thomson in developing the implant procedure, and R. Hassinger in computer programming. This work was supported in part by Liberty Mutual Insurance Co.

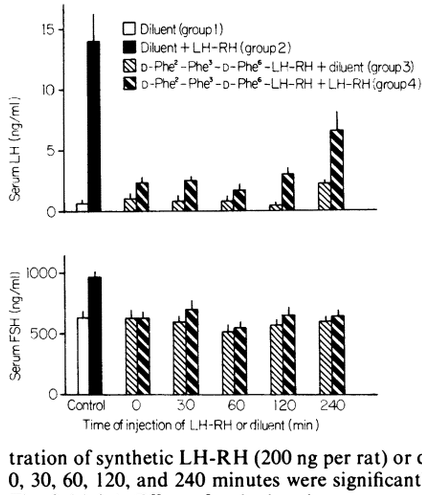
29 September 1975

Blockade of Ovulation in Rats by Inhibitory Analogs of Luteinizing Hormone–Releasing Hormone

Abstract. *An antagonist of luteinizing hormone-releasing hormone (LH-RH), [D-Phe²-Phe³-D-Phe⁶]-LH-RH (Phe, phenylalanine), suppressed luteinizing hormone (LH) and follicle-stimulating hormone (FSH) release in male rats in response to LH-RH for at least 4 hours. Three subcutaneous injections of 1 milligram of this antagonist into rats during proestrus completely suppressed ovulation, while a single injection of 1.5 milligrams per rat inhibited 95.3 percent of the preovulatory surge of LH, 84.2 percent of the FSH surge, and suppressed ovulation by 86.4 percent.*

Several in vivo and in vitro systems for measuring the antigonadotropin releasing activities of inhibitory analogs of luteinizing hormone-releasing hormone (LH-RH) have been described (*1, 2*). We have reported that [D-Phe²-D-Leu⁶]-LH-RH (Phe, phenylalanine; Leu, leucine), an inhibitory analog of LH-RH, suppressed the preovulatory surge of luteinizing hormone (LH) in proestrous hamsters and partially blocked ovulation (*3*). We also synthesized more potent antagonists of LH-RH, as judged by their powerful and prolonged suppression of LH and follicle-stimulating hormone (FSH) release in immature male rats (*4*). This report deals with blockade of the response to LH-RH in male rats as well as with suppression of the preovulatory surge of LH and ovulation in cycling rats by some of the most effective inhibitory analogs.

In the first experiment, the antigonadotropin releasing activity of [D-Phe²-Phe³-D-Phe⁶]-LH-RH was tested in vivo in immature male rats (Simonsen Laboratories), weighing 60 to 75 g (*1*). The animals were first injected subcutaneously with this analog (500 μg per rat) or with diluent alone (20 percent propylene glycol in saline). Simultaneously, or at different times thereafter, the rats were injected subcutaneously with 200 ng (per rat) of synthetic LH-RH or 0.2 ml of diluent. This quantity of LH-RH gave nearly maximum responses in immature male rats after careful dose-response studies. Animals were decapitated 30 minutes after the injection of LH-RH or diluent, and blood was collected for measurement of LH and FSH by radioimmunoassays. Serum samples were separated after centrifugation and stored at -20°C until assayed.



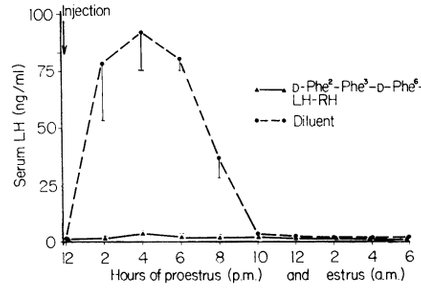


Fig. 1 (left). Time course in immature male rats of blockade of LH and FSH release in response to LH-RH by [D-Phe²-Phe³-D-Phe⁶]-LH-RH (500 μg per rat) injected subcutaneously. The values are expressed as means ± S.E. (standard error). The analog was injected at time 0. The rats were decapitated 30 minutes after administration of synthetic LH-RH (200 ng per rat) or diluent. Values for means ± S.E. for LH and FSH at 0, 30, 60, 120, and 240 minutes were significantly different from the values of group 2, respectively. Fig. 2 (right). Effect of a single subcutaneous administration of [D-Phe²-Phe³-D-Phe⁶]-LH-RH (1.5 mg) on the preovulatory surge of LH in proestrous rats. The differences in LH levels between animals treated with diluent (20 percent propylene glycol in saline) and analog were significant at 1400, 1600, 1800, and 2000 hours ($P < .01$).

The LH was determined in duplicate (*5*), and expressed as nanograms per milliliter in terms of the standard, NIH-ovine-LH-S_{17}. The FSH was determined in duplicate (*6*), and expressed as nanograms per milliliter in terms of the standard, NIAMD-rat-FSH-RP_1. Mean serum LH and FSH levels after treatment with analog or diluent at each time interval were calculated and compared with each other by means of Duncan's new multiple range test (*7*). The peptide significantly suppressed LH and FSH release up to 4 hours after administration, when the magnitude of suppression of LH and FSH was 67 and 88 percent, respectively (Fig. 1). The greatest inhibition, 92.6 percent for LH and 94.4 percent for FSH, was observed at 1 hour after administration of this analog. Recent, similar experiments have revealed that this peptide effectively inhibits LH and FSH release for up to 8 hours in the rat. The peptide showed no significant gonadotropin-releasing activity during this time.

In the second experiment, mature female rats (Charles River Breeding Laboratories), weighing 190 to 210 g, were maintained in groups of eight animals per cage in animal quarters with controlled temperature and light (lights on between 0700 and 1800). After a week of adaptation to our local conditions (C.S.T.), the estrous cycles were monitored by daily vaginal smears. Animals that showed at least two consecutive 4-day cycles were selected.

One single or several subcutaneous injections of [D-Phe^2-Phe^3-D-Phe^6]-LH-RH, [D-Phe^2-D-Leu^6]-LH-RH, [des-His^2-D-Phe^6]-LH-RH (His, histidine) or diluent were given in an attempt to block ovulation. On the following morning, the oviducts were checked under a microscope for the presence of ova. Three subcutaneous injections of 1 mg of [D-Phe^2-Phe^3-D-Phe^6]-LH-RH completely blocked ovulation (Table 1). A single subcutaneous injection of 1.0 mg or of 1.5 mg of the same analog caused an 86.4 percent suppression of ovulation. A similar suppression was obtained after three subcutaneous injections of 2 mg of [D-Phe^2-D-Leu^6]-LH-RH, but three subcutaneous injections of 4 mg of [des-His^2-D-Phe^6]-LH-RH had no effect.

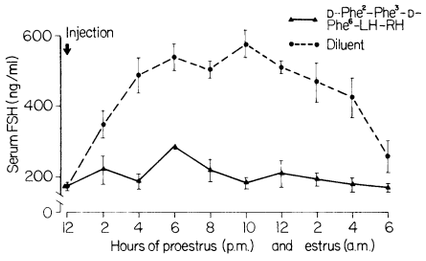


Fig. 3. Effect of single subcutaneous administration of [D-Phe^2-Phe^3-D-Phe^6]-LH-RH (1.5 mg) on the preovulatory surge of FSH. The differences in FSH levels between animals treated with diluent and analog were significant at all time intervals ($P < .01$), except at 0600 ($P < .05$).

The effect of [D-Phe^2-Phe^3-D-Phe^6]-LH-RH on the preovulatory LH surge was studied as follows: 50 rats were injected subcutaneously with diluent alone at 1200 noon on the third proestrous day and divided into ten groups of five rats each. Each rat was bled once (1 ml) from the jugular vein under light ether anesthesia at either 1200, 1400, 1600, 1800, 2000, 2200, or at 2400 of the day of proestrus, or at 0200, 0400, or 0600 of the following day so that at each time interval five blood samples were collected. Each of the same 50 animals in their fourth day of proestrus were injected subcutaneously with 1.5 mg of [D-Phe^2-Phe^3-D-Phe^6]-LH-RH and blood was collected in a similar fashion. In addition to comparison of the mean serum LH concentrations after diluent or analog, the integrated serum LH and FSH concentrations were also determined for the entire gonadotropin surge (Figs. 2 and 3) as described (*8*).

The integrated LH levels of the rats treated with this analog were reduced by 95.3 percent as compared with control rats and those of FSH by 84.2 percent when the FSH levels of the late proestrus and early estrus were included and compared. The elevation of FSH in control animals continued well into the morning of estrus, long after the LH peak had disappeared and increased pituitary sensitivity to LH-RH had returned to normal (Fig. 3). This phenomenon has been observed by many groups and no satisfactory explanation appears to have been formulated. Our results seem to rule out two explanations suggested by Zeballos and McCann (*9*): (i) that the late release of FSH is caused by a separate releasing factor, unless it has receptor sites virtually identical to those for LH-RH, and (ii) that it is produced directly by steroid hormones which should be unaffected by an LH-RH antagonist. A more likely explanation would seem to be that LH-RH, as well as releasing stored FSH, also promotes the synthesis of fresh hormone which, in turn, is also released with some delay. No rebound phenomena in serum LH concentrations were observed up to 0600 of estrus.

The fact that treatment with [D-Phe^2-Phe^3-D-Phe^6]-LH-RH effectively suppressed the preovulatory surge of LH, which has been considered the obligatory ovulating hormone in rats (*10*), and suppressed also that of FSH, followed by a blockade of spontaneous ovulation in rats suggests that this compound or related ones may be useful in the development of a new method of birth control.

Table 1. Suppression of ovulation in rats by inhibitory analogs of LH-RH. The rats had a 4-day estrus cycle and weighed 202.6 ± 1.6 g. Three subcutaneous injections were administered at 1200, 1430, and 1700 hours, or a single subcutaneous injection was given at 1200 of the proestrous day (C.S.T.). The diluent was 20 percent propylene glycol in saline. Abbreviation: N.S., not significant.

Treatment	Dose (mg)		Animals (No.)	Animals ovulating (No.)	No. of ova (mean ± S.E.)	Suppression (%)	*P*
Diluent		(× 3)	6	6	13.3 ± 0.8		
[D-Phe^2-Phe^3-D-Phe^6]-LH-RH	1	(× 3)	5	0	0.0 ± 0.0	100.0	.001
[D-Phe^2-D-Leu^6]-LH-RH	2	(× 3)	5	1	2.4 ± 2.4	82.8	.01
[des-His^2-D-Phe^6]-LH-RH	4	(× 3)	5	5	11.8 ± 0.9	11.3	N.S.
Diluent		(× 1)	5	5	13.2 ± 0.5		
[D-Phe^2-Phe^3-D-Phe^6]-LH-RH	1.0	(× 1)	5	1	2.2 ± 2.2	82.0	.01
	1.5	(× 1)	5	1	1.8 ± 1.8	86.4	.01

ANTONIO DE LA CRUZ, DAVID H. COY
JESUS A. VILCHEZ-MARTINEZ
AKIRA ARIMURA, ANDREW V. SCHALLY
Department of Medicine, Tulane University School of Medicine, and *Endocrine and Polypeptide Laboratories, Veterans Administration Hospital, New Orleans, Louisiana 70146*

References and Notes

1. D. H. Coy, E. J. Coy, A. V. Schally, J. A. Vilchez-Martinez, L. Debeljuk, W. H. Carter, A. Arimura, *Biochemistry* **13**, 323 (1974); J. A. Vilchez-Martinez, A. V. Schally, D. H. Coy, E. J. Coy, M. C. Miller III, A. Arimura, *Endocrinology* **96**, 1130 (1975).
2. M. W. Monahan, M. S. Amoss, H. A. Anderson, W. Vale, *Biochemistry* **12**, 4616 (1973); J. Spona, *FEBS Lett.* **48**, 89 (1974).
3. A. de la Cruz, D. H. Coy, A. V. Schally, E. J. Coy, K. G. de la Cruz, A. Arimura, *Proc. Soc. Exp. Biol. Med.* **149**, 576 (1975).
4. J. A. Vilchez-Martinez, D. H. Coy, E. J. Coy, A. Arimura, A. V. Schally, in preparation.
5. G. D. Niswender, A. R. Midgley, S. E. Monroe, L. E. Reichert, *Proc. Soc. Exp. Biol. Med.* **128**, 807 (1968).
6. T. A. Daane and A. R. Parlow, *Endocrinology* **88**, 653 (1971).

7. R. G. D. Steel and H. J. Torrie, *Principles and Procedures of Statistics* (McGraw-Hill, New York, 1960).
8. A. Arimura, J. A. Vilchez-Martinez, D. H. Coy, E. J. Coy, Y. Hirotsu, A. V. Schally, *Endocrinology* **95**, 1174 (1974).
9. G. Zeballos and S. M. McCann, *ibid.* **96**, 1377 (1975).
10. N. B. Schwartz, S. B. Cobbs, W. L. Talley, C. A. Ely, *ibid.*, p. 1171.
11. We thank Abba J. Kastin for his help in the review of the manuscript, W. H. Carter for assistance, G. D. Niswender, D. Ward, and the National Institutes of Health for the materials used in radioimmunoassays. Supported by the Veterans Administration, NIH contract 72-2741, PHS research grants HD-06555 and AM-07467, and the Population Council, Inc.

5 September 1975; revised 22 October 1975

Nucleus Suprachiasmaticus: The Biological Clock in the Hamster?

Abstract. *Destruction of the suprachiasmatic nuclei in the golden hamster by bilateral radiofrequency lesions abolishes three well-documented circadian rhythms—locomotor activity, estrous cyclicity, and photoperiodic photosensitivity. Entrainment of these rhythms by light cycles fails in lesioned hamsters; females become persistently estrous; in both sexes locomotor activity becomes sporadic, confined primarily to the light instead of darkness, and is totally arrhythmic when lesioned animals are exposed to continuous darkness; the photoperiodic gonadal response (gonadal regression induced by short day lengths) is abolished; lesioned animals remain reproductively mature irrespective of photoperiodic treatment.*

Several papers have described the effects in rats of ablation of the suprachiasmatic nuclei on rhythms of locomotor activity, drinking, pineal *N*-acetyltransferase activity, and adrenal corticosterone content (*1, 2*). Pittendrigh (*3*) suggested that the suprachiasmatic nuclei comprise a central pacemaker (biological clock) regulating circadian oscillations in various target organs. To investigate this possibility in the golden hamster we monitored three circadian rhythms—locomotor activity (wheel running), estrous cyclicity, and photosensitivity. Numerous studies have documented the precision and stability of the rhythm of locomotor activity (*4*), which, like others, is free-running in the absence of any entraining agents or zeitgebers; for this rhythm, the only documented entraining agent is the photoperiod. Thus, in constant conditions such as total darkness (DD) or constant, but dim, light (LL) the free-running activity rhythm has a period length of approximately 24 hours, thought to represent the periodicity of the endogenous oscillator driving the rhythm. In the presence of a light cycle of, for example, 14 hours of light (L) in 24 hours (LD 14:10), the oscillator and the rhythm that it drives assume the period length (24 hours) of the entraining agent. In this entrained state the hamster's activity is nearly entirely confined to the hours of darkness (Fig. 1, A and B). Interruption of the entraining signal from its receptor, which in this case is the retina (*5*), allows the oscillator to express its own endogenous periodicity and the rhythms it drives are accordingly free-running. If the oscillator could somehow be turned off, by electrocoagulation, for example, then the expresion of its driven rhythms, in the absence of a cyclic "driver" may be aperiodic.

The circadian rhythm of locomotor activity can serve as a marker for the less easily assayable rhythm of photosensitivity, which is the basis of the hamster's photoperiodic reproductive response (*6*). The two discrete phases of this rhythm, the photosensitive and the photoinsensitive, are each approximately 12 hours in duration (*6*). Light perceived during the photosensitive phase is interpreted as a long day and maintains gonadal function, whereas light perceived only during the insensitive phase is interpreted as a short day and promotes gonadal regression. The two rhythms of locomotor activity and photoperiodic photosensitivity appear to bear a fixed phase relationship such that the onset of activity corresponds closely to the onset of photosensitivity (*6*). If the hamster's clock entrains to a light cycle (for example, a short day of LD 6:18) such that light is presented during the photoinsensitive phase of the rhythm only, the reproductive system ceases to function; testes and sex accessory structures regress and estrous cyclicity ceases (*7*)—females enter a continuous diestrous condition. However, if the clock entrains so that light is present during at least a portion [as little as 1 hour is sufficient (*8*)] of the photosensitive phase of the rhythm, the reproductive system remains functional.

The least investigated circadian rhythm that we examined is estrous cyclicity. The periodic release of luteinizing hormone in both rats and hamsters is regulated by a circadian oscillator and depends on a differential sensitivity to estrogens (*9*). High concentrations of plasma estrogen (during the afternoon of proestrus) appear to sensitize the hypophysiotropic region of the hypothalamus to a signal from the clock and result in the release of sufficient luteinizing hormone to cause ovulation. Therefore, in females with estrogen implants that assure continuous high levels of plasma estrogen, there is a "preovulatory" release of luteinizing hormone at the same time every day (*9*). In the entrained state the hamster's estrous cycle repeats once every 4 days (96 hours), whereas in continuous dim light the period length of free-running estrous cycles is significantly greater than 96 hours (*10*). We have reproduced these results in a group of hamsters whose locomotor activity we also monitored. Our data demonstrate: (i) the free-running period length of estrus is a multiple of four of the free-running period length of locomotor activity; that is, estrus occurs once every 4 "days," as in the entrained state, but the length of the hamsters' "day" differs from 24 hours; (ii) arrhythmicity arises coincidentally in both rhythms after long-term subjection to continuous light—locomotor rhythmicity breaks down at the same time as the animals become persistently estrous (assessed by continuous vaginal cornification and continuous lordosis in the presence of a male). We suggest that estrous cyclicity and locomotor rhythmicity may be related in the same way as the photosensitivity and locomotor rhythms; we also suggest that all three rhythms may be driven by the same circadian clock.

We therefore attempted to locate and identify this clock by making radiofrequency lesions in the brains of adult, reproductively mature, male and female hamsters (*11*). If we could destroy the clock, (i) locomotor activity would be random with no indication of entrainment; (ii) estrous cyclicity would be replaced by persistent estrus; and (iii) animals without this clock would maintain gonadal function irrespective of photoperiod.

Histological studies were made in every hamster with a lesion (*12*), and the animals were placed into one of five groups (Table 1) according to the location and extent of the lesions. Those hamsters (groups 3 and 5) in which the suprachiasmatic nuclei were severely damaged or entirely destroyed failed to entrain to the ambient light cycle (LD 14:10); after 1 to 10 days of near total inactivity following the operation, such hamsters displayed patterns of running in the wheel that varied from sporadic bursts of activity throughout each 24-hour period (Fig. 1A) to more concentrated activity during the daylight hours, with little activity during darkness (Fig. 1B). When exposed to continuous darkness all animals were arrhythmic (Fig. 1, A and B). In addition, females with extensive lesions (group 5) quickly became persistently estrous, as judged by constant vaginal cornification, and these animals