Rev. Inst. Med. trop. São Paulo
24(3):148-154, *maio-junho*, 1982

UDC 593.161.13
616.937.3

LUTEINIZING HORMONE SECRETION IN MEN WITH CHRONIC CHAGAS' DISEASE (*)

A. C. MOREIRA (1), J. ANTUNES-RODRIGUES (2), E. C. LIMA FILHO (3), M. C. FOSS (1), N. IAZIGI (1) and J. M. T. VERÍSSIMO (1)

SUMMARY

Luteinizing hormone (LH) secretion was investigated in men with chronic Chagas' disease as well as in control subjects, under basal (pulsatile pattern) and stimulating conditions (LH-RH and clomiphene citrate tests). The chagasic group exhibited a greater dispersion of integral LH levels under basal conditions that the control. The frequency of secretory spikes and the mean concentration of testosterone measured in 12 blood samples drawn over a 3-hour period, were normal. No functional disorder of pituitary LH secretion following acute and continuous LH-RH infusions was detected. However, in the chagasic group the values for individual LH release under clomiphene citrate treatment were abnormally scattered. These results suggest the existence of a hypothalamic dysfunction in Chagas' disease.

INTRODUCTION

A loss of efficiency of the mechanisms concerned with the homeostasis of circulating thyroid hormone seems to occur in patients with chronic Chagas'disease [12]. KIMACHI et al. [10] concluded that there seems to be some abnormality in the regulation of the pituitary-adrenal axis. However, no functional disorders of the thyroid gland or of the adrenal cortex could be demonstrated. They suggested that the control mechanisms for secretion of thyroid-stimulating hormone (TSH) and adrenocorticotropic homone (ACTH) were disturbed in Chagas' disease. This could be a result of the neuronal lesions characteristic of the disease [11], which is particularly evident in the visceral autonomic innervation, and also demonstrable upon post mortem in the anterior region of the hypothalamus of patients with chronic Chagas' disease [4].

The present study was designed to explore some aspects of the regulation of luteinizing hormone (LH) secretion in patients with chronic Chagas' disease.

MATERIAL AND METHODS

Subjects

The subjects were 10 healthy male volunteers and 11 male patients with chronic Chagas' disease aged 20 to 50 years. The controls age range matched to the chagasic patients. The diagnosis of Chagas' disease was based on a positive Machado-Guerreiro complement fixation reaction [9] and fluorescent antibody test [6] in patients from endemic areas with treated megaesophagus and megacolon or chronic chagasic cardiopathy without heart failure [11]. All

(*) (1) Departments of Clinical Medicine
(2) Physiology
(3) Mathematics
Faculty of Medicine, Ribeirão Preto, São Paulo, Brasil
Supported by FINEP (Financiadora de Estudos e Projetos) B/39/79/349/00/00

the subjects exhibited no clinical evidence of endocrine disease or undernutrition. They were not receiving medical treatment at the time of the study.

Procedures

All tests were performed between 8:30 and 9:00 a.m. without fasting. In the studies involving frequent blood sampling, a heparinized scalp vein needle was inserted into an antecubital vein. Small amounts of heparinized saline were injected after each blood collection. Blood samples (3-4 ml) were kept in heparinized glass tubes on ice. The plasma was separated by centrifugation and stored at -20°C. Three experiments were carried out on the subjects on different days.

1) Determination of the individual pulsatile pattern of basal LH release. Blood samples were drawn every 15 minutes over a 3-hour period for LH and testosterone measurements.

2) Determination of the LH responses to LH releasing hormone (LH-RH). Synthetic LH-RH (Escola Paulista de Medicina — São Paulo) was injected as in i.v. bolus (200 μg) and blood was sampled at -5, 0, 5, 15, 30, 45, 60 and 120 min from the time of injection. On a subsequent day, LH-RH was administered through a Harvard constant — infusion pump at a rate of 0.2 μg/min for 4 hours and blood was collected at -15, 0, 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 minutes from the start of the infusion.

3) Determination of LH release after clomiphene citrate. Clomid-Merrel, 3 mg/kg/day but not more than 200 mg/day was administered orally in two doses for 8 days. Blood was collected on days 0, 1, 4, 6 and 8.

Hormone Assays

Plasma LH was determined in duplicate using the double antibody radioimmunoassay method described by ODELL et al.[15]. Reagents supplied by the National Pituitary Agency, USA (anti-LH batch No. 2 antisera and LER-960 HLH for radioiodination) and produced in our laboratory (sheep anti-rabbit gamma globulin) were utilized. The standard preparation (CALSTAN-II) was supplied by Calbiochem and expressed in mIU/ml using the Second International Reference Preparation for Human Menopausal Gonadotropins (HMG-IRP-2). The sensitivity of the method was 0.50 mIU/tube. The within-assay coefficient of variation was 6.3% at 50.0% binding on the standard curve. At this level, between-assay variability was 14.0%.

Plasma testosterone was measured in duplicate by the radioimmunoassay method[1] using commercial kits supplied by Trilab, São Paulo. The sensitivity of the assay was 25 pg/tube. The within-assay coefficient of variation was 11.0%. Between-assay variability at 50.0% binding was 17.0%.

All samples obtained for each patient for each experiment were analyzed in the same assay to avoid interassay variability. The data were analyzed according to the ratio SD.[2]chagasic/SD.[2] control, for the significance level at 5.0% [16]. In the figures we also utilized the range, the 10th (P_{10}) and 90th (P_{90}) percentile.

Quantitative parameters of LH pulses

An LH secretory episode (pulse) was defined as a series of values consisting of two or more constant or decreasing concentrations followed by two or more higher values[14]. The increment from nadir to peak was equal to or greater than 1.0 mIU/ml (3 times the coefficient of variation of LH assay at the 5.0 mIU/ml level). The quantitative parameters determined were the mean concentration of LH from 12 blood samples drawn over a 3-hour period, the number of pulses in 3 h and initiation (low set-point) and cessation (high set-point) of LH secretory activity. The parameters were determined as described by BOYAR et al.[3] and SANTEN & BARDIN [18].

RESULTS

Pattern of Basal LH Release

The results are shown in Table I and Fig. 1. The mean and median LH values for controls and chagasic patients were similar. However, in the chagasic group the individual data are abnormally scattered, above as well as below the limits of the control interval. The men with Chagas' disease exhibited a significantly greater ($P < 0.05$) dispersion of individual mean concentration of LH in relation to the control limits. A wider range of variation than that of the control group is also apparent in the chagasic subjects when the values for

TABLE I

Pattern of basal LH secretion and testosterone concentration during a 3-hour study period in control and chagasic men

Control subjects	Secretory spikes/3h	Low set-point LH mIU/ml	High set-point LH mIU/ml	Mean*/3h LH mIU/ml	Mean /3h Testosterone ng/100ml	Chagasic subjects	Secretory spikes/3h	Low set-point LH mIU/ml	High set-point LH mIU/ml	Mean*/3h LH mIU/ml	Mean/3 h Testosterone ng/100ml
1	2	1.9 2.4	6.0 5.2	4.0±1.4	298.3	1	2	3.1 5.0	5.8 9.9	6.8±2.0	-
2	1	2.3	7.7	4.7±1.8	332.8	2	2	2.6 2.0	3.7 3.7	3.3±0.7	392.5
3	2	3.8 5.0	10.1 9.9	6.7±2.0	234.8	3	1	5.1	11.0	6.4±2.0	406.0
4	1	3.5	5.7	3.7±0.7	236.1	4	-	-	-	3.7±0.4	138.0
5	-	-	-	4.2±0.5	396.2	5	1	1.8	10.8	3.6±2.5	571.0
6	1	3.0	4.9	4.3±1.2	639.6	6	1	1.6	2.7	2.8±0.6	291.6
7	1	3.1	6.9	3.7±1.3	144.3	7	1	7.2	8.8	8.5±0.9	462.3
8	1	4.3	6.1	5.0±0.8	574.0	8	1	5.6	8.9	7.0±1.1	957.5
9	-	-	-	5.2±0.6	-	9	1	5.4	10.0	5.7±1.8	634.7
10	2	2.7 3.1	4.7 4.7	3.8±0.7	281.1	10	2	2.1 2.1	4.0 4.5	3.3±1.2	-
						11	1	5.8	13.2	7.4±2.3	278.3
Median	1	3.1	6.0	4.2	298.3	Median	1	3.1	6.0	5.7	406.0
Mean±SD.	1.4±0.5	3.2±0.9	6.5±1.9	4.5±0.9	348.5±162.9	Mean±SD.	1.3±0.4	3.8±1.9	7.4±3.5	5.3±2.0	459.1±240.5
$SD.^2$	0.25	0.81	3.6	0.81	26536.4	$SD.^2$	0.16	3.6	12.2	4.0	57840.2
P_{10}-P_{90}	1-2	2.3-4.3	4.7-9.9	3.7-5.2	234.8-574.0	$\frac{SD.^2 \text{ chagasic}}{SD.^2 \text{ control}}$	0.64	4.4**	3.4	4.9**	2.2

* Mean ± SD. (n = 12) ** $P < 0.05$

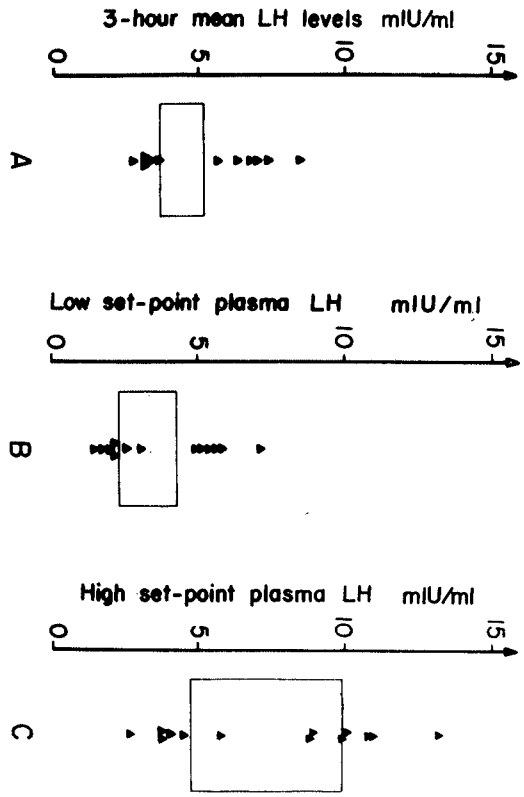

Fig. 1 — Pattern of basal LH release in chagasic men. (A), 3-hour mean LH levels, (B), low, and (C) high set-points for initiation and cessation of LH secretory activity. The horizontal lines on columns show the control 10th and 90th percentile

the low and high set-points are considered. However, the difference between the respectives variances is significant ($P < 0.05$) only for the low set-points.

The frequency of LH secretory spikes, the relation high/low set-point, the mean concentration of testosterone (from 12 blood samples drawn over a 3-hour period) and the frequency of episodic testosterone spikes in the chagasic group fell within the control range.

LH Responses to LH-RH

In the chagasic patients the biphasic pattern of LH secretion during the four-hour infusion of LH-RH was within the control range (Fig. 2). The responses to one-bolus intravenous LH-RH were also similar in both the chagasic and control groups.

LH Release during Clomiphene Citrate Administration

Plasma LH levels rose progressively during administration of clomiphene in both groups (Table II). The mean values reached in the chagasic group were not significantly different from the corresponding controls. However, in the chagasic patients the individual data are abnormally scattered, above as well as below the control range (Fig. 3), but only on day 8 the difference between the respective variances is statistically significant ($P < 0.05$).

DISCUSSION

The present study indicates that men with chronic Chagas' disease exhibit a peculiar abnormality of the pattern of basal LH release. This dysfunction is apparent as a considerable dispersion of the individual mean concentration of LH per 3 h as compared with the control limits. Since the individual mean LH plasma levels determined from multiple samples might be a useful index of integral LH secretion [18], we attempted to identify the site of LH dysfunction in chagasic men.

The individual mean concentration of testosterone blood levels per 3 h were not abnormally scattered in the chagasic men. These data suggest that these abnormal but subtle changes in plasma LH levels were not accompanied by testicular testosterone secretion dysfunction in Chagas' disease.

In order to get a better understanding of the mechanism of altered LH secretion in this disease, the pituitary responses to LH-RH were studied. The release of LH in response to acute or continuous LH-RH stimuli were identical both in chagasic and control groups and in agreement with earlier observations in normal men [5,7,19].

These results indicate that the adenohypophyseal LH secretion mechanisms were not probably disturbed in Chagas' disease.

However, when the effect of clomiphene citrate on LH plasma levels was examined, the results showed that chagasic men had LH variations with a wider scatter than those of the controls. Since this drug interferes with hypothalamic and adenohypophyseal feedback mechanisms for testosterone [2,13,17] we believe

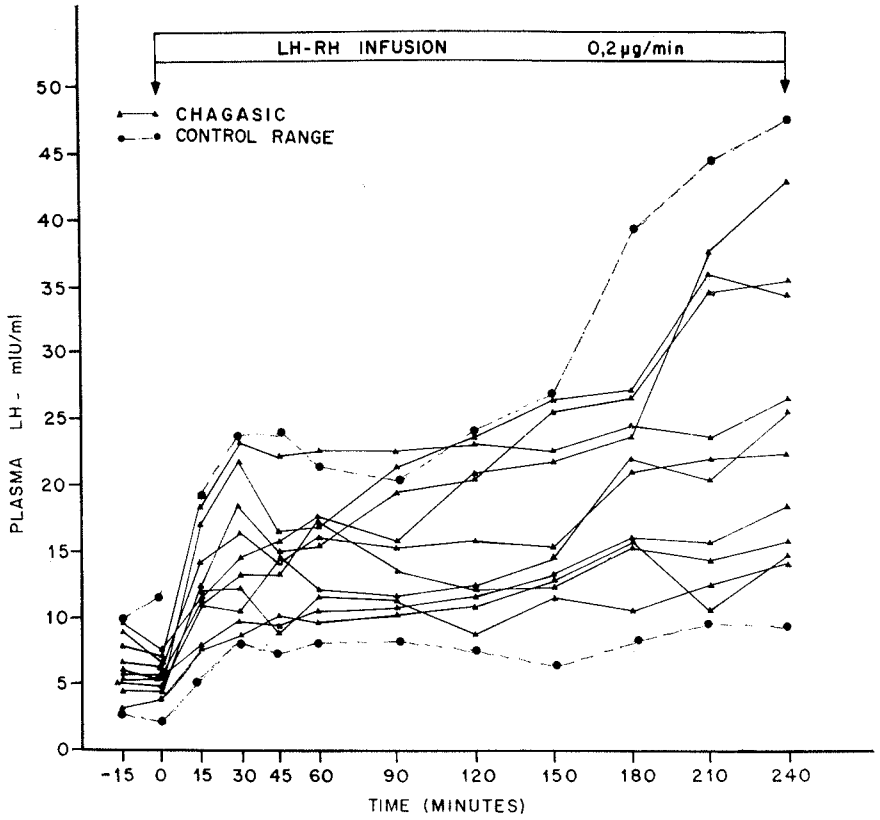

Fig. 2 — Plasma LH concentrations prior and during 4-hour iv infusion of synthetic LH-RH (0.2 µg/min) in ten chagasic patients (Δ-Δ). The control range obtained in ten normal subjects indicated by 0—0

TABLE II
Plasma LH levels during clomiphene citrate administration (3 mg/kg/day) to control and chagasic men

	Day of Test				
	0	1	4	6	8
	Plasma LH mIU/ml				
Control (n = 9) Mean ± SD.	5.7±1.4	5.1±2.3	8.8±2.7	11.2±2.6	11.4±1.9
Chagasic (n = 11) Mean ± SD.	5.4±2.3	6.8±3.4	11.7±4.2	13.4±4.1	14.4±6.6
SD.² chagasic	2.8	2.1	2.4	2.5	12.0*
SD.² control					

* $P < 0.05$

that the abnormal responses to clomiphene in the chagasic subjects were determined by a hypothalamic dysfunction.

These results suggest that men with chronic Chagas' disease exhibit an alteration in integral basal LH secretion determined by a derangement in quantitative hypothalamic regulatory mechanisms. The additional finding of altered low and high set-points but in the same direction as that of the mean individual LH basal secretion, is compatible with this idea.

It was also noted that alterations in frequency of LH secretory pulses could not be seen in Chagas' disease. These findings are in agreement with the hypothesis that the central regulatory mechanism governing pituitary gonadotropin release is made up of a pulsatile and a quantitative component, independent of each other [8,20].

Our results are also in agreement with others obtained for chronic Chagas' disease. These include the probably altered secretion of the TSH [12] and ACTH [10]. A common denominator for all these dysfunctions seems to be the decreased number of neurons in hypothalamic and extrahypothalamic structures in chronic Chagas' diseases [4,11]. Although the relationship between the subtle hormonal altera-

tions and the clinical manifestations was not evident, it is clear that chronic Chagas' disease has a some form of neuroendocrine dysfunction.

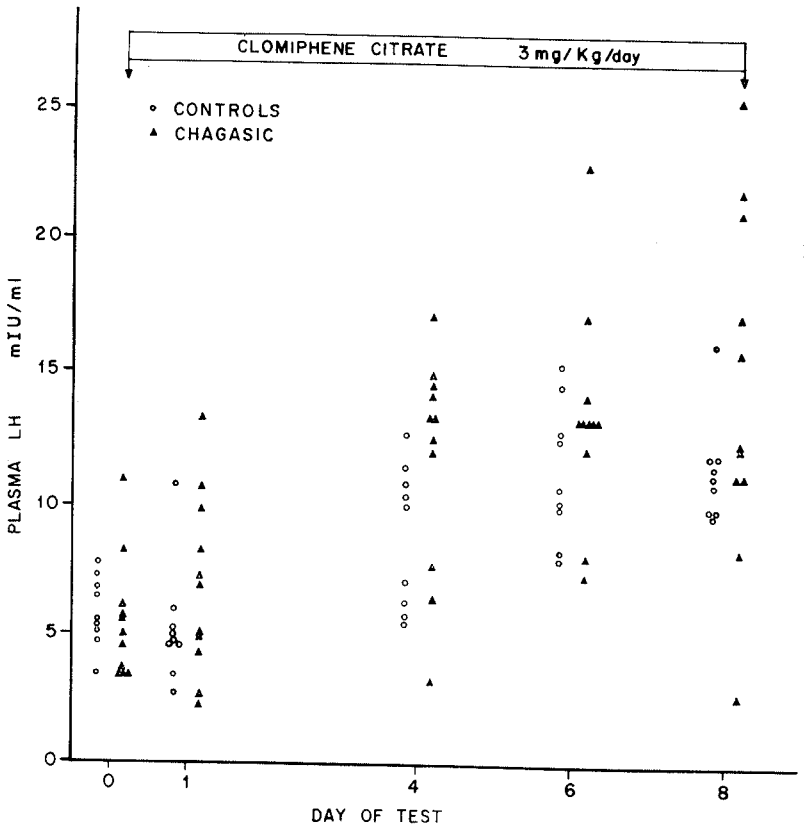

Fig. 3 — Response of plasma LH to clomiphene citrate (3 mg/kg/day) in control and chagasic men. Individual values are shown. The horizontal bar indicates the duration of clomiphene treatment.

RESUMO

Secreção do hormônio luteinizante no homem com moléstia de Chagas crônica

A secreção do hormônio luteinizante foi realizada em pacientes do sexo masculino com doença de Chagas crônica e indivíduos controles, sob condições basais (secreção basal pulsátil) e de estímulo (testes do LH-RH e citrato de clomifene). O grupo chagásico mostrou uma maior dispersão dos níveis do LH integral em condições basais que os apresentados pelo grupo controle. A freqüência dos pulsos e a concentração média de testosterona de 12 amostras por um período de 3 horas, foram normais. Alterações funcionais da secreção do LH hipofisário seguindo-se infusões aguda ou contínua de LH-RH não foram demonstradas. Entretanto, a liberação de LH durante o teste do citrato de clomifene mostrou valores com maior dispersão, principalmente no 8.° dia no grupo chagásico em relação ao controle. Esses resultados, provavelmente, sugerem a existência de uma disfunção hipotalâmica na doença de Chagas crônica.

ACKNOWLEDGEMENTS

The Authors are deeply grateful to MIYOKO A. OWA and MARINA HOLLANDA for their technical aid and to Professor HELIO LOURENÇO DE OLIVEIRA and Mrs. E. GREENE for assistance with the manuscript. We also thank the National Institute of Arthritis, Metabolism and Digestive Diseases, National Pituitary Agency, USA, for supplying the materials for radioimmunoassay.

REFERENCES

1. ABRAHAM, G. E. — Radioimmunoassay of steroids in biological materials. **Acta Endocr. Copenh. 183:** 7-41, 1974.

2. BARDIN, C. W.; ROSS, G. T. & LIPSETT, M. B. — Site of action of clomiphene citrate in men: a study of the pituitary-Leydig cell axis. **J. Clin. Endocr. Metab. 27:** 1558-1564, 1967.

3. BOYAR, R.; PERLOW, M.; HELLMAN, L.; KAPEN, S. & WEITZMAN, E. — Twenty-four pattern of luteinizing hormone secretion in normal men with sleep state recording. **J. Clin. Endocr. Metab. 35:** 73-81, 1972.

4. BRITTO-COSTA, R. & GALLINA, R. A. — Hipotálamo anterior na moléstia de Chagas humana. **Rev. Inst. Med. trop. São Paulo 13:** 92-98, 1971.

5. BREMNER, W. J. & PAULSEN, C. A. — Two pools of luteinizing hormone in human pituitary: evidence from constant administration of luteinizing-releasing hormone. **J. Clin. Endocr. Metab. 39:** 811-815, 1974.

6. CAMARGO, M. E. — Fluorescent antibody test for the serum diagnosis of american trypanosomiasis. Technical modification empolying preserved culture forms of **Trypanosoma cruzi** in a slide test. **Rev. Inst. Med. trop. São Paulo 8:** 227-234, 1966.

7. DeKRETSER, D. M.; BURGER, H. G. & DUMPYS, R. — Serum LH and FSH response in four-hour infusions of luteinizing hormone-releasing hormone in normal men, Sertoli cell only syndrome and Klinefelter's syndrome. **J. Clin. Endocr. Metab. 41:** 876-886, 1975.

8. ELLICOT, A. R.; BENOIT, R. J.; BORTH, R.; STRICKLER, R. C. & WOOLEVER, C. A. — Effect of pentobarbital anaesthesia on luteinizing hormone levels in intact and ovariectomized ewes. **Acta Endocr. Copenh. 80:** 32-41, 1975.

9. FREITAS, J. P. L. — Reação de fixação do complemento para o diagnóstico da moléstia de Chagas pela técnica quantitativa: vantagens do método e sua aplicação em Saúde Pública. **Hospital** (Rio) **41:** 256-267, 1952.

10. KIMACHI, T.; LOMONACO, D. A. & VERÍSSIMO, J. M. T. — Exploração funcional do eixo hipotálamo-adenohipófise-córtex adrenal na forma crônica da moléstia de Chagas. **Rev. Ass. Med. Brasil. 20:** 57-76, 1974.

11. KÖBERLE, F. — Chagas' disease and Chagas' syndrome: The pathology of American trypanosomiasis. **Adv. Parasitol. 6:** 63-116, 1968.

12. LOMONACO, D. A.; OLIVEIRA, H. L.; KIEFFER, J. & PIERONI, R. R. — Abnormal regulation of thyroid function in patients with chronic Chagas' disease. **Acta Endocr. Copenh. 53:** 162-176, 1966.

13. MARSHALL, J. C.; ANDERSON, D. C.; BURKE, C. W.; GALVÃO-TELES, A. & RUSSELL FRASER, T. — Clomiphene citrate in men: increase of cortisol, luteinizing hormone, testosterone and steroid-binding globulins. **J. Endocr. 53:** 261-276, 1972.

14. NANKIN, H. R. & TROEN, P. — Repetitive luteinizing hormone elevations in serum of normal men. **J. Clin. Endocr. Metab. 33:** 558-560, 1971.

15. ODELL, W. D.; ROSS, G. T. & RAYFORD, P. L. — Radioimmunoassay for luteinizing hormone in human plasma or serum: Physiological studies. **J. Clin. Invest. 46:** 248-255, 1967.

16. PEARSON, E. S. & HARTLEY, H. O. — Biometrika Tables for Statisticians. 2 nd. Cambridge, University Press, 1962, p. 179.

17. SANTEN, R. J.; LEONARD, J. M.; SHERINS, R. J.; GANDY, H. M. & PAULSEN, C. A. — Short and long-term effects of clomiphene citrate on the pituitary-testicular axis. **J. Clin. Endocr. Metab. 33:** 970-979, 1971.

18. SANTEN, R. J. & BARDIN, C. W. — Episodic Luteinizing hormone secretion in man. **J. Clin. Invest. 52:** 2617-2668, 1973.

19. WOLLENSEN, F.; SWERDLOFF, R. S. & ODELL, W. D. — LH and FSH responses to luteinizing-releasing hormone in normal adult human males. **Metabolism 25:** 845-863, 1976.

20. YEN, S. S. C.; REBAR, R.; VANDENBERG, G. & JUDD, H. — Hypothalamic amenorrhea and hypogonadotropinism: Responses to synthetic LRF. **J. Clin. Endocr. Metab. 36:** 811-816, 1973.

Recebido para publicação em 13/2/1981.