(*5, 6*). Since the present results demonstrate that this amount is more than enough to lyse isolated malaria parasites, we propose that the selective antimalarial action of chloroquine is due to the accumulation of a toxic chloroquine-heme complex. Although the mechanism underlying this toxicity has not yet been studied, it may be similar to that observed in the erythrocyte model (*10*).

AUGUSTINE U. ORJIH
H. S. BANYAL
REKHA CHEVLI
COY D. FITCH
Department of Internal Medicine, St. Louis University School of Medicine, Saint Louis, Missouri 63104

References and Notes

1. S. R. Meshnick, K.-P. Chang, A. Cerami, *Biochem. Pharmacol.* **26**, 1923 (1977).
2. A. C. Chou and C. D. Fitch, *J. Clin. Invest.* **66**, 856 (1980).
3. H. S. Jacob and K. H. Winterhalter, *Proc. Natl. Acad. Sci. U.S.A.* **65**, 697 (1970); *J. Clin. Invest.* **49**, 2008 (1970); T. G. Gabuzda, in *Drugs and Hematologic Reactions*, N. V. Dimitrov and J. H. Nodine, Eds. (Grune & Stratton, New York, 1974), p. 49.
4. J. D. Fulton and C. Rimington, *J. Gen. Microbiol.* **8**, 157 (1953); K. A. Yamada and I. W. Sherman, *Exp. Parasitol.* **48**, 61 (1979).
5. A. C. Chou, R. Chevli, C. D. Fitch, *Biochemistry* **19**, 1543 (1980); C. D. Fitch and R. Chevli, *Antimicrob. Agents Chemother.* **19**, 589 (1981).
6. C. D. Fitch, N. G. Yunis, R. Chevli, Y. Gonzalez, *J. Clin. Invest.* **54**, 24 (1974); C. D. Fitch, R. Chevli, Y. Gonzalez, *J. Pharmacol. Exp. Ther.* **195**, 389 (1975).
7. C. D. Fitch, R. Chevli, Y. Gonzalez, *Antimicrob. Agents Chemother.* **6**, 757 (1974).
8. A stock solution of 1 m*M* hemin was prepared freshly on the day of an experiment by dissolving 16.3 mg of hemin in 25 ml of 0.02*N* NaOH and kept on ice until final dilutions were made with the standard medium. After dilution the *p*H of solutions containing hemin was 7.4
9. The method of B. W. Langer, Jr., P. Phisphumvidhi, and D. Jiampermpoon [*Exp. Parasitol.* **28**, 298 (1970)] was used to measure glutamic and dehydrogenase activity.
10. A. C. Chou and C. D. Fitch, *J. Clin. Invest.* **68**, 672 (1981). In the erythrocyte model, exposure to hemin impairs the ability of the cell membrane to maintain cation gradients, and there is massive loss of potassium, the cell swells, and lysis eventually occurs.
11. This work was supported by the United Nations/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases.

30 June 1981; revised 6 August 1981

Thymosin Stimulates Secretion of Luteinizing Hormone–Releasing Factor

Abstract. *Partially purified thymosin fraction 5 and one of its synthetic peptide components, thymosin β_4, but not thymosin α_1, stimulated secretion of luteinizing hormone–releasing factor from superfused medial basal hypothalami from random cycling female rats. In addition, luteinizing hormone was released from pituitary glands superfused in sequence with hypothalami. No release of luteinizing hormone in response to thymosin was observed from pituitaries superfused alone. These data provide the first evidence of a direct effect of the endocrine thymus on the hypothalamus and suggest a potentially important role for thymic peptides in reproductive function.*

Experimental evidence supports the concept that the thymus gland participates in the development of the neuroendocrine system in mammals (*1*). With regard to reproductive function, we have previously documented that congenitally athymic nude mice have reduced pituitary concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and that these hormones can be restored to normal by thymic transplantation on the first day of life (*2*). Furthermore, hypothalamic concentrations of luteinizing hormone–releasing factor (LRF) appear reduced in athymic animals whereas gonadal function in vitro seems intact (*3*). In the experiments reported here, we attempted to determine if thymosin fraction 5, a partially purified thymic preparation known to play an important regulatory role in the function of the thymus-dependent lymphoid system (*4*), and two of its component peptides, thymosin α_1 and β_4 (*5*), might also be important in reproductive function. To investigate the role of thymic peptides in the regulation of gonadotropin secretion, we used medial basal hypothalami (MBH) or pituitary glands that were obtained from randomly cycling female rats and were superfused in a sequential double chamber system.

Sprague-Dawley rats were decapitated at 0900 hours and the MBH and pituitary gland were removed. In some studies, the MBH was placed into the first 0.1-ml plastic chamber of a double chamber superfusion system, and the pituitary was placed into the second chamber. In other studies, either the MBH or pituitary was perfused singly in the corresponding chamber of the series. The superfusion system, including the anatomical boundaries of the dissected MBH tissue, have been described (*6*). The sequential chambers were perfused with Medium 199 (Gibco) saturated with 95 percent O_2 and 5 percent CO_2 at 37°C at a flow rate of 3 ml per hour. Bacitracin (5 μl, 2 m*M*; Sigma) was added to the collection tubes to prevent the enzymic degradation of LRF. The dissected tissue was allowed to equilibrate for 2 hours, and then the collection of 0.5-ml fractions of media was initiated. After fractions were collected for 1 hour, 20 μg of thymosin fraction 5, or 4×10^{-11} *M* synthetic thymosin α_1 or β_4 in a volume of 10 μl in Medium 199, or Medium 199 alone as a control, was injected into the first chamber and samples were collected for an additional 2 hours. The fractions were stored at − 20°C until assay. The LH or LRF in these effluent samples was measured by radioimmunoassay as described (*2, 7*). All samples from a given study were measured in the same assay. For LH the intra-assay coefficient of variation was 6.4 percent when approximately 50 percent of the hormone was bound and for LRF it was 3.8 percent. The sensitivities of these assay systems were 10 ng/ml for LH (with NIH RP-1 being used as the reference preparation) and 2.5 pg/ml for LRF (with a synthetic preparation being used as standard). Thymosin fraction 5, thymosin α_1, thymosin β_4, and Medium 199 did not displace iodinated hormone in either assay system. Statistical significance of the changes in the hormone concentrations was determined by the Student's *t*-test for unpaired data.

Figure 1A shows that the injection of either thymosin fraction 5 or Medium 199 caused no change in LH when the pituitaries from female rats were superfused without MBH. In contrast, when the MBH and the pituitary from individual female rats were superfused in sequence, the LH released from the pituitaries into the efflux increased in response to thymosin in comparison to the LH concentrations in the effluent from those receiving only Medium 199 ($P < .05$). The final effluent concentrations of LH from thymosin-treated pituitaries superfused with MBH's were 200 percent increased over basal levels. The administration of thymosin fraction 5 produced significant increases in mean LRF concentrations in the effluents from MBH's in comparison to control groups that received only Medium 199 (Fig. 1B).

In other experiments (Fig. 1C), injection of thymosin β_4 but not thymosin α_1 or Medium 199 alone elicited release of LH from pituitaries superfused together with MBH. Furthermore, thymosin β_4 stimulated a greater than 100 percent increase in secreted LRF over basal levels (Fig. 1D).

To our knowledge this is the first time that thymosin fraction 5 and at least one of its component peptides, thymosin β_4, have been shown to directly affect the reproductive system by inducing release

0036-8075/81/1106-0669$01.00/0 Copyright © 1981 AAAS

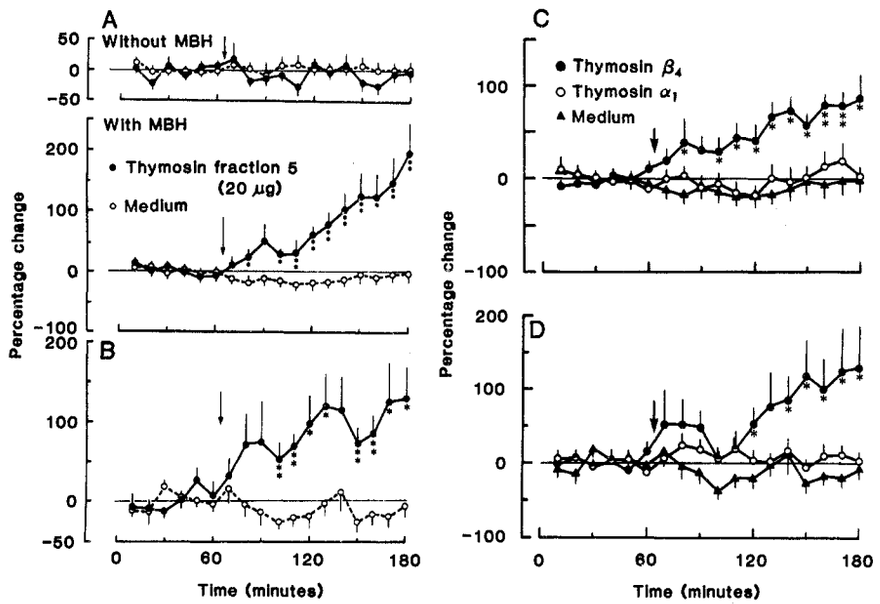

Fig. 1. (A) Release of LH into the efflux from pituitary glands superfused alone (top panel) or in sequence with MBH (bottom panel) from random cycling female rats in response to thymosin fraction 5 (20 μg) or Medium 199 (injected at the arrows). For each experiment, the mean LH concentration in the fractions collected for 1 hour prior to injection was calculated. Each value during the experiment was then calculated as the percentage change from this baseline mean. The average percentage change (± S.E.M.) at each point for the seven experiments is plotted; significant changes from the control group are indicated by asterisks: *, $P < .05$; **, $P < .01$. The mean basal concentration (± S.E.M.) of LH was 323.4 ± 29.5 ng/ml. (B) Release of LRF from superfused MBH from random cycling female rats in response to thymosin fraction 5 (20 μg) (●) or Medium 199 (○) (injected at the arrows) expressed as the percentage change from the mean basal levels. The mean basal concentration (± S.E.M.) of LRF was 30.7 ± 6.6 pg/ml. (C) Mean percentage changes (± S.E.M.) of LH from basal levels by pituitary glands superfused in sequence with MBH in response to synthetic thymosin α_1 ($N = 8$), β_4 ($N = 6$), and Medium 199 alone ($N = 7$). The synthetic thymosin or Medium 199 was injected at the arrow. The mean basal concentration of LH was 403.1 ± 45.6 ng/ml. (D) Mean percentage changes (± S.E.M.) of LRF from basal levels by superfused MBH in response to synthetic thymosin α_1 ($N = 8$) (○), β_4 ($N = 8$) (●), and Medium 199 alone ($N = 7$) (▲). The mean basal concentration of LRF was 34.3 ± 3.7 pg/ml.

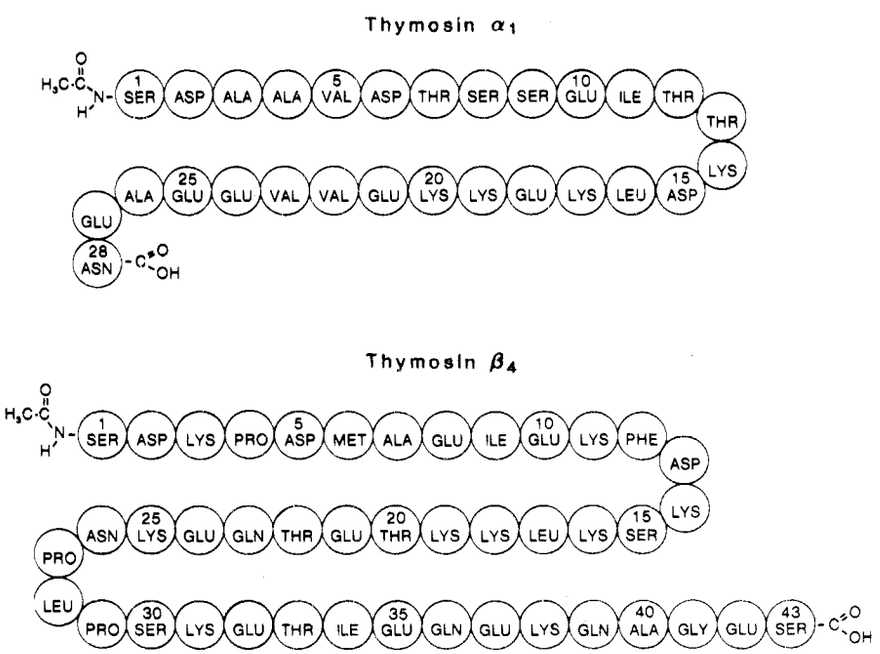

Fig. 2. The primary structures of thymosin α_1 and thymosin β_4. Thymosin α_1 has a molecular weight of 3108 and an isoelectric point of 4.2; thymosin β_4 has a molecular weight of 4982 and an isoelectric point of 5.1.

of hypothalamic LRF. Such data are consistent with older studies documenting interaction between the thymus gland and the reproductive system (*1*, *8*). These findings are also consistent with our previous observations that gonadotropin is reduced in athymic mice (*2*, *3*) as well as in neonatally thymectomized mice (*9*). Athymic rodents have a number of recognized reproductive defects including delayed vaginal opening, reduced fertility, ovarian dysgenesis, accelerated follicular atresia, and premature ovarian failure (*8*). Many of these defects can be eliminated by replacement with thymic or other lymphoid tissues early in development (*2*, *10*). Our findings provide an explanation for the observation that the administration of thymosin fraction 5 to normal prepubertal rats leads to premature vaginal opening (*11*).

Thymosin β_4 (Fig. 2) is composed of 43 amino acid residues with an acetyl group at the NH_2 terminus and is rich in glutamic acid and lysine (*5*). A computer search of the sequence of thymosin β_4 against other protein sequences published to date (*12*) failed to reveal any homologies. Thymosin β_4 is active in inducing expression of the enzyme terminal deoxynucleotidyl transferase (DNA nucleotidylexotransferase, E.C. 2.7.7.31) in transferase-negative murine thymocytes in vivo and in vitro (*5*, *13*). Thus, it appears that thymosin β_4 is important in the early stages of T-cell differentiation, acting on lymphoid stem cells to form prothymocytes. To its functions, we now add the ability of thymosin β_4 to release LRF. In contrast, thymosin α_1 demonstrated no ability to elicit LRF, even though it is a far more potent immunopotentiating agent than β_4 with 10 to 1000 times the activity of thymosin fraction 5 in a number of bioassay systems designed to measure the maturation and function of T lymphocytes and appears to act on prothymocytes to form more mature T cells (*5*). The possibility that other thymic peptides can stimulate or inhibit the release of LRF (or other neuropeptides) remains to be ascertained. Thymosin β_4 alone may, but need not, be solely responsible for the ability of crude thymosin fraction 5 to elicit LRF release. Furthermore, whether thymosin β_4 or structurally similar peptides are synthesized within the brain as well as in the thymus gland remains to be determined.

The physiologic significance of these observations is unclear. Removal of the thymus in rodents subsequent to 96 hours after birth does not result in any

apparent acute alterations in either endocrine or immune functions (*14*). The same is true of other species, although the time period when the thymus can be removed without altering function varies among species (*15*). Such data support the concept that there is interdependence of the immune and endocrine systems early in ontogeny only when critically important steps in the development and programming of neuroendocrine functions are occurring (*16*).

Our findings provide evidence linking neuroendocrine and immune functions, and suggest a new area of research with implications to the understanding and control of reproductive function. Since we show that synthetic thymosin β_4 can release LRF it should be possible to examine the mechanism by which LRF secretion is stimulated and to characterize the role of thymosin β_4 (and possibly other thymic peptides) within the reproductive system. Furthermore, the potential clinical usefulness of thymosin β_4 in eliciting LRF release in women in whom endogenous LRF secretion is diminished for the purpose of inducing ovulation is obvious as is the possible effectiveness of analogs of this peptide for the same (or perhaps the opposite?) purpose.

ROBERT W. REBAR*
AKIRA MIYAKE
Department of Reproductive Medicine, University of California, San Diego, School of Medicine, La Jolla 92093
TERESA L. K. LOW
ALLAN L. GOLDSTEIN
Department of Biochemistry, George Washington University, School of Medicine and Health Sciences, Washington, D.C. 20037

References and Notes

1. W. Pierpaoli and H. O. Besedovsky, *Clin. Exp. Immunol.* **20**, 323 (1975); W. Pierpaoli, H. G. Kopp, E. Bianchi, *ibid.* **24**, 501 (1976).
2. R. W. Rebar, I. C. Morandini, K. Benirschke, J. E. Petze, *Endocrinology* **107**, 2130 (1980); R. W. Rebar, I. C. Morandini, G. F. Erickson, J. E. Petze, *ibid.* **108**, 120 (1981).
3. R. W. Rebar, I. C. Morandini, M. F. Silva de Sa, G. F. Erickson, J. E. Petze, in *Dynamics of Ovarian Function*, N. B. Schwartz and M. Hunzicker-Dunn, Ed. (Raven, New York, 1981), p. 285.
4. J. A. Hooper, M. McDaniel, G. B. Thurman, G. H. Cohen, R. S. Schulof, A. L. Goldstein, *N.Y. Acad. Sci.* **249**, 125 (1975); A. L. Goldstein, T. L. K. Low, G. B. Thurman, M. M. Zatz, N. Hall, J. Chen, S.-K. Hu, B. P. Naylor, J. E. McClure, *Recent Prog. Horm. Res.* **37**, 369 (1981). The thymosin fraction 5 used in this study was a nonpyrogenic clinical quality fraction (lot No. C100496) prepared by Hoffmann–La Roche Inc.
5. T. L. K. Low, G. B. Thurman, M. McAdoo, J. McClure, J. L. Rossio, P. H. Naylor, A. L. Goldstein, *J. Biol. Chem.* **254**, 981 (1979); T. L. K. Low and A. L. Goldstein, *ibid.*, p. 987; T. L. K. Low, S.-K. Hu, A. L. Goldstein, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 1162 (1981).
6. L. W. L. Kao and J. Weisz, *Endocrinology* **100**, 1723 (1977); M. M. Wilkes and S. S. C. Yen, *Life Sci.* **27**, 1387 (1980); *ibid.* **28**, 2355 (1981); A. Miyake and S. S. C. Yen, in preparation.
7. R. M. Kobayashi, K. H. Lu, R. Y. Moore, S. S. C. Yen, *Endocrinology* **102**, 98 (1978); S. E. Monroe, A. F. Parlow, A. R. Midgley Jr., *ibid.* **83**, 1004 (1968).
8. D. H. Rolleston, *The Endocrine Organs in Health and Disease* (Oxford Univ. Press, London, 1936), p. 435; I. F. Dougherty, *Physiol. Rev.* **32**, 379 (1952); S. P. Flanagan, *Genet. Res.* **8**, 295 (1966); Y. Nishizuka and T. Sakakura, *Science* **166**, 753 (1969); H. O. Besedovsky and E. Sorkin, *Nature (London)* **249**, 356 (1974).
9. S. D. Michael, O. Taguchi, Y. Nishizuka, *Biol. Reprod.* **22**, 343 (1980).
10. T. Sakakura and Y. Nishizuka, *Endocrinology* **90**, 431 (1972); S. D. Michael, *Arthritis Rheum.* **22**, 1241 (1979); P. Deschaux, B. Massengo, R. Fontanges, *Thymus* **1**, 95 (1979).
11. S. D. Michael, L. S. Allen, J. E. McClure, A. L. Goldstein, M. S. Barkley, *Abstracts of the 63rd Annual Meeting of the Endocrine Society*, Cincinnati, Ohio, 17 to 19 June 1981, p. 159, Abstr. 308.
12. M. O. Dayhoff, L. T. Hunt, W. D. Barker, R. M. Schwartz, B. C. Orcutt, *Atlas of Protein Sequence and Structure* (National Biomedical Research Foundation, Washington, D.C., 1978), vol. 5, Suppls. 1, 2, and 3.
13. N. H. Pazmino, J. N. Ihle, R. N. McEwan, A. L. Goldstein, *Cancer Treat. Rep.* **62**, 1749 (1978).
14. Y. Nishizuka and T. Sakakura, *Endocrinology* **89**, 886 (1971).
15. J. B. Solomon, *Foetal and Neonatal Immunology* (North-Holland, Amsterdam, 1971).
16. R. A. Gorski, in *Frontiers in Neuroendocrinology*, L. Martini and W. F. Ganong, Eds. (Oxford Univ. Press, New York, 1971), p. 237.
17. We thank the National Pituitary Agency, NIAMDD, for the reagents for the rat LH radioimmunoassay; R. Guillemin for the synthetic LRF; Hoffmann–La Roche Inc. for the synthetic thymosin α_1 and β_4; S. S. C. Yen for critical evaluation of this work; A. Latham and J. E. Petze for technical assistance; and D. Crowe for preparing the manuscript. This research was supported by NIH grants HD-14362, HD-12303, AG-01531, and CA-24974.
* Reprint requests should be addressed to R.W.R.

8 July 1981; revised 2 September 1981

Intraventricular Calcitonin Inhibits Gastric Acid Secretion

Abstract. *Parenteral and intracerebroventricular administration of calcitonin in rats resulted in the suppression of gastric acid secretion. This suppression also occurred in rats with insulin-induced hypoglycemia and after the administration of thyrotropin-releasing hormone. Intracerebroventricularly administered calcitonin was 1000 times more effective than parenterally administered calcitonin in suppressing gastric acid secretion. Calcitonin also inhibited the development of stress-induced ulcers in rats.*

Calcitonin, a peptide hormone secreted from C cells within the mammalian thyroid, promotes absorption of calcium and phosphate into bone and acts in the conservation of skeletal calcium (*1*). A role for calcitonin as a neuromodulator in the central nervous system was suggested by the observations that radioactively labeled calcitonin that is administered parenterally binds specifically to sites in the hypothalamus (*2*) and that immunoreactive calcitonin is present in the hypothalamus and cerebrospinal fluid (*3*). Intracerebroventricular (ICV) administration of calcitonin potently suppresses feeding in rats (*4*), and parenterally administered calcitonin in animals and man inhibits gastric secretion (*5*). Physiological and pharmacological evidence indicates that the central and autonomic nervous systems play an important role in the modulation of gastric secretion (*6*), and several endogenous brain oligopeptides have been implicated as chemical messengers involved in the central nervous system modulation of

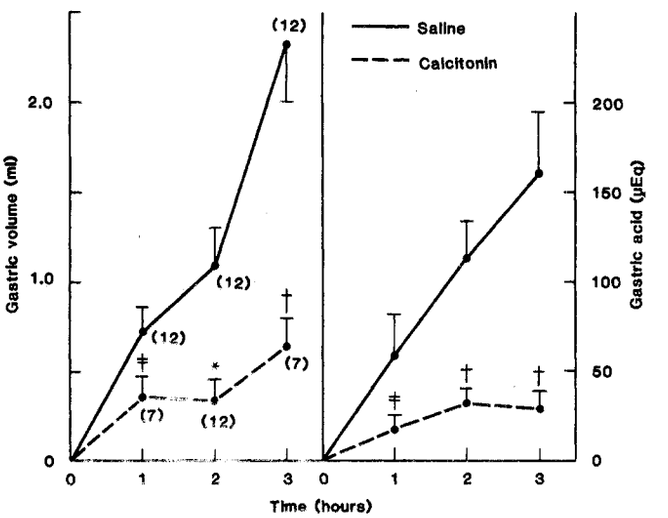

Fig. 1. Temporal effect of ICV administration of calcitonin (2 U, 416 ng) on gastric secretion. All results are expressed as means ± standard error (*$P < .001$, †$P < .01$, ‡$P < .05$). Numbers in parentheses refer to the number of animals at each time point. All animals were anesthetized with urethane (100 mg/kg) and the pylorus was ligated 1 hour later. Then intraventricular cannulas were inserted by using a stereotactic apparatus as described (*7*), and calcitonin (salmon, synthetic; Armour Pharmaceutical Co.) or saline was administered. The rats were killed 60, 120, or 180 minutes after the injection of calcitonin or saline. A two-tailed Student's *t*-test was used to determine statistical significance in all studies.

0036-8075/81/1106-0671$01.00/0 Copyright © 1981 AAAS