2. Yu. A. Berzin'sh, in: The Physiology and Pathology of the Small Intestine [in Russian], Riga (1970), pp. 121-122.
3. O. V. Volkova and S. B. Tarabrin, Arkh. Anat., No. 7, 5 (1975).
4. Yu. K. Eletskii and L. K. Lindenberg, Byull. Éksp. Biol. Med., No. 3, 93 (1974).
5. S. G. Mamontov, in: Regulation of Regeneration and Cell Division [in Russian], Moscow (1977), pp. 34-39.
6. S. G. Mamontov and V. V. Sinel'shchikova, Zh. Obshch. Biol., No. 1, 100 (1977).
7. G. G. Polyanskaya, Vestn. Leningrad. Univ., No. 15, 117 (1972).
8. Yu. A. Romanov, "Some problems in the diurnal rhythm of cell division and the action of hormones on cell multiplication," Author's Abstract of Doctoral Dissertation, Moscow (1970).
9. A. Yu. Tsibulevskii, "Morphological and functional analysis of the state of the jejunal mucosa after bilateral subdiaphragmatic vagotomy," Author's Abstract of Candidate's Dissertation, Moscow (1975).
10. A. Yu. Tsibulevskii and E. N. Orlova, Byull. Éksp. Biol. Med., No. 2, 236 (1975).
11. E. Burns and L. Scheving, J. Cell Biol., 56, 605 (1973).
12. J. Liavag and S. Vaage, Scand. J. Gastroent., 7, 23 (1972).
13. F. Musso, J. Lackat, A. Cruz, et al., Cell Tissue Res., 163, 395 (1975).
14. W. Silen, O. Peloso, and B. Jaffe, Surgery, 60, 32 (1966).
15. P. Tutton, J. Anat. (London), 121, 405 (1976).

IMMUNOREACTIVE LULIBERIN IN THE VISCERAL ORGANS OF RATS

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The distribution of luliberin (luteinizing hormone-releasing hormone, LH-RH) in various organs and tissues of rats was investigated. In the liver, kidneys, duodenum, pancreas, adrenals, and heart of rats a factor analogous to LH-RH in its immunochemical and chromatographic properties was found. The concentration of immunoreactive LH-RH in the liver, kidneys, duodenum, pancreas, and adrenals was about equal (5-7 pg/mg methanol extract obtained from an acetate extract of acetone powder), whereas in the heart it was a little less (2 pg/mg extract). In the blood cells this factor was present in trace amounts. The immunoreactive LH-RH of the visceral organs is either hypothalamic in origin or is synthesized in these organs.

KEY WORDS: luliberin; visceral organs.

Considerable progress has been made in recent years in the study of the peptide hormones of the hypothalamus: thyroliberin (TRH), luliberin (luteinizing hormone-releasing hormone — LH-RH), and somatostatin. They have been isolated in the pure form, their chemical structure has been established, and they have been synthesized chemically [4]. Studies of the mechanism of regulation of secretion and synthesis of anterior pituitary hormones by these hypothalamic factors are making rapid progress [4, 14].

Ever-increasing attention is nowadays being paid to investigations of the extrahypothalamic localization of TRH, somatostatin, and LH-RH and their effect on regulatory processes unconnected with pituitary activity. Recently published work has shown that somatostatin may be found in the pancreas, intestine, and other organs [5, 6] and that this hormone regulates various physiological processes in the body [13]. As regards TRH it is known that, besides in the hypothalamus, it is also found in various parts of the CNS [7, 8, 11], and for that reason a mediator function has been suggested for this tripeptide [7, 8, 11]. Somatostatin and TRH are thus evidently characterized by a broad spectrum of biological action.

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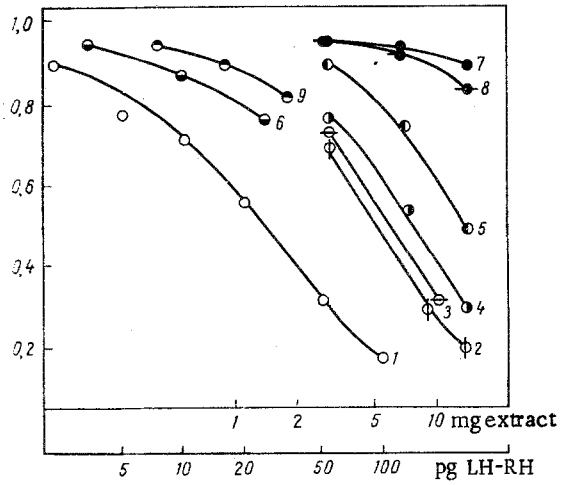

Fig. 1. Radioimmunochemical analysis of LH-RH in extracts of visceral organs of rats. Displacement of [^{125}I]LG-RH from complex with antibodies by synthetic LH-RH (1) and by extracts of liver (2), duodenum (3), kidney (4), heart (5), adrenals (6), blood cells (8),* and plasma (9). Abscissa, quantity of extracts (in mg) and of LH-RH (in pg); ordinate, ratio of radioactivity of [^{125}I]LH-RH bound with antibodies in presence (B) or in absence (B_0) of extracts or synthetic hormone.

The least studied in this respect is LH-RH. Information on LH-RH is restricted mainly to investigations of the hypophyseotrophic effect of this hormone [4] and its localization in the hypothalamus, pineal gland, and periventricular formation [1, 3, 4, 10, 14].

In the present investigation the distribution of this hormone was studied in the organs and tissues of the body, an essential preliminary to the study of the biological role of LH-RH.

EXPERIMENTAL METHOD

Female albino rats weighing 120-150 g were used. Visceral organs (the liver, kidneys, duodenum, pancreas, adrenals, and heart) and the blood (cells and plasma) were frozen in liquid nitrogen. The organs and blood were homogenized at the temperature of liquid nitrogen and treated with acetone. The acetone powder (15-20% of the wet weight of the tissue) was extracted with 2 M acetic acid. The extract (20-25% of the weight of the acetone powder) was lyophilized and extracted with 70% aqueous methanol. The methanol extract (50% of the weight of the acetate extract) was dried in a stream of nitrogen and used for immunochemical and chromatographic investigations. Details of the radioimmunochemical method of determination of LH-RH were published previously [2]. Free and antibody-bound [^{125}I]LH-RH were fractionated by the use of sheep's antibodies against rabbit γ globulin. The extracts were chromatographed on a column with Sephadex G-25 (medium): column 1.2 × 30 cm, elution with 1 M acetic acid at the rate of 7.5 ml/h, volume of fraction 1 ml.

EXPERIMENTAL RESULTS

The results of radioimmunochemical analysis of the extracts of the rat organs and blood are given in Fig. 1. This figure shows that all organs tested contained a factor competing with [^{125}I]LH-RH for binding with antibodies against LH-RH. Curves reflecting the process of displacement of labeled hormone from the complex with antibodies by synthetic LH-RH and by extracts of liver, kidneys, and other organs were parallel. This course of the curves is evidence that the chemical structure of the synthetic hormone is similar to or identical with that of the immunoreactive LH-RH of the visceral organs.

*Curve 7 not mentioned in Russian original — Consultants Bureau.

TABLE 1. Content and Concentration of LH-RH in Visceral Organs of Rats (mean values for group of 80 animals)

Organs tested	Concn. of LH-RH, pg/mg extract		Content in LH-RH in organ or in 1 ml blood plasma, pg†
	radioimmunochemical analysis of extracts	Radioimmunochemical analysis of extracts after chromatography*	
Liver	7,3	6,8	666
Duodenum	6,0	—	230
Kidneys	5,9	9,4	460
Adrenals	5,3	—	1,5
Pancreas	—	5,0	—
Heart	1,9	2,3	46,6
Plasma	2,2	—	4,6
Blood	0,07	—	0,25

*The results allow for losses during chromatography (the losses were about 50%, as determined by chromatography of synthetic LH-RH)
†Losses of LH-RH during extraction were not less than 58%, as was determined with [^{125}I]LH-RH added to the acetone powder, with subsequent monitoring of the radioactivity of all fractions obtained.

Data on the content and concentration of immunoreactive LH-RH in the visceral organs are given in Table 1. They show that the concentration of immunoreactive LH-RH in the liver, kidneys, duodenum, pancreas, and adrenals was about the same (5-7 pg/mg extract), whereas in the heart it was a little lower (about 2 pg/mg extract). In the blood this factor was present in trace amounts. Consequently, the presence of immunoreactive LH-RH in the visceral organs was not due to the presence of a certain quantity of blood in these organs.

Besides the immunochemical properties, the chromatographic properties of immunoreactive LH-RH of the visceral organs of the rats also were studied. For this purpose the extracts were subjected to chromatography on a column with Sephadex G-25. As Fig. 2 shows, immunoreactive LH-RH of the liver, kidneys, and heart and the synthetic hormone possessed equal chromatographic mobility.

The results thus demonstrate that the liver, kidneys, duodenum, pancreas, adrenals, and heart contain a factor similar to LH-RH in its immunochemical and chromatographic properties. This factor is most probably identical with the hypothalamic hormone. Nevertheless, the possibility cannot be ruled out that the chemical structures of this factor and of LH-RH differ slightly. It can tentatively be suggested that immunoreactive LH-RH is a product of degradation of the natural hormone by tissue peptidases. However, special experiments showed that products of degradation of LH-RH by peptidases of the liver, heart, and other visceral organs are, first, not immunoreactive and, second, are easily separated from the synthetic hormone on chromatography on a column with Sephadex G-25.

These results raised the question of the origin and biological role of LH-RH in the visceral organs. One suggestion concerning this problem is that the factor discovered is probably hypothalamic in nature: The hormone secreted from the hypothalamus into the blood stream is taken up by visceral organs by means of nonspecific adsorption or specific reception. This hypothesis is supported by the observations of Takahashi et al. [12], which indicate rapid uptake of [H^3]LH-RH by the liver, kidneys, pancreas, and other organs after intravenous injection of the labeled hormone, and also by results published by Marshall et al. [9], who showed that the plasma membranes of the liver, kidneys, and other organs possess specific LH-RH receptors characterized by high capacity and a low association constant. An

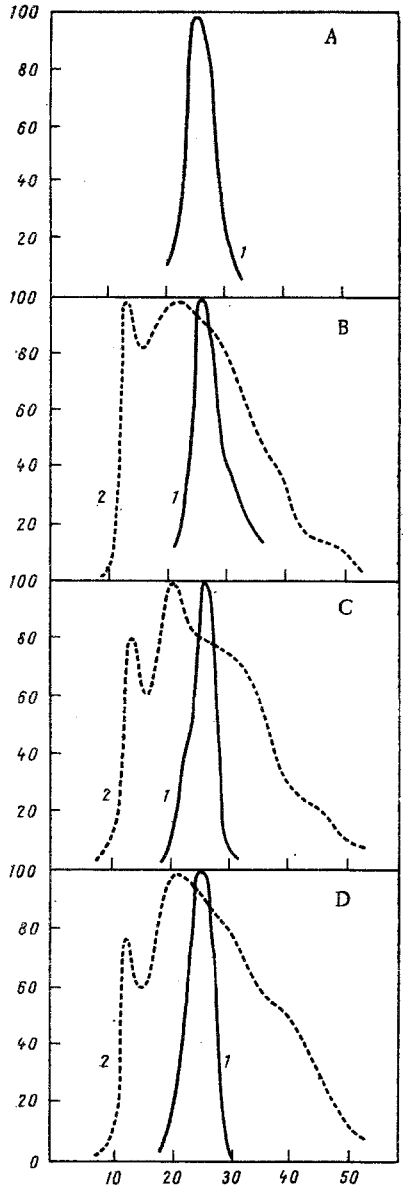

Fig. 2. Distribution of immunoreactive LH-RH among fractions after chromatography of synthetic LH-RH (A) or extracts of heart (B), liver (C), and kidneys (D) on column with Sephadex G-25. 1) LH-RH determined by radioimmunochemical method (in % of maximal value); 2) optical density at 280 nm (in % of maximal value). Abscissa, Nos. of fractions; ordinate, quantity of immunoreactive LH-RH and optical density at 280 nm (in % of maximal value).

alternative explanation assumes the endogenous origin of LH-RH in the visceral organs. However, it is impossible at present to express any preference for either view.

Regarding the biological role, all that can be said is that, despite the lower content of LH-RH in the visceral organs than in the hypothalamus, this hormone possibly plays an important role in the regulation of the physiological functions of the organ tested. Further research will be devoted to the study of the concrete role and mechanism of action of LH-RH in the visceral organs.

LITERATURE CITED

1. A. S. Antonov, G. Ya. Bakalkin, O. G. Krivosheev, et al., Probl. Éndokrinol., No. 5, 51 (1977).
2. G. Ya. Bakalkin, K. N. Yarygin, and V. A. Isachenkov, Probl. Éndokrinol., No. 6, 72 (1976).
3. E. I. Chazov et al., in: Evolutionary Aspects of Neuroendocrinology. (7th International Symposium on Neurosecretion) [in Russian], Leningrad (1976).
4. N. Fleisher and R. Guillemin, in: Peptide Hormones, Baltimore (1976), p. 317.
5. T. Hökfelt, S. Efendic, C. Hellström, et al., Acta Endocrinol. (Copenhagen), Suppl. No. 200, 5 (1975).

6. T. Hökfelt, L. G. Elfvin, R. Elde, et al., Proc. Natl. Acad. Sci. USA, 74, 3587 (1977).
7. I. M. D. Jackson, R. Saperstein, and S. Reichlin, Endocrinology, 100, 97 (1977).
8. F. Kardon, R. J. Marcus, A. Winokur, et al., Endocrinology, 100, 1604 (1977).
9. J. C. Marshall, R. A. Shakespear, and W. D. Odell, Clin. Endocrinol. (London), 5, 671 (1976).
10. E. Okon and Y. Koch, Nature, 268, 445 (1977).
11. J. M. Schaeffer, M. J. Brownstein, and J. Axelrod, Proc. Natl. Acad. Sci. USA, 74, 3579 (1977).
12. T. Takahashi, M. Yoshikawa, S. Honda, et al., Pharmacometrics, 8, 1319 (1974).
13. W. Vale, P. Brazeau, C. Rivier, et al., Rec. Prog. Hormone Res., 31, 365 (1975).
14. W. F. White, M. T. Hedlund, G. F. Weber, et al., Endocrinology, 94, 1422 (1974).

CHANGES IN PLATELET AGGREGATION AND FIBRINOLYTIC ACTIVITY OF THE BLOOD IN HEALTHY SUBJECTS DURING A 23-DAY PHYSICAL CYCLE

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It is shown that throumbocyte aggregation and the fibrinolytic activity of the blood of healthy individuals change over the 23-day physical cycle: In the negative phase of the cycle the quantity of thrombocytes, their aggregation, the process of deaggregation of thrombocyte aggregates, and the fibrinolytic activity of the blood are higher than in the positive phase.

KEY WORDS: biological rhythms; platelets; aggregation; fibrinolytic activity of the blood.

The rhythmic character of biological functions and of the main biophysical, biochemical, and physiological processes forming the basis of vital activity is one of the conditions of existence of animals and plants. From unicellular organisms to man, from processes in the cell to the function of organs and systems — everything is oriented in time, so that the rhythm of activity of the organism is determined by its own "living clock." The system of hemostasis and its various components have their own biorhythm of function (circadian [3, 4], polydian [5, 8], and seasonal [1], and changes in the activity of the hemostasis system depending on solar activity [1, 6-7]).

According to one theory [10, 11], the life of every person, starting from birth, proceeds in accordance with three separate cycles: physical (duration 23 days), emotional (duration 28 days), and intellectual (duration 33 days). Each cycle has its positive and negative phases, which are characterized by a definite difference in the functional state of the systems of the body.

In this paper, data are given on the functional state of the platelets and fibrolytic system of the blood of healthy persons during different phases of the physical, emotional, and intellectual cycles.

MATERIALS AND METHODS

Observations were made on 100 healthy persons (men and women aged 18-40 years). The number of platelets and their aggregation under the influence of ADP [2] were determined and the fibrinolytic activity of the blood was estimated according to lysis of fibrin on slides [9] in different phases of the physical, emotional, and intellectual cycles. The tests were carried out in the morning under standard conditions.

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