COMPARISON OF THE SPECIFIC ACTIVITY OF SOME ANALOGS OF THYROTROPIN-RELEASING HORMONE

L. G. Morozova, A. P. Smirnova,
Yu. P. Shvachkin, and V. P. Fedotov

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The effect of substitution of the second and third amino acid residues in the molecule of thyrotropin-releasing hormone (TRH) on manifestation of its specific biological activity, determined as the quantity of immunoreactive thyrotropic hormone, was studied in rats. Substitution of histidine for alanine caused a 100-fold drop in activity, but its substitution for phenylalanine caused only a tenfold drop. The biological activity of the Glu—Phe—$ProCH_3$ TRH analog was only ten times less than that of the standard. The other twice-modified analog of this hormone (Glu—Glu—$SerNH_2$) had no effect on the release of thyrotropic hormone by the pituitary.

KEY WORDS: thyrotropin-releasing hormone; structural analogs; biological activity.

The question of the role of individual functional groups of the thyrotropin-releasing hormone (TRH) molecule in the manifestation of its specific biological activity is being discussed in the literature. Replacement or blocking of individual groups in the TRH molecule is reflected variously in its hormonal properties [3, 5, 6]. Peptide compounds with no effect on the liberation of thyrotropic hormone do not interact likewise with specific TRH-binding receptors of the pituitary [9, 10]. The role of individual substituted components and groups in the manifestation of specific biological activity can be judged by comparing the results of tests with different TRH modifiers.

This paper describes a study of the ability of five TRH analogs, modified at positions 2 and 3, to stimulate the liberation of thyrotropic hormone (TSH) from the rat pituitary.

EXPERIMENTAL METHOD

Thyrotropin-releasing hormone derivatives (Glu—Phe—$ProNH_2$, Glu—Ala—$ProNH_2$, Glu—Glu—$ProNH_2$, Glu—Phe—$ProCH_3$, and Glu—Glu—$SerNH_2$) were synthesized by the solid-phase method. The biological activity of these tripeptides was determined by comparing doses causing the same increase in the blood TSH level of rats as 10 ng of a standard preparation of TRH (Hoechst, West Germany). The TSH concentration was determined by a radioimmune method [8], using highly purified rat TSH as the standard and the preparation for iodination (rat TSH, National Institute of Arthritis and Medical Disease, National Institutes of Health, Bethesda, USA). Antibodies against bovine TSH, prepared as described previously [1], were used in the reaction. Special experiments showed that a stable and marked increase in the blood TSH level develops in rats 30 min after subcutaneous injection of TRH. This time interval was accordingly chosen for testing. Male Wistar rats weighing 100-120 g received injections of the test preparations in doses of 10, 100, 1000, 10,000, and 100,000 ng. The animals were decapitated 30 min after the injection and blood was collected in heparinized tubes.

Laboratory of Biological Standardization of Hormones and Laboratory of Protein Hormone Chemistry, Institute of Experimental Endocrinology and Hormone Chemistry, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Yudaev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 6, pp. 745-747, June, 1976. Original article submitted July 3, 1975.

EXPERIMENTAL RESULTS AND DISCUSSION

The biological tests showed that the TRH derivative with replacement of histidine by a phenylalanine amino acid residue exhibited about 10% of the biological activity of the standard. Introduction of alanine into position 2 of the TRH molecule yielded a product with low activity (1% of the standard), and tests of the glycine modifier showed no specific biological effect whatever.

In the opinion of some workers the conformation of the TRH molecule is one of the leading factors in its interaction with the pituitary cell receptors [4, 7]. The low activity of the analog in which the histidine was replaced by alanine, and the absence of biological activity in the glycine analog evidently resulted from the sharp difference in structure of the aliphatic amino acids alanine and glycine, and that of histidine.

This hypothesis is also confirmed by the fact that introduction of phenylalanine, with definite conformational and structural similarity to the imidazole ring of histidine, into position 2 yielded an analog with a biological effect only one order of magnitude lower than that of the standard. Evidently aromatic properties in position 2 of the TRH molecule are more important for its interaction with the receptors than the other properties of the imidazole ring (π electrons and the ionic charge of the imidazole ring).

The prolinamide moiety of the TRH molecule is important for the exhibition of its biological effect. In the writers' view, the results of biological tests of the derivative in which histidine was replaced by phenylalanine and the amino group of proline by a methyl group are very interesting. Since we know that analogs substituted at only one of these two positions retain up to 10% of the biological activity of the standard [5], it was natural to expect that with this double modification, a product of low activity would result. However, the ability of the newly synthesized preparation to affect TSH release was only one order of magnitude lower than that of releasing hormone itself.

The biological activity of another double substitution product of TRH in which histidine was replaced by glutamine and proline by serine also was studied. This analog was found to have no effect, in any of the doses used, on the release of TSH by the pituitary.

The results of biological tests of the first and third TRH analogs by the use of a radioimmune method of determining TSH were identical, it will be noted, with those obtained by McKenzie's method [2]. This indicates that both methods of investigation of the hormonal activity of thyrotropin-releasing hormone and its analogs are effective.

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