INHIBITION OF THE ACTION OF EXOPHTHALMIC FACTOR BY ANAHORMONE

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The presence in the pituitary of a factor causing exophthalmos in animals and man has now been demonstrated conclusively [6, 9, 10]. This factor has been obtained in a reasonably pure form, and its distinction from thyrotropic hormone has been proved [7].

However, the biological significance of exophthalmic factor has not been made clear, and that may be the reason why an individual substance of hormonal nature is still described as a factor and not as a hormone.

One of the methods which may be used to study the physiological role of hormones is the inhibition of their action. For this purpose an attempt was made to obtain a competitive preparation with an action based on the antimetabolite principal.

The protein molecule of the hormone is distinguished by a number of characteristics: a) the polyspecificity of the hormonal effects, b) its ability to be fixed by the effector organ, c) antigenic properties (in certain conditions), and d) participation in hormonal self-regulation (for some hormones). These properties may be separate, i.e., for some hormones preparations can be obtained in which, despite abolition of the specific hormonal effect, either the affinity for the effector organ, permitting the phenomenon of competition, or the initial antigenic characteristics are preserved. Such preparations, called "anahormones" [1], can be used as antimetabolites to counteract the effect of an unchanged hormone.

Acetylation has proved to be a very effective means of obtaining anahormones. In this way an acetylated thyrotrophin and somatotrophin have been obtained, capable of competing with the active hormone [2, 11].

The object of the present investigation was to attempt to obtain a competitive inhibitor of the exophthalmic factor by acetylation.

EXPERIMENTAL METHOD

The exophthalmic factor was isolated from unpurified preparations of bovine thyrotrophic hormone [4, 8], and after purification, only traces of thyrotrophic activity could be found. The exophthalmic factor was tested by a radiometric method based on absorption of S^{35} by the dorsal lacrimal gland or Harder's gland of a guinea pig [5]. In each animal the absorption of S^{35} in both glands was determined and the mean value obtained.

Inactivation of the exophthalmic factor was carried out by acetylation with acetic anhydride in a half-saturated solution of sodium acetate in the presence of detergents—nonylphenol and alkylbenzenesulfonate.

In each series of experiments four groups of animals were used: controls (group 1); animals receiving acetylated exophthalmic factor in a dose of 7.5 mg protein (group 2); receiving active exophthalmic factor in the same dose of protein (group 3); and receiving active exophthalmic factor combined with acetylated hormone in five times the dose of active hormone (group 4).

The preparation was administered for 4-5 days. On the last day the animals received an intraperitoneal injection of S^{35} in a dose of 60-80 μCi.

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Effect of Acetylated Exophthalmic Factor on the Action of the Active Hormone

Groups of animals	Factor	Number of animals	Absorption of S^{35} (pulses min/100 mg gland tissue)	P
1	Control	15*	242 ± 20	
2	Acetylated exophthalmic	11	226 ± 26	
3	Active exophthalmic	10	363 ± 23	<0.01 (between 1st and 3rd groups)
4	Active exophthalmic plus acetylated †	12	285 ± 17	<0.01 (between 3rd and 4th groups)

*In each animal the absorption was determined in two glands.

†In one series of experiments, not included in the table, the active hormone was injected not with five times, but with three times the dose of the acetylated hormone; in this case the absorption of S^{35} fell by 50% compared with the action of five times the dose.

EXPERIMENTAL RESULTS

The results obtained are shown in the table, illustrating that acetylated exophthalmic factor does not increase the absorption of sulfur by comparison with the control. However, the simultaneous administration of active and acetylated exophthalmic factors essentially inhibited the action of the former. Three times the dose partially, and five times the dose almost completely abolished the visible signs of the action of the untreated hormone.

In contrast to the usual experiments for revealing competition, in the present experiments the active factor was administered first, followed after 1 h by the inactivated factor, thus demonstrating the competition more reliably.

Acetylation of the exophthalmic factor thus led not only to loss of its biological activity, but also to the creation of a preparation capable of preventing the action of the active exophthalmic factor. The acetylated exophthalmic factor may thus be classed as an anahormone.

The term "anahormone" should not be confused with "antihormone" (an antibody produced against an uncharged hormone).

It is likewise not equivalent to the term "hormone derivative," for in this case it is uncertain whether the derivative is biologically active in relation to the characteristics of the protein hormone, i.e., whether it may be used as an antimetabolite for specific immunization or for acting on regulatory processes. The term "anahormone" is in some respects analogous to the term "anatoxin," or toxoid, but it is wider in its application than the latter because it applies not only to the immunological characteristics [1, 3].

Like other anahormones, the preparation obtained above may be investigated in relation to several properties [3], and primarily as a therapeutic factor in pituitary exophthalmos in man and to study the biological significance of the exophthalmic factor.

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