OVERCOMING ARTIFICIAL TOLERANCE TO SOMATOTROPIN BY HUMAN SOMATOTROPIN ANAHORMONE WITH ADDITIONAL ANTIGENICITY

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A reaction of coupling diazotized novocainamide with human somatotropin (SH) followed by acetylation leads to the formation of an SH derivative which, although it has no hormonal action, retains the original immunological characteristics and acquires additional antigenicity. The derivative can thus be used to overcome artificial tolerance to SH experimentally, when the induced antibodies neutralize the biological action of native SH. It is postulated that azo-SH, as an anahormone-antigen, can be used to induce antibodies capable of blocking human endogenous SH.

The fundamental biological properties of protein hormones can be separated by chemical modification and, in particular, their hormonal action can be abolished while preserving their original immunological specificity [2, 3]. The name anahormone-antigens was given by the writers previously to these derivatives of protein hormones on the grounds that this class of anahormones can be used for active immunization in order to obtain immunological neutralization of the corresponding endogenous hormone [5].

The main advantage of immunization by anahormones is that hormonal side effects are absent during immunization. However, for neutralization of the endogenous hormone to take place the anahormone-antigen must possess two properties: it must have certain antigenic differences from the endogenous hormone in order to be able to induce antibodies, and at the same time it must have a similarity to the endogenous hormone so that the antibodies can neutralize the action of the hormone. Preparations obtained from most species of animals are unsuitable for human immunization for the purpose of obtaining antibodies neutralizing endogenous SH because of substantial antigenic differences [11]. The use of human SH does not as a rule lead to the formation of antibodies because of natural tolerance to this homologous protein. In order to obtain an immunizing preparation the antigenic properties of human SH must be so modified that the antigenic similarity with the native hormone remains; yet, at the same time, additional tolerance is obtained, so that the natural tolerance can be overcome.

The object of the present investigation was to obtain an anahormone-antigen of human SH with additional antigenicity and to demonstrate that by means of this preparation artificial tolerance to SH can be overcome.

EXPERIMENTAL METHOD

SH was obtained from human pituitary glands [13]. Its biological activity was assessed from the level of nonesterified fatty acids (NEFA) [9]. To study the immunological properties of the SH and its derivatives,

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TABLE 1. Properties of SH and of Its Acetylated Azo-Derivative (A−SH)*

Index	Preparation		Numerical results
Biological effect (increase in NEFA level) in percent	SH		100—170
	A-SH†		10—30
Neutralizing power of antisera: volume of serum in ml neutralizing 1 dose of SH	Antiserum against SH		0,25
	Antiserum against A-SH		0,25
Titer of antibodies against SH	Antiserum against SH		1:80 000±20 000
	Antiserum against A-SH		1:240 000±46 000
Titer of antibodies after exhaustion of SH antisera	Antiserum against SH	with SH	1:30±5,8
		with A-SH	1:30±11,2
	Antiserum against A-SH	with SH	1:40±9,2
		with A-SH	1:6 000±3 000
Overcoming of tolerance (titers of antibodies in rabbits tolerant to SH)	After immunization with SH		1:20±6
	After immunization with A-SH		1:128 000±3 800

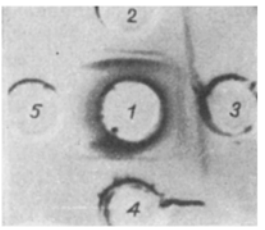
*A−SH denotes acetylated azosomatotropin obtained by coupling with diazonovocainamide.

† Dose of A−SH was 10-15 times greater than dose of SH injected.

the delay of passive hemagglutination test [14] and the agar-diffusion test [8, 12] were used. SH anahormone was obtained by coupling SH with diazotized novocainamide and acetylating the product [1]. To produce tolerance, rabbits were injected intraperitoneally with 5 mg native growth hormone immediately after birth and 24 h later. During the next 3 months the antigen was injected once a month; absence of antibodies against SH was determined, revealing the development of artificial tolerance. Starting with the 4th month, two groups of tolerant rabbits were immunized; the rabbits of group 1 received injections of native SH, while those of group 2 received azo-SH. Both preparations were injected mixed with alumina. For biological assay of the resulting antisera their ability to neutralize the hormonal effect of native SH was tested.

EXPERIMENTAL RESULTS

Table 1 shows that the azo-derivatives of SH possessed no hormonal activity when tested for their effect on the blood NEFA level, but they completely retained their original antigenic properties. Meanwhile immunization with hormonally inactive azo-SH led to the induction of antibodies in the tolerant rabbits, i.e., tolerance was overcome. In control animals immunized with native SH, tolerance remained. This shows that coupling SH with diazonovocainamide created additional antigenicity enabling tolerance to native SH to be overcome. The neutralizing power of sera against azo-SH was identical with that of antisera obtained against native SH. After exhaustion of antisera against azo-SH with native SH, they still preserved antibodies against azo-SH (from 1:2000 to 1:51,200 in the passive hemagglutination test), whereas antisera obtained from intact rabbits immunized with native SH lost the ability to react after exhaustion both with native SH and with azo-SH. The presence of additional antigenicity in azo-SH also was demonstrated by the agar diffusion test (Fig. 1). Antisera against azo-SH formed two precipitation bands with this preparation, one of them a common band with active SH, whereas antsera against native SH formed only one precipitation



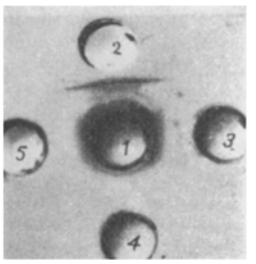


Fig. 1 Fig. 2

Fig. 1. Agar diffusion test of antiserum against A-SH (1) with A-SH in concentrations of 1 mg/ml (2) and 3 mg/ml (3) and with native SH in concentrations of 1 mg/ml (4) and 3 mg/ml (5).

Fig. 2. Agar diffusion test of antiserum against A-SH exhausted by native SH (1) with A-SH in concentrations of 3 mg/ml (2) and 1 mg/ml (4) and with SH in concentrations of 3 mg/ml (3) and 1 mg/ml (5).

band. After exhaustion with native SH the antisera against this preparation formed no precipitation bands either with native or with azo-SH, while antisera against azo-SH formed only one precipitation band with the homologous antigen (Fig. 2), reflecting the presence of additional antigenicity.

The results show that attachment of a hapten, in the form of diazonovocainamide, to the protein molecule of human SH bestows additional antigenicity in a similar manner to that observed in experiments with nonhormonal proteins [10]. The writers have shown that azo-SH, when acetylated, possesses no hormonal action but fully retains its original immunological characteristics and also acquires additional antigenicity, so that experimentally it can overcome artificial tolerance to native SH. It can accordingly be concluded that the azo-SH can also overcome natural human tolerance to human SH, i.e., it can be used as an immunizing anahormone to induce antibodies in order to neutralize the hormonal action of the endogenous SH. The neutralizing power of antibodies against azo-SH has also been demonstrated by the writers in a study of passive serum therapy in two patients with, in particular, an insulin-resistant for of diabetes mellitus [6].

The theoretical grounds for the use of SH anahormone have been examined by the writers previously [4].

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