PRODUCTION AND PROPERTIES OF A HORSE ANTISERUM AGAINST HUMAN GROWTH HORMONE

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The authors previously described a method of immunizing rabbits with microdoses of growth hormone, thereby producing an antiserum practically free from contamination by antibodies against possible secondary antigens [1]. The specificity of such a serum is so high that it can be used to determine whether the hormone has been obtained by saline extraction or by extraction with glacial acetic acid, a procedure which has been shown to modify the hormone molecule [4].

It is now necessary to obtain large amounts of antihormonal antisera, so that the range of experimental research may be broadened and the possibility of using these antisera therapeutically may be investigated [2, 3]. In this connection the development of methods of immunization of larger animals and the study of the properties of the antibodies thus obtained are of considerable interest.

This paper describes the preparation of a horse antiserum against human growth hormone, its species, hormonal, and tissue specificity, and also the ability of this serum to cause biological neutralization of the hormone.

EXPERIMENTAL METHOD

Horses weighting 340-400 kg and aged from 3 to 8 years, used for work on the farm, were immunized. Well purified preparations of human growth hormone were obtained by Raben's method [9]. The preparation of the antigen, the scheme of immunization, and the verification of the antibody titer were carried out in principle in the same way as during immunization of rabbits [1]. Between 8 and 10 ml of a stable emulsion of a mixture of equal parts of a solution of somatotropic hormone (STH) in physiological saline (p H 8.0-8.5) and a solution of autoclaved BCG with 3 parts of mineral oil was prepared for the injections. The dose of hormone given at one injection was 5 mg/100 kg body weight. In all two injections were given at an interval of 10 days. The antigen mixture was injected subcutaneously, in fractions of 2-3 ml, into 3-4 different parts of the body. Between 7 and 10 days after the second injection the animals were bled in sterile conditions. The serum thus obtained was heated for 30 min at 56°, preserved with chloroform, and kept at 4-6°.

The titer of antibodies was verified by the agar gel diffusion reaction [1], by passive hemagglutination [10], and by the flocculation test. The species, hormonal, and tissue specificity were investigated by the gel diffusion reaction also. Animal STH was obtained by Raben's method [9], human ACTH by the method as Astwood and co-workers [5], and thyrotropic hormone (TTH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and LTH by Ellis's method [6]. The human and animal organs and tissues (brain, liver, lung, muscle) were extracted with physiological saline (ph 8.0). Precipitation was carried out directly with the extracts or with solutions of tissue proteins isolated from the extracts with acetone at pH 5.5. In another series of experiments the organs and tissues, dired with acetone, were extracted with glacial acetic acid, and the proteins were isolated from the extract with a mixture of acetone and ether.

The ability of the antiserum to produce biological neutralization of the growth hormone was investigated by means of the tibia test [7]. Serial dilutions of antiserum in buffered physiological saline (pH 7.4) were added to a series of test tubes containing equal amounts of STH. The mixture was kept for 30 min at 40° and than 4-6° unite required. Daily for 4 days 0.2 ml of the serum–hormone mixture, and in the control the same volume of normal horse serum or physiological saline, was injected into hypophysectomized

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TABLE 1. Antisomatotropic Activity of Horse Antiserum

Mixture No.	Test solution	Total dose per rat over 4 days: STH (in μg)	Total dose per rat over 4 days: serum (in ml)	Width of cartilage (in μ)
1	Normal serum (n/s)	0	0,24	168,3±4,3
2	Antiserum (a/s)	0	0,24	157,1±3,4
3	STH	40	0	255,4±1,9
4	STH+n/s	40	0,24	252,2±4,7
5	STH+a/s	40	0,04	226,2±11,3
6	STH+a/s	40	0,08	213,9±9,7
7	STH+a/s	40	0,12	179,2±0,7
8	STH+a/s	40	0,24	163,6±3,9
9	Physiological saline	0	0	157,9±2,9

rats. Each group consisted of 5-7 rats. The animals were sacrificed on the 5th day and the width of the cartilage was measured in the usual manner.

EXPERIMENTAL RESULTS

All three horses reacted in general similarly to injection of growth hormone. Precipitins began to appear 7-9 days after the second injection of the antigen. Just as with the rabbits, no further increase in titer took place after a third, booster injection, and it was equivalent to a 1:5-1:7 dilution of the hormone solution with an initial concentration of 1 mg/ml. In other words, the antiserum of one of the horses formed a precipitate with STH solution in a concentration of 63 μg/ml, whereas the titer of the antiserum of another horse was equivalent to the limiting concentration of 16 μg/ml STH. The antibody titer estimated by the hemagglutination reaction was 1:6400-1:12,800. The titer of γ-globulin isolated from the antiserum by alcoholic fractionation was 1:26,000-1:52,000 respectively, while the titer of antiserum purified by the fermentation method reached 1:200,000. The titer of the crude serum remained unchanged for over a year when kept in sterile conditions in the cold; observations are still continuing.

The method of immunizing animals with microdoses of antigen, initially developed in rabbits, thus proved more effective still when used on horses. By this method as much hormone was used on one horse as on the immunization of 6 or 7 rabbits by the macrodose method [6, 8], but the yield of serum was the same as could be obtained from 240-280 rabbits, and moreover the serum was more specific.

Table 1 shows that the horse antiserum, in the tibia test, neutralized the stimulant action of growth hormone on the width of the epiphyseal cartilage of the hypophysectomized rats. Besides the relative increase in the antibody content of the serum-hormone mixture injected into the rats, the difference between the width of the cartilage in the control and experimental series was reduced. This pattern was observed, however, up to a certain limit, which was evidently the equilibrium point of biological neutralization of the antigen–antibody system. In the experiment under discussion this point corresponded to the ratio between 40 μg STH and 0.12 ml antiserum, although a further increase in the dose of serum to 0.24 ml still further reduced the difference between the control and experiment by a few microns. A special test was carried out to determine the presence of free STH or free antiserum in the serum–hormone mixtures. The use of the same immunochemical methods showed that mixtures Nos. 5 and 6 contained an excess of hormones, and mixture No. 8 had such an excess of antiserum that it could fix another 40 μg STH. Neither free STH nor free antiserum was found in mixture No. 7.

With a decrease in the dose of STH injected into the hypohysectomized rats complete neutralization was much more easily attained (complete agreement between the control and experimenta results), whereas with an increase in the dose of STH not even a large excess of antiserum was able to reduce the width of the cartilage to its control value.

The somatotropic horse antiserum obtained as described above possessed high species, hormonal, and tissue specificity, as Table 2 shows. The serum formed a precipitate only with human and monkey's growth hormone, but not with the growth hormones of other species of animals or with other protein hormones. The exception to this rule was acetylated human thyrotropic hormone, which formed a precipitate

TABLE 2. Specificity of Antisomatotropic Horse Antiserum

Antigen	Initial concentration of antigen (in mg/ml)	Results of precipitation of antiserum with serial dilutions of antigens						
		1	2	4	8	16	32	64
Human STH	1.0	+	+	+	+	+	+	+
Monkey STH	1.0	+	+	+	+	+	+	+
STH of animals (ox, horse, shepp, pig)	4.0	–	–	–	–	–	–	–
Human ACTH	2.0	–	–	–	–	–	–	–
Insulin of animals	4.0	–	–	–	–	–	–	–
TTH, FSH, LH, LTH, of animals	4.0	–	–	–	–	–	–	–
Human TTH (acetylated)	4.0	+	+	–	–	–	–	–
Saline extract of human organs and tissues*	4.0	–	–	–	–	–	–	–
Acetic acid extract of human organs and tissues*	4.0	–	–	–	–	–	–	–
Saline extract of monkey's organs and tissues*	4.0	–	–	–	–	–	–	–
Acetic acid extract of monkey's organs and tissues*	4.0	–	–	–	–	–	–	–

*Brain, lungs, liver, and muscles; legend: + precipitation present, - precipitation absent.

with somatotropi antiserum in a concentration of 2 mg/ml or more. This fact may evidently be explained by contamination of the preparation of acetylated TTH with growth hormone, for purification was limited to reprecipitation in metaphosphoric acid [3]. The suggestion may also be made that acetylated derivatives of thyrotropic and somatotropic hormones possess some degree of group specificity. All these observations concur with the results of investigation of the specificity of the rabbit somatotropic antiserum obtained by immunization with microdoses of antigen [1].

Bearing in mind the possibility of using somatotropic antiserum experimentally for neutralizing the stimulant action of STH on tumor development, its tissue specificity also was investigated. It was found (see Table 2) that the horse antiserum against human growth hormone precipitated neither with saline nor with acetic acid extracts of human or monkey's brain, lungs, liver, and muscles.

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