Production of Monkey Antiserum against Mouse Growth Hormone

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Synopsis

The antiserum against mouse growth hormone (MGH) was produced by immunizing monkeys and some basic immunological properties of the antiserum were studied. The antiserum formed single precipitin lines in agar gel double diffusion plate against both MGH preparation and the crude extract of mouse anterior pituitaries, but reacted with neither normal mouse serum nor the crude extracts of several viscera of mouse. Immunoelectrophoresis of the antiserum with MGH preparation showed a single precipitin arc at the corresponding site of MGH. The precipitin lines between MGH preparation and monkey antiserum and rabbit antiserum to MGH produced previously by us fused completely. Furthermore, the biological activity of MGH was significantly inhibited by the antiserum. All the results have demonstrated that the antiserum obtained in the present experiment contains the antibody specific only to MGH.

The cross-reaction test of the antiserum indicates that MGH shares immunologically reactive site identical to that contained in growth hormones of rats, rabbits, goats and cows.

There have been some immunological studies on rodent pituitary growth hormone; mouse (Kosugiyama et al., 1970), rat (Hayashida and Contopoulos, 1967; Ellis et al., 1968) and rabbit (Ellis et al., 1968). Previously, the antiserum to mouse growth hormone (MGH) preparation induced in the rabbit was found to inhibit slightly the biological activity of MGH and to contain the antibody to mouse prolactin as well as to MGH. Hayashida and Contopoulos (1967) produced the antiserum to rat growth hormone (RGH) by immunizing monkeys which had a high potency, whereas they could not obtain the antiserum to RGH by immunizing rabbits. Ellis et al. (1968) also produced the antiserum to either RGH or rabbit growth hormone by immunizing monkeys.

The present study was undertaken to obtain the antiserum to MGH which had a high potency, using monkeys as the immunization animal.

Materials and Methods

Animal

Approximately 3–5 year old female cynomolgus monkeys (Macaca irus) weighing 2.5–3.5 kg were used. Two were immunized with MGH preparation and one remained as the control. These three animals were imported from Cambodia and conditioned at Department of Veterinary Science, National Institute of Health, Tokyo, for 6 weeks before arrival to our laboratory. They were maintained in an air-conditioned (24 ± 1°C temperature and 65–68% relative humidity) and artificially illuminated (12 hr light from 8:00 am to 8:00 pm) room and offered commercial chow (Oriental Yeast Co. Ltd., Tokyo) supplemented with fresh sweet potatoes.

Immature male rats hypophysectomized at the age
of 28 days were used two weeks postoperatively for the test of inhibition of the biological activity of MGH by the antiserum obtained.

**Hormone**

MGH preparation was extracted by the method of Ellis (1961) from anterior pituitaries of mice. Bovine growth hormone (BGH: NIH-GH-B₄), the lyophilized crude extracts of anterior pituitaries of the rat, rabbit and caprine were used for the test of the possible cross-reactions of the antiserum with growth hormones of mouse and of these species.

**Immunization procedure**

Two mg MGH preparation dissolved in 1 ml physiological saline was emulsified with equal volume of Freund’s complete adjuvant (Difco Labs., Detroit, Mich.). The mixture was injected subcutaneously at the dorsal site of each of two monkeys nine times at two weeks interval. Each animal received a total dose of 18 mg MGH preparation and was bled 14 days after the last injection. Since the antisera induced in two monkeys were quite similar in the immunological characteristics except the precipitin titer checked by precipitin ring test, the pooled antiserum was used for several serological tests and for the inhibition test of the biological activity of MGH. The remaining one monkey was served as the control from which the normal serum was obtained.

**General methods of immunological tests of the antiserum**

The characteristics of the antiserum was studied by means of precipitin ring test, double-diffusion test of Ouchterlony (1957) and immunoelectrophoresis applying disc electrophoresis. All the procedures were essentially the same as described previously (Kosugiyama et al., 1970). Our favorite plate in double-diffusion test contained 6 wells of 6 mm in diameter with 3 mm well distance. MGH preparation dissolved in physiological saline at the concentration of 200 μg/ml was used for the immunological tests unless otherwise stated. The lyophilized crude extracts of several viscera of mice used were dissolved in physiological saline at the concentration of 10 mg/ml. The rabbit antiserum against MGH used for examining the immunological identity of the antiserum was previously produced by us (Kosugiyama et al., 1970). This antiserum was proven to be specific only to MGH after absorption with mouse prolactin.

**Inhibition test of the biological activity of MGH**

The method of Greenspan et al. (1949) was employed for the test. Forty μg MGH preparation dissolved in 0.2 ml physiological saline was mixed with 0.8 ml of the antiserum or normal monkey serum (NMS). The mixture was kept at 4°C for 12 hr and centrifuged for 10 min at 3,000 rpm. Each supernatant was injected intraabdominally once a day for 4 days to the experimental or control rats. The rats were killed by decapitation 24 hr after the last injection and the width of uncalcified cartilage was measured.

**Results and Discussion**

**MGH preparation**

Figure 1 shows the disc electrophoretic pattern of MGH preparation. The major band was identified previously as growth hormone (Yanai et al., 1968). Any hormone other than growth hormone was not detected.

**Precipitin titer**

Precipitin titer of the antiserum estimated by precipitin ring test was 1: 8 against MGH preparation.

**Reaction of the monkey antiserum with normal mouse serum and several viscera**

The antiserum formed one precipitin line against MGH preparation (Fig. 2) and the crude extract of mouse anterior pituitaries (Figs. 3 and 6) in double-diffusion test, but did not react with normal mouse serum (Fig. 2) and with the crude extracts of several viscera of mouse (Fig. 3). These results indicated that

![Fig. 1. Disc electrophoretic pattern of mouse growth hormone used in the present experiment. AP: Anterior pituitary. MGH: Mouse growth hormone preparation. GH: growth hormone. Al: Albumin. PL: Prolactin. IF: Ion front.](image-url)
the antiserum was specific to mouse anterior pituitary hormone(s).

**Immunoelectrophoresis**

Figure 4 shows the immunodiffusion pattern of the antiserum with mouse anterior pituitary homogenate after electrophoresis on polyacrylamide gel. One precipitin arc at the corresponding site of growth hormone band was observed.

**Reaction of the monkey antiserum and rabbit antiserum with MGH preparation**

Figure 5 shows the results. Single precipitin lines were observed between MGH preparation and the rabbit antiserum absorbed with mouse prolactin preparation as well as between MGH preparation and monkey antiserum. These two lines fused completely.

These results strongly suggested that the antiserum was specific to MGH.

**Inhibition test of the biological activity of MGH**

As illustrated in Table 1, the average width of uncalcified cartilage of the experimental rats was significantly smaller than that of the control (P < 0.01), indicating that the anti-
serum inhibited clearly the biological activity of MGH.

Cross-reaction
All the results have demonstrated that the antiserum obtained in the present experiment has a specific antibody only against MGH. Single precipitin lines were observed between the antiserum and MGH preparation, crude extracts of anterior pituitaries of the mouse, rat, rabbit and caprine and BGH preparation (Fig. 6). The immunologically wide cross-reactivity of rodent growth hormone has been suggested by Hayashida and Contopoulos (1967) and Ellis et al. (1969). The present results showed that MGH shared immunologically reactive site identical with that contained in growth hormone of other species.

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References