Some Immunological Properties of Rabbit Antiserum to Mouse Growth Hormone

MOTOAKI KOSUGIYAMA, JUNICHI MORI*, REIKO YANAI, TATSUO HOSODA* AND HIROSHI NAGASAWA

National Cancer Center Research Institute, Tokyo and *National Institute of Animal Industry, Chiba-shi

Synopsis

As a fundamental experiment for the study on the role of growth hormone in mouse mammary gland, some immunological properties of rabbit antiserum to mouse growth hormone (MGH) were studied.

Antiserum was obtained by immunizing New Zealand White male rabbits with MGH preparation extracted from mouse anterior pituitaries. The precipitin titer of the antiserum by precipitin ring test against MGH preparation was 1:16. The antiserum did not react with mouse serum, and extracts of brain, liver, kidney, spleen and heart. The immunoelectrophoretic pattern of polyacrylamide gel after electrophoresis of anterior pituitary with the antiserum or the reaction of antiserum with MGH and mouse prolactin (MPL) preparations on the Ouchterlony agar plate showed two precipitin arcs or lines; one appeared to be due to MGH and another due to MPL. The absorption of the antiserum with MPL preparation resulted in the development of a single precipitin line between the absorbed antiserum and MGH preparation on the Ouchterlony agar plate. Further, immunoelectrophoretic pattern of polyacrylamide gel after electrophoresis of mouse pituitary homogenate with the absorbed antiserum showed one precipitin arc at the corresponding site of MGH band. Inhibition by the antiserum of the biological activity of MGH was proven in rat tibia test and in body and mammary growth of a mouse.

All the results have demonstrated that the antiserum absorbed with MPL preparation is specific only to MGH.

It is well known that anterior pituitary hormones, especially growth hormone and prolactin are essential for mammary gland growth and function, although the mechanisms of their roles are not always understood yet. It would be of much interest to study the effects of inhibition of biological activities of endogenous growth hormone and/or prolactin on mammary growth and function by using the specific antiserum, as one step of clarifying the mechanisms of participation of either hormone.

Many studies have been reported in the immunological characteristics of anterior pituitary hormones; growth hormone in human (Hayashida and Li, 1958a, 1959; Grumbach et al., 1960), rat (Furth and Moy, 1967: Hayashida and Contopoulos, 1967; Takizawa et al., 1967; Ellis et al., 1968), rabbit (Ellis et al., 1968), dog (Hashimoto et al., 1969), ovine (Moudgal and Li, 1961), porcine (Papkoff et al., 1960; Parker et al., 1965), bovine (Hayashida and Li, 1958b, 1959; Moudgal and Li, 1961; Zaan, 1962; Wallace and Sobey, 1965; Kwa et al., 1965; Johke et al., 1966; Sundaram and Sonenberg, 1969), prolactin in mouse (Kwa and Verhofstad, 1967; Kwa et al., 1967a), rat (Furth and Moy, 1967; Kwa et al., 1967b; Takizawa et al., 1967; Ellis et al., 1969); thyrotropin in mouse (Moy et al., 1964). Immunological properties of anterior pituitary hormones in various species were

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reviewed by Li et al. (1962) and Hayashida (1966). So far as we are aware, however, few reports are available on mouse growth hormone (MGH).

The present experiment was carried out in order to investigate the immunological characteristics of rabbit antiserum to MGH.

Materials and Methods

Hormones

Anterior pituitary MGH preparation used for immunization and serological tests was extracted by the method of Lewis et al. (1965). Mouse prolactin (MPL) preparation was extracted from the residue of MGH extraction by the method of Bates and Riddle (1935).

Immunization procedure

Antiserum was prepared by immunizing three New Zealand White male rabbits weighing approximately 3.5 kg with an emulsion of 1.5 mg of MGH preparation each time in complete Freund's adjuvant (Difco Labs., Detroit, Michigan). Injection was given firstly to foot pads and subsequently intramuscularly and intraperitoneally once a week for 26 weeks with a total dose of 39 mg of the preparation per rabbit. The rabbits were bled several times during the later stage of immunization period just before immunization, and pooled antisera were used for serological tests.

Disc electrophoresis (Polyacrylamide gel electrophoresis)

The procedure of analytical poyvacrylamide gel electrophoresis was the same as in our previous paper (Yanai et al., 1968) except that 10% separating gel was used instead of 7.5% gel, because it was found in the anterior pituitary of mouse that albumin and prolactin bands were separated very well by employing 10% gel (Cheever et al., 1969).

Serological test of antiserum

The titer of antiserum was estimated by precipitin ring test in which the doubling dilution technique was employed in successive tubes. The double-diffusion technique of Ouchterlony (1953) (1.2% agar in physiological saline) was used for the characterization of antiserum. The agar plate was prepared as follows; petri dishes of standard size containing 15 ml of 1.2% agar were employed. In most cases, holes of 7 mm in diameter with 2.5 mm well distance were made in the set agar. The MGH preparation used for all serological tests was dissolved at the concentration of 200 µg/ml of physiological saline.

Immunodiffusion using polyacrylamide gel after electrophoresis

The longitudinally sliced polyacrylamide gel was embedded in agar gel plate immediately after electrophoresis of MGH preparation or mouse pituitary homogenate. Two 2.5 cm slits with 0.3 cm width were cut parallel to the polyacrylamide gel at the distance of 0.5 cm from the gel. The slits were filled with antisera. This procedure was substituted for immunoelectrophoresis.

Inhibition of biological activity of MGH

Inhibition test of biological activity of MGH by the antiserum was firstly performed by tibia test of Greenspan et al. (1949) using the hypophysectomized rat. The mixture of MGH preparation (10 µg per day dissolved in physiological saline) with antiserum (0.7 ml per day) or with normal rabbit serum (NRS) (0.7 ml per day) was kept in a refrigerator for 12 hr and the supernatant was injected intraperitoneally once daily for 4 days in the experimental and control rats, respectively. The inhibitory effects of the antiserum were estimated by the difference in the width of uncalcified proximal epiphyseal cartilage of tibia between the experimental and control rats. Furthermore, inhibitory effects of the antiserum on endogenous MGH were investigated using the body growth and mammary DNA content of mouse as the indices. The antiserum or NRS was injected intraperitoneally for 10 days to 60-day-old intact female C3H/He mice. The daily dose of 0.5 ml of antiserum or NRS was given at 8 hr interval in three injections a day. On the next morning of the last injection, % increase of body weight was calculated and DNA content in the bilateral inguinal glands was determined as described previously (Nagasawa et al., 1966).

Results and Discussion

Properties of antigen

Disc electrophoretic pattern of MGH preparation used in the present experiment is shown in Figure 1. The major band was identified previously as growth hormone (Yanai et al., 1968).

Precipitin ring test

Precipitin titer of the antiserum against MGH preparation (200 µg/ml physiological saline) was 1:16.

Reaction of the antiserum with normal
mouse serum and with the extracts of some viscera

The antiserum did not react with normal mouse serum (NMS), but reacted with MGH preparation, in which two precipitin lines developed in the agar gel plate (Fig. 2). The antiserum did not react with crude extracts of mouse liver, kidney, spleen, brain and heart, but reacted only with the pituitary extract (Fig. 3). These results indicated that the antiserum obtained was specific to mouse anterior pituitary hormones.

Immunodiffusion using polyacrylamide gel after electrophoresis

Figure 4 shows the immunodiffusion patterns of 10% polyacrylamide gels after electrophoresis of MGH preparation and mouse pituitary homogenate with the antiserum. Two precipitin arcs were observed; one was located at the corresponding site of MGH band and another at MPL band.

The reaction of the antiserum with MPL preparation

The reaction of the antiserum with MGH and MPL preparations is presented in Figure 5. The antiserum had two precipitin lines with MPL preparation; one was a distinct line (external) and another a feeble one (internal). They fused completely to the two precipitin lines with MGH preparation, respectively.

These results suggested that the antiserum obtained contained antibody against MPL as well as against MGH, which might be due
Fig. 4. Immunodiffusion pattern of 10% polyacrylamide gels after electrophoresis of mouse growth hormone (MGH) and mouse pituitary homogenate (Pit.) with the antiserum.

GH: Growth hormone band, PL: Prolactin band
i.f.: Ion front

Fig. 5. Ouchterlony plate showing the reaction of the antiserum with mouse growth hormone (MGH) and mouse prolactin (MPL) preparations.

Absorption of the antiserum with MPL preparation

The antiserum (1 ml) was absorbed with MPL preparation (0.2 mg). The absorbed antiserum developed the single precipitin line with MGH preparation on the Ouchterlony agar plate, while the preabsorbed antiserum had two precipitin lines with MGH preparation (Fig. 6). The internal line between the center and the right wells which was due to MPL contamination disappeared between the center and left wells. Moreover, in the im-
Table 1. Inhibition by the antiserum of the biological activity of mouse growth hormone*  

<table>
<thead>
<tr>
<th>Group</th>
<th>Saline</th>
<th>BGH</th>
<th>MGH</th>
<th>MGH + NRS</th>
<th>MGH + Antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Daily dose (μg)</td>
<td></td>
<td>10</td>
<td>10</td>
<td>10 + 0.7 ml</td>
<td>10 + 0.7 ml</td>
</tr>
<tr>
<td>Width of uncalcified</td>
<td>169</td>
<td>182</td>
<td>222</td>
<td>182</td>
<td>170</td>
</tr>
<tr>
<td>cartilage (μ)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BGH: Bovine growth hormone, MGH: Mouse growth hormone, NRS: Normal rabbit serum  
* The tests were repeated three times by using the different batches of MGH preparation and the antiserum, and the similar results were obtained. One of the results is presented in this table.

munodiffusion of 10% polyacrylamide gel after electrophoresis of mouse pituitary homogenate with the absorbed antiserum, the single precipitin arc was observed at the corresponding site of MGH band (Fig. 7).

All the results provide that the pre-absorbed antiserum has the antibody against MPL as well as MGH which was ascribed to the existence of prolactin contamination in MGH preparation, and that the antiserum specific only to MGH was obtained by absorption of the antiserum with MPL preparation.

**Inhibition test of biological activity of MGH**

As shown in Table 1, the addition of the antiserum had a tendency to decrease the biological activity of MGH. The width of uncalcified cartilage was also made smaller by the addition of NRS to MGH, which was probably attributed to the nonspecific inhibition by NRS.

Table 2 shows the inhibitory effects of the antiserum on endogenous MGH employing body growth rate and mammary DNA content as the indices. Body growth rate and mammary DNA content were distinctly smaller in the experimental mice than in the control, although the differences were not statistically significant, indicating the antiserum inhibited

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of mice</th>
<th>Body growth rate (%)</th>
<th>Mammary DNA content (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. (Antiserum)</td>
<td>8</td>
<td>9.7 ± 1.5</td>
<td>608 ± 70</td>
</tr>
<tr>
<td>Cont. (NRS)</td>
<td>9</td>
<td>14.0 ± 1.5</td>
<td>789 ± 75</td>
</tr>
</tbody>
</table>

Mean ± S.E.M.
NRS: Normal rabbit serum
apparently the biological activity of endogenous MGH.

Acknowledgement

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References