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IDENTIFICATION OF A NONIMMUNOREACTIVE BUT HIGHLY BIOACTIVE FORM OF PROLACTIN IN THE MOUSE PITUITARY BY GEL ELECTROPHORESIS

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SUMMARY

Polyacrylamide gel electrophoresis of fresh mouse pituitary extracts in 10% acrylamide reveals three closely situated bands of protein in the area where prolactin usually migrates. The fastest migrating band constitutes only 10-30% of the total, but it is twice as active in the pigeon crop-sac bioassay as the major band and has little or no immunologic cross-reactivity against an antiserum to the major constituent. This newly recognized band may be a hitherto unrecognized molecular variant of prolactin.

INTRODUCTION

Despite high levels in the pituitary gland, serum levels of radioimmunoassayable prolactin in mice show a lack of positive correlation with the incidence of mammary tumors (1). The reason for this may be that some of the prolactin undergoes changes in its immunologic properties during or after secretion and thus escapes detection (2). This possibility has led us to search for forms of prolactin in the sera and pituitary glands of mice that do not cross-react with the antibody used in the current radioimmunoassay. Mouse pituitary prolactin was originally identified (3) and isolated for antibody production (4) by the use of polyacrylamide gel electrophoresis. Such electrophoresis using a 10% concentration of acrylamide shows a major band of prolactin, which was used primarily in the development of the radioimmunoassay, and one or two additonal minor bands, which were considered to be deamidation products of the major component (3, 5). In this study, we have examined the bioactivity and immunoactivity of the three protein bands migrating in the area where prolactin usually migrates.

MATERIALS AND METHODS

Pituitary glands were removed following decapitation. The posterior lobe was separated from the anterior and discarded. The anterior lobe was homogenized

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in 0.05M Na_2CO_3-$NaHCO_3$ buffer, pH 10, in the ratio of 10 mg/ml and centrifuged. One hundred microliters of the supernatant were applied to each gel column of size 0.5 x 5.5 cm (for analytical purposes) or 0.5 x 8 cm (for isolation purposes). Pituitary glands from 225 mice were processed for isolation of the protein bands and electrophoresed within an hour of removal from the animal.

Disc electrophoresis was performed according to the method of Davis (6) with a 10% concentration of acrylamide (3). The gels were stained with 0.25% Coomassie Blue G-250 and 12% trichloroacetic acid mixture (1:20 vol/vol) and destained by diffusion in distilled water. The amount of protein in a band was estimated by scanning in a Gilford densitometer.

Protein was eluted from the gels as described by Lewis and Clark (7). With one stained gel from each run as a guide, gel segments containing the three bands designated here PRL I, PRL II and PRL III (Fig. 1) were cut out, minced and allowed to elute in a small volume of 0.01M NH_4HCO_3, pH 7.8, at 4°C. The gel fragments were removed from the eluate by centrifugation.

The protein concentration of the eluates was determined by the method of Bensadoun and Weinstein (8). The biologic activity of the eluates was determined by the pigeon crop-sac assay, as described by Nicoll (9). The immunologic activity was estimated by a mouse prolactin radioimmunoassay previously described (10). Immunodiffusion was carried out by the method of Ouchterlony (11).

RESULTS

Three closely situated bands in the area where prolactin usually migrates (3) were observed. A diagramatic representation of the location of these bands is given in Figure 1. The slowest migrating band, designated PRL I, was predominant. Immediately ahead of it appeared a faintly staining, diffuse band, designated PRL II. Most anodal and well-separated from the second was a third, very distinct band, designated PRL III.

PRL I constituted 48 to 80%, PRL II 11 to 22% and PRL III 9 to 33% of the total protein in the various pituitaries examined (Fig. 2). Male mice had the highest concentrations of the PRL III band (20 to 33%); virgin C3H/St females, the lowest (9%). Mice of the high-mammary tumor C3H/St strain had higher proportions of PRL I and lower proportions of PRL III band compared with mice of the low-mammary tumor C57BL/St strain.

Elution of the proteins from the gels yielded preparations of only partial purity. Re-electrophoresis of the three eluates showed that the PRL I eluate contained 45% of PRL I, 37% of PRL II and 19% of PRL III; the PRL II eluate had 46% of PRL II and 32% and 22%, respectively, of PRL I and PRL III; and the PRL III eluate had only PRL III.

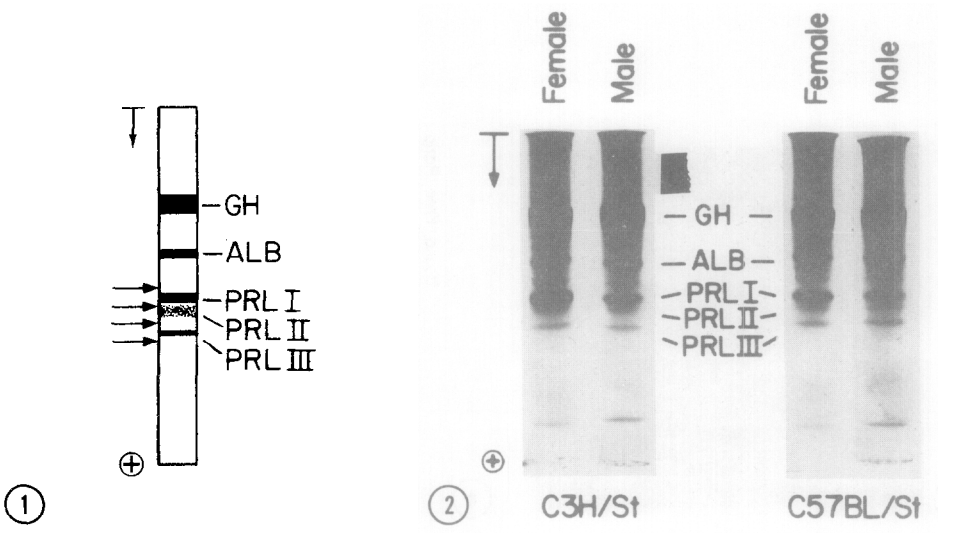


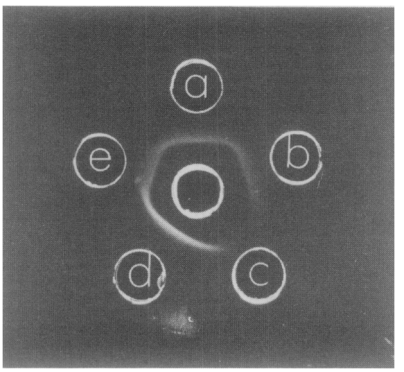
Fig. 1. Disc electrophoretic pattern of mouse pituitary extract on a 0.5 x 8 cm gel. The arrows indicate the points where the gels were cut in order to isolate the three protein bands.

Fig. 2. Disc electrophoresis of anterior pituitaries from mice of a high-mammary tumor (C3H/St) and a low-mammary tumor (C57BL/St) strain. One hundred microliters of the pituitary extract containing 1 mg equivalent of tissue were applied on a 0.5 x 5.5 cm gel column in each case.

PRL I and PRL II each elicited a single precipitin line on the Ouchterlony plate, which was confluent with the line produced by the standard mouse prolactin (Fig. 3). PRL III produced no visible precipitin line. None of the three proteins produced any precipitation against an antiserum to mouse growth hormone.

All three components produced dose-response curves parallel to the standard in the mouse prolactin radioimmunoassay (Fig. 4). PRL I and PRL II were quite active and similar in activity; PRL III was the least active of all. A quanti tative estimate of immunologic potency is given in Table I, which shows that PRL III had only one-sixth as much immunoactivity as PRL I. None of the three proteins produced significant inhibitions in a mouse growth hormone radioimmunoassay.

The results of the pigeon crop-sac bioassay are given in Table I. The PRL I and PRL II eluates had similar biologic potencies, which were lower (1/3 to 1/4), however, than that of the standard mouse prolactin. The PRL III band had the highest activity--more than twice as much as either PRL I or PRL II.



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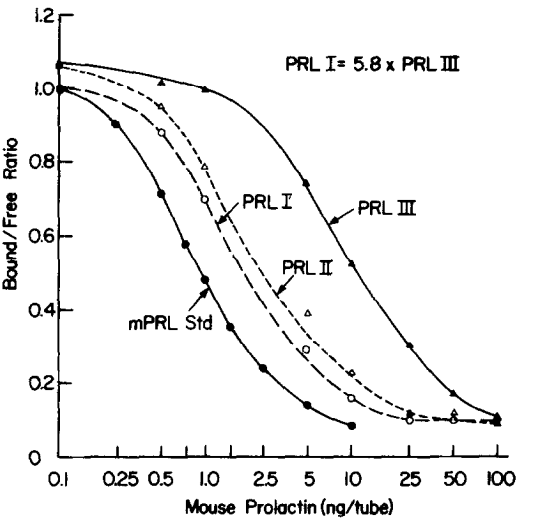

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Fig. 3. Immunodiffusion of prolactin bands against an antiserum to mouse prolactin. The center well contained 10 µl of the prolactin antiserum used in the radioimmunoassay. The peripheral wells contained in 10-µl volumes the quantities indicated below of the following substances: a) PRL I--0.4 µg, b) PRL II--0.4 µg, c) PRL III--0.4 µg, d) mouse prolactin standard--1 µg and e) mouse pituitary extract--100 µg.

Fig. 4. A plot of the dose-response curves of the three prolactin bands in the mouse prolactin radioimmunoassay. Final dilution of antibody used was 1:25,000. Note that the PRL III band produced a parallel inhibition only at high concentrations. mPRL Std = mouse prolactin standard.

Table 1. Biological and immunological potencies of the three electrophoretic bands of mouse prolactin.

Band No.	Pigeon crop-sac activity[1]	Activity in the radioimmunoassay[2]
	(IU/mg protein)	(IU/mg protein)
PRL I	7.0 (0.9 - 17.1)	12.8
PRL II	5.3 (1.8 - 10.0)	9.0
PRL III	14.8 (2.3 - 33.1)	2.2

[1]A 2 x 2 bioassay, 10 birds per point. Values in parenthesis indicate 95% Confidence Interval of the potency estimate. Standard used was NIH-P-S_{12}-ovine, potency = 35 IU/mg. The average index of precision (λ) for the three assays was 0.42.

[2]Computed from the data in Figure 4. Standard used was mouse prolactin, potency = 25 IU/mg.

DISCUSSION

The results of this study indicate that at least three identifiable charge isomers of prolactin are present in fresh mouse pituitary extracts. A similar finding of three bands in organ cultures of mouse pituitary was reported by Kohmoto (5). Although the concentrations of the two minor components in the pituitary extracts are small, the more acidic of these (PRL III) has distinct immunologic characteristics. It does not cross-react with an antibody to the major prolactin component. (The slight (17%) cross-reactivity seen in the radioimmunoassay was most likely due to its contamination with PRL I and/or PRL II). This band also has a greater pigeon cros-sac stimulating bioactivity. Thus, PRL III represents a new form of prolactin in the mouse pituitary gland. Asawaroengchai et al. (2) observed a faster migrating component with a high bioassay:immunoassay ratio in a 4-day culture medium of rat pituitary.

Whether PRL III is a deamidation or proteolytic conversion product of the major component (PRL I) or whether it represents a structural variant of prolactin is not known. Since it is found in fresh pituitary extracts and its relative proportions in male and female mice are different, it is most likely a naturally occurring substance in the mouse pituitary. Lewis et al. (12) have identified a structural variant of growth hormone in the human pituitary gland that has poor immunologic reactivity against antibody to the major human growth hormone but is sufficiently bioactive. Some recent studies (13, 14) have shown that rat prolactin is synthesized as a larger molecule that is enzymatically cleaved before secretion. However, since antibody to the major pituitary prolactin was used to identify the synthetic product, a nonimmunoreactive entity, such as PRL III, would not be recognized. Thus, the biosynthetic relationship of this new band to the secretory process of prolactin awaits characterization.

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