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A Radioimmunoassay for Human Prolactin

(affinity chromatography/galactorrhea/amniotic fluid/menstrual cycle/growth hormone)

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ABSTRACT A radioimmunoassay for primate prolactin has been developed, with [^{131}I] monkey prolactin, and antibodies to monkey or human prolactin. The assay is specific for prolactin; human growth hormone, and human and monkey placental lactogen show no significant crossreaction. The assay is sensitive enough to measure prolactin concentrations in the sera of most humans studied. The concentration of prolactin in the serum of normal children and adults of either sex was usually below 30 ng/ml, while very high concentrations (up to 500 ng/ml) were observed in newborn infants. The serum prolactin concentration during the menstrual cycle showed no definite increase in the luteal phase. Of 24 patients with galactorrhea, 20 had prolactin concentrations above 30 ng/ml; the highest value observed was 1500 ng/ml. In contrast, 12 of 13 patients with acromegaly had concentrations within the normal range. During pregnancy, the concentration of prolactin in serum rose progressively from an average of 30 ng/ml in the first trimester to 200 ng/ml at term. Postpartum, prolactin concentrations fell to normal levels after 1–2 weeks. Suckling was a potent stimulus to prolactin release, increasing its concentration in serum some 10- to 20-fold.

The existence of prolactin in primates has been questioned (1) because all attempts to isolate it by chemical means have failed. All preparations from primate pituitary glands possessing lactogenic activity have been shown to be contaminated by growth hormone to varying degrees (2); this observation has led some investigators to suggest that perhaps in primate species growth hormone and prolactin are one and the same hormone. Many observations, however, point to the existence of prolactin as a separate hormone in primates; this evidence has been reviewed recently (3). We have demonstrated that both monkey (4) and human (5) pituitary glands incubated *in vitro* synthesize and secrete proteins that are immunologically related to sheep prolactin. These proteins, which on the basis of indirect evidence would appear to represent prolactin, can be clearly distinguished from growth hormone by immunological methods. The immunological difference between primate prolactin and growth hormone enabled us to completely and reproducibly separate monkey prolactin (MPr) and growth hormone (6). With this crude MPr, we obtained antibodies to MPr that bound sheep [^{131}I]prolactin (7). In the present study, we describe a radioimmunoassay for prolactin with which we have studied prolactin concentration in the sera of normal subjects and sera from a number of patients.

Abbreviations: MPr, monkey prolactin; HPr, human prolactin; HPL, human placental lactogen; HGH, human growth hormone.

MATERIALS AND METHODS

Preparation of immunoadsorbents

Antibodies to sheep prolactin and to human placental lactogen (anti-HPL) were coupled to Sepharose (6) by the method of Cuatrecasas *et al.* (8).

Preparation of labeled hormone

Crude MPr was iodinated by the method of Hunter and Greenwood (9), and purified by affinity chromatography with anti-sheep prolactin. The [^{131}I]MPr was eluted with 4 M sodium thiocyanate and immediately placed on a Sephadex G-100 column to remove the thiocyanate and any damaged [^{131}I]MPr.

Standards

A postpartum serum sample, kindly supplied by Dr. A. Frantz, containing 161 ng/ml of sheep-prolactin equivalents by bioassay (10) and less than 5 ng/ml of human growth hormone (HGH), was used as a standard in our assay. Wilhelmi HGH HS-1394 was used as standard for the HGH assay.

Antisera and assay procedure

Rabbit antiserum to MPr, generated as described (7), was used at a final dilution of 1:15,000 in the assay. Among eight rabbit antisera to HGH (anti-HGH) examined, two were found to bind significant amounts of labeled MPr, presumably because they also contained antibodies to human prolactin (HPr). One of these antisera, when used in the prolactin radioimmunoassay at a 1:500 dilution, gave results comparable to those obtained when anti-MPr was used. A double-antibody radioimmunoassay procedure (11) was used with sheep anti-rabbit globulin serum as the second antibody.

RESULTS

Preliminary characterization of the assay

Various substances were tested for crossreactivity in the assay (Fig. 1). Sheep prolactin crossreacts, but gives a nonparallel inhibition curve. The parallelism observed between the inhibition curves of the standard serum and the human pituitary extract indicates that the crossreacting materials in the serum and the pituitary are immunologically indistinguishable. The weak crossreaction shown by HGH is probably due to contaminating HPr, as passage through an anti-sheep prolactin–Sepharose column completely removed the crossreactivity. Human pituitary gonadotropin LER 907 (1 μg), human neurophysin (5 μg), oxytocin (0.5 μg), lysine vaso-

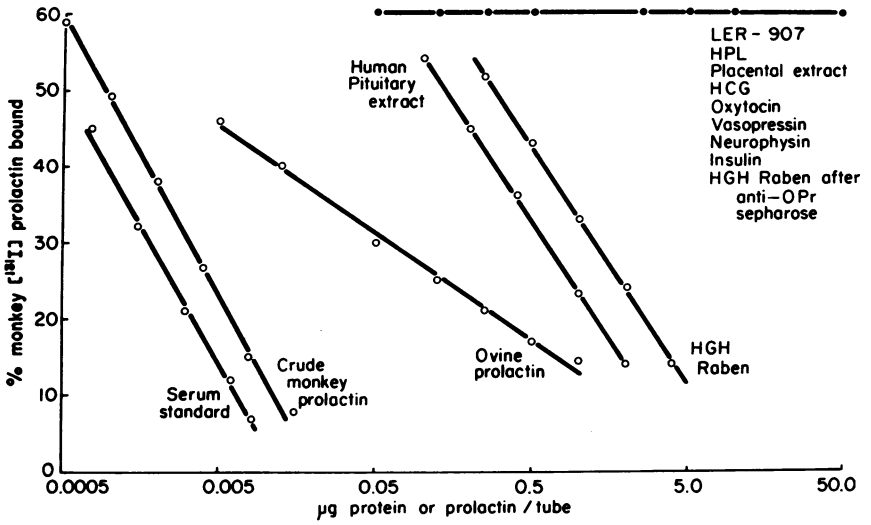

FIG. 1. Crossreactivity of different materials in the assay: the curve of the standard serum is plotted on the assumption that it contained 161 ng/ml of human prolactin. Other curves are plotted on the basis of the amount of protein added. Among the substances showing no crossreaction, only some (see text) have been tested up to a concentration of 50 μg. Frozen human pituitary or placental tissue was extracted with 1 ml of 0.1 M NH_4HCO_3/20 mg of wet weight. LER-907, human pituitary gonadotropin; HCG, human chorionic gonadotropin; anti-OPr Sepharose (see *Methods*, OPr = sheep prolactin).

pressin (10 μg), a placental tissue extract, HPL (50 μg) and its reduced and alkylated derivative (50 μg), human chorionic gonadotropin (100 IU), and insulin (10 μg) all do not cross-react in the assay.

The binding of [^{131}I]MPr to the antiserum is readily inhibited by serum from a patient with galactorrhea, but not from sera from normal or acromegalic subjects, indicating that the assay system specifically measures a circulating antigen present in galactorrheic sera. This antigen is related to sheep prolactin immunologically, as it is completely removed by passing such serum through a column of anti-sheep prolactin–Sepharose, whereas passage of the same serum through an anti-HPL–Sepharose column failed to remove any of the crossreacting material. Sera from pregnant and postpartum subjects, and from cord blood, gave inhibition curves parallel to that of the serum from the galactorrheic patient, showing that the immunoreactive substance(s) in these types of sera are identical. The sensitivity of the assay was 0.25–0.5 ng, and the useful range up to 3 ng. The reproducibility of the assay was satisfactory in the range from 0.5 to 2.5 ng; intra- and interassay variations rarely exceeded 10 and 20%, respectively, if duplicate assays were performed.

TABLE 1. *Recovery of HPr in BSA* and human serum*

Amount of serum standard (161 ng/ml) added		Observed HPr	
		in BSA	in human serum
μl	HPr (ng)	(ng/ml)	(ng/ml)
0	0	0	5
10	2	2	6
25	4	4	10
50	8	8	12
75	12	11	17
100	16	18	22
150	24	27	30
300	48	45	56

* BSA, bovine-serum albumin.

As undiluted sera were assayed in most instances, and concentrations were determined from standards diluted in 2.5% bovine-serum albumin, it was necessary to determine whether this difference would produce any systematic errors due to nonspecific effects of serum. Different amounts of the serum standard were diluted to 1 ml with bovine-serum albumin or a human-serum sample that contained 5 ng/ml of HPr. The concentrations of HPr in both sets of tubes were measured; the results shown in Table 1 indicate that the presence of serum proteins did not significantly affect the assay results.

Serum prolactin concentration

Normal Adults. This group consisted of hospitalized patients with no suspected endocrinopathies. Of 42 males, aged 16–84, only seven (16%) had serum HPr concentrations above 15 ng/ml; the range of all values in the group was 0–28 ng/ml. Among 47 females, aged 16–85, 15 (30%) had concentrations above 15 ng/ml; the range was 0–30 ng/ml.

Menstrual Cycle. Fig. 2 shows the serum prolactin concentration in 9 women from whom daily samples were obtained throughout a 30-day period. No ovulatory prolactin peak was observed and no increase was observed in the luteal phase of the cycle. The serum prolactin concentrations in women in the

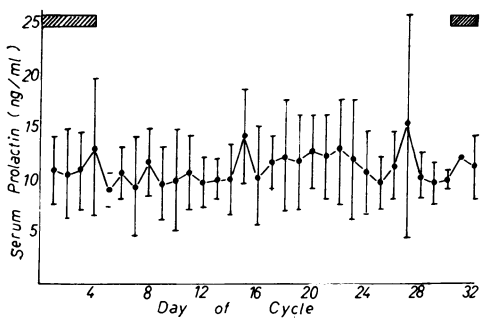

FIG. 2. Serum prolactin concentration during the menstrual cycle. The mean ±SD is shown for 9 subjects.

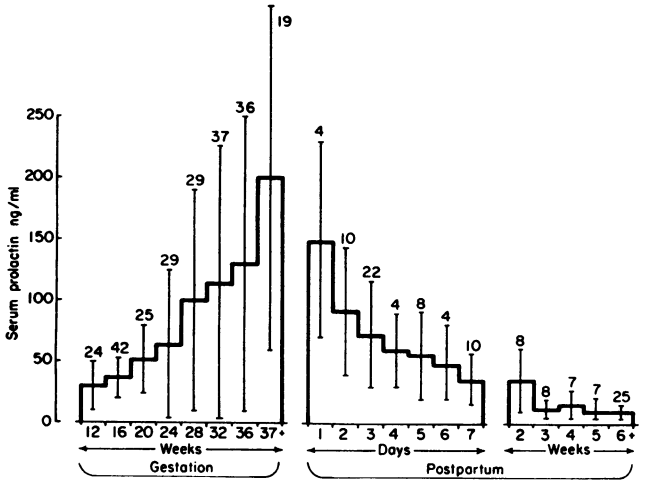

FIG. 3. Serum prolactin concentrations during pregnancy and postpartum period. The prolactin concentration is given as mean ±SD. The number of subjects in each group is indicated.

postmenopausal period were identical to those seen during a menstrual cycle.

Pregnancy and the Puerperium. Fig. 3 shows the HPr concentration in sera during various stages of normal pregnancy and in the immediate postpartum period in women who were not breast feeding. There was a progressive rise from the first trimester concentration of 30 ± 20 ng/ml (SD) to a mean of about 200 ng/ml at term. Postpartum, the decline was fairly rapid; by the end of the first week the mean concentration had dropped to about 30 ng/ml. There were large variations in the HPr concentrations at each stage of pregnancy, particularly in the third trimester where values ranged from 40 to 600 ng/ml. Amniotic fluid concentrations of prolactin were often 5- to 10-fold greater than the maternal serum concentrations. Both HPr and HPL concentrations decline postpartum but HPL is usually undetectable by 24 hr (12), whereas HPr concentrations remain elevated much longer.

Effect of Suckling. Fig. 4 shows that the serum prolactin concentration increased by 10 to 20-fold 30 min after the start of breast feeding. The subsequent fall was equally rapid, suggesting that the half-life of prolactin in such serum is less than 30 min.

Newborn Infants and Children. Fig. 5 shows that the HPr concentrations in newborn serum are similar to maternal levels at term. A progressive fall is observed after delivery, so that by 6 weeks the HPr concentrations have declined to

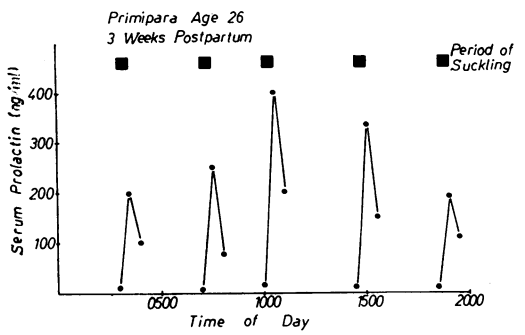

FIG. 4. Effect of suckling on serum prolactin concentration.

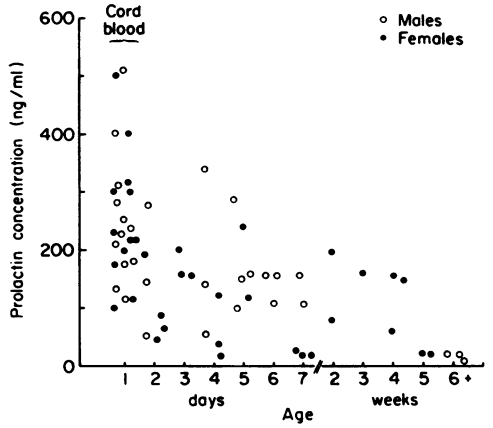

FIG. 5. Serum prolactin concentrations in the newborn.

those of the normal adult. The HPr concentrations in serum of 37 hospitalized children (aged 2 months to 17 years) showed no significant difference from that of adults.

Patients with Galactorrhea and Acromegaly. The acromegalic group included subjects who had been treated by conventional pituitary irradiation. Among the 24 patients with galactorrhea, eight had pituitary tumors, four developed galactorrhea after contraceptive medication, and one after hysterectomy; in the rest there was no obvious cause. The one male subject was a young man with mild gynecomastia and moderate galactorrhea whose serum HPr concentration was 60 ng/ml. The cause of the galactorrhea is not known.

It is clear from Fig. 6 that, while HPr concentrations were high and HGH concentrations low in patients with galactorrhea, the opposite was true for all acromegalics except one. In the group with galactorrhea, no correlation was observed between HPr concentrations and the presumed etiology. Three cases of galactorrhea (one in whom contraceptive therapy was implicated and two idiopathic) had serum HPr concentrations below 20 ng/ml.

Patients with Hypopituitarism. Table 2 summarizes the serum HPr and HGH concentrations of six patients with different degrees of hypopituitarism. The surprising finding was that prolactin concentrations were detected in all pa-

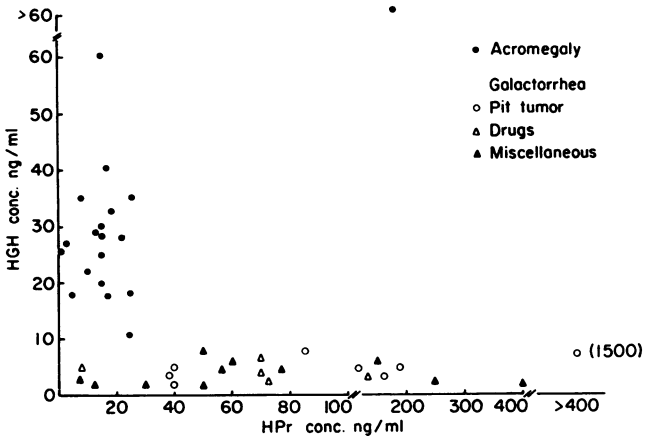

FIG. 6. Serum growth hormone and prolactin concentrations in patients with galactorrhea and acromegaly. Patients with galactorrhea have been subdivided into several groups, according to the presumed etiology.

TABLE 2. *Patients with hypopituitarism*

Age	Sex	Clinical status	Maximum HGH after insulin infusion (ng/ml)	Serum HPr (ng/ml)		
				before immuno-adsorption	after anti-HPL Sepharose	after anti-OPr* Sepharose
13	M	Growth retardation	<0.5	15	14	<2.5
12	F	Growth retardation	<0.5	8	9	<2.5
14	M	Growth retardation	<0.5	32	30	<2.5
44	F	Hypopituitarism after excision of craniopharyngioma	<0.5	60	65	<2.5
15	F	Hypopituitarism after excision of craniopharyngioma	—	10	8	<2.5
50	F	Breast cancer	—	10	—	—
		before hypophysectomy	—	<2.5	—	—
		after hypophysectomy				

* OPr, sheep prolactin.

tients except one, with breast cancer, whose pituitary gland was surgically removed for palliative reasons. That prolactin was being measured in the serum of these patients was shown by the virtually complete removal of the crossreacting substance when the sera of these patients were passed through an anti-sheepprolactin–Sepharose column, whereas an anti-HPL–Sepharose column had no effect. The serum HPr concentration (60 ng/ml) observed in the patient who had a craniopharyngioma removed was well above the concentrations observed in normal adults. Hypophysectomy in the patient with breast carcinoma clearly led to a fall in the concentration of serum HPr.

DISCUSSION

The radioimmunoassay has been carefully evaluated with regard to its specificity. No other hormones examined, including HGH and HPL, crossreact significantly. Moreover, by acrylamide gel electrophoresis at an acid pH, it was possible to separate HGH and HPr and to demonstrate that the crossreacting material in the HPr assay was prolactin (13). Nonspecific serum factors do not inhibit the binding of the tracer to the antibody; passage of a serum sample with a low concentration of HPr through an anti-sheep prolactin column removed all crossreacting material in the prolactin assay, whereas an anti-HPL column did not affect the prolactin concentration upon reassay.

A bioassay for prolactin that has been reported by Frantz and Kleinberg (10) is sufficiently sensitive to detect circulating prolactin at elevated concentrations, but both HPL and HGH produce positive responses in the bioassay. Postpartum lactating women and patients with nonpuerperal galactorrhea had high prolactin activity in their plasma (range 15–130 ng/ml) that was not neutralized by antiserum to HGH; it was concluded (10) that the residual biologic activity exhibited by these sera was not due to HGH, but to a separate HPr. The uniformly low HGH concentrations and elevated HPr values measured by a specific assay in our series of galactorrheic patients support this conclusion. The acromegalic patients studied by us generally have HPr concentrations within normal limits; however, in one case due to a pituitary tumor, the HPr concentration was found to be 150 ng/ml, although clinically the patient had no abnormal breast development or function. This certainly suggests that in some cases of acromegaly prolactin is secreted in increased amounts as well, and that prolactin alone is insufficient to induce abnormal breast changes. It is conceivable that the pituitary tumor, while causing acromegaly by excessive HGH production, also interfered with hypothalamic function, thus leading to a decrease in prolactin-inhibiting factor and a consequent rise in HPr secretion from residual normal pituitary tissue. Alternatively, some pituitary tumors functioning autonomously may secrete excessive amounts of both HGH and HPr. It should be possible to differentiate between these two possibilities with the use of L-dihydroxyphenylalanine, which we have found consistently shuts off prolactin secretion in normal subjects (unpublished observations).

Among the patients with "hypopituitarism", the three children with growth failure had detectable concentrations of HPr in serum, although after insulin-induced hypoglycemia their serum HGH concentration did not increase. The finding of detectable prolactin concentrations in these patients suggests that some functional pituitary tissue was present. It is likely that in patients with "idiopathic" hypopituitarism, the disease process primarily involves the hypothalamus if high concentrations of prolactin are found. Our data suggests that such a high concentration is common in patients with "idiopathic hypopituitarism". The therapeutic implications of this hypothesis are obvious; the administration of appropriate releasing factors might correct the disease in many patients of this type.

During pregnancy the concentration of HPr increases progressively, whereas in the rhesus monkey, MPr concentrations remain low (14). Possibly the difference in prolactin secretion in the two species is accounted for by the very great difference in estrogen metabolism during pregnancy. In the human being at term, the daily urinary estrogen excretion is 30 mg, compared to 30 μg in the monkey. Perhaps in primates, as in rats (15), there is a direct correlation between serum prolactin concentration and circulating estrogen. Moreover, in both rats and primates, stress is a potent stimulus to the release of prolactin (14). One of the interesting questions posed by the high prolactin concentrations in serum throughout pregnancy is what factors inhibit lactation until after delivery. The very high concentration of prolactin in the newborn serum, and especially in the amniotic fluid, raises the possibility that prolactin might serve an important function during fetal or neonatal life. The role of prolactin during stress and the menstrual cycle remains to be clarified.

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