Development of a radioimmunoassay for *Cebus apella* prolactin

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Prolactin (PRL) is a pituitary hormone that plays important roles in mammalian reproductive physiology, specially lactation. The regulation of PRL secretion shows important species differences. To study PRL regulation in a subhuman primate, the ***Cebus apella****, we developed an heterologous radioimmunoassay using an antibody against rhesus PRL (anti-m5PRL) and a* ***Cebus apella*** *pituitary extract as PRL standard. The assay has a sensitivity that allows measurements of cebus PRL in small amounts of* ***Cebus apella*** *plasma obtained from animals in different physiological conditions. Plasma cebus PRL concentrations (± SEM) varied in different reproductive stages. PRL concentration in adult* ***Cebus apella*** *females that have regular menstrual cycles (161.6 ± 15.0 mIU/ml) was similar to that found in adult (100.3 ± 7.6 mIU/ml) and prepuberal males (101.2 ± 3.9 mIU/ml). PRL concentration was higher in pregnant (677.8 ± 11.8 mIU/ml) and in nursing (625.0 ± 47.0 mIU/ml)* ***Cebus apella*** *females than in 15-d post-partum non-nursing (369.0 ± 19.0 mIU/ml) and cycling females. PRL concentration in* ***Cebus apella*** *newborns (719.0 ± 49.2 mIU/ml) was similar to that found in pregnant and nursing females, and higher than in the other females as well as adult and prepuberal males. These differences in PRL concentration in different physiological conditions are similar to that observed in humans and other primates. A PRL response to thyrotropin releasing hormone (TRH) was demonstrated in 2 nursing* ***Cebus apella*** *females, similar to the response found in nursing woman and rhesus.*

Altogether, the data presented support the proposal that the assay developed to measure PRL in ***Cebus apella*** *is an adequate tool to study the physiology of PRL in this species.*

Key terms: ***Cebus apella****, prolactin, PRL, radioimmunoassay.*

INTRODUCTION

Prolactin (PRL) is a pituitary hormone present in most vertebrates species. PRL functions are manifold and span from osmoregulation in fishes, growth and development in amphibians, maternal behavior in pigeons and many others (Nicoll, 1974). In mammals, PRL serves at least two important functions: it is involved in the regulation of immune system (Berczi *et al*, 1981; Reber, 1993) and it plays a decisive role in the preparation, maintenance and secretory activity of the mammary gland

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during lactation (Neill & Nagy, 1994). Thus, in most mammals, PRL is essential for the survival of the young.

The regulation of PRL during lactation is not well understood. During lactation, the stimulus for PRL release is provided by the young suckling the maternal nipple. Suckling generates a neural reflex that releases PRL from the maternal pituitary. The available data show important differences between species in the mechanisms involved in PRL regulation. As a consequence, results obtained in rodents (the most studied species) can not be extrapolated to the humans (Neill & Nagy, 1994). Since many experiments cannot be done in the human, non human primates provide an important alternative model for research in this area. The *Cebus apella* monkey, a New World primate, shares several reproductive and endocrine features with the human (Nagle & Denari, 1983) and may provide a useful model to study regulation of PRL during lactation. Since an assay for the measurement of PRL in this species is not available, our objective was to establish and validate a reproducible, specific and sensitive radioimmunoassay to measure PRL in small volumes of *Cebus apella* plasma.

PRL is a protein hormone formed by a single chain of 197 to 199 aminoacids that can be recognized by specific antibodies. PRL from several species has been purified allowing to develop antibodies and set up radioimmunoassays using these antibodies and the respective purified PRL as standard. This hormone is highly conserved across several species (Neill & Nagy, 1994) and antibodies against PRL from one species may recognize PRL from other. As cebus PRL has not been purified yet, standards and antibodies against this hormone are not available. To overcome this difficulty we developed an heterologous assay for cebus PRL using a polyclonal antibody against rhesus PRL. This antibody recognizes PRL of several species of primates (rhesus, *cynomolgus* and human) and does not present cross reaction with other human or rhesus pituitary hormones (Bethea & Papkoff, 1986). We prepared a *Cebus apella* pituitary extract to be used as standard. We report here the development of the assay and its validation in the *Cebus apella*, in different physiological situations known to modify PRL secretion.

Part of this work was presented as a communication (Vásquez & Vergara, 1995) to the 38th Annual Meeting, Society of Biology of Chile, Viña del Mar, Chile.

MATERIALS AND METHODS

Radioimmunoassay (RIA) reagents

We used an antiserum against rhesus monkey PRL (anti-m5PRL) prepared in rabbits and supplied by Dr Cynthia Bethea, Department of Reproductive Biology and Behavior, Oregon Regional Primate Center, Beaverton, Oregon. This antibody cross-reacts with human PRL (hPRL) and rhesus monkey PRL (rhPRL), but not with human or rhesus GH or human FSH, LH or TSH (Bethea & Papkoff, 1986). Human PRL from NIDDK-NIH was used to prepare a iodinated tracer and hPRL from WHO (IRP75/504) was used as standard. Goat anti-rabbit antibody (2nd antibody) was purchased locally (LMNI/PUC/CHILE 659, Lot 4/91) and normal rabbit serum was obtained from WHO (kit 478410). Chemicals were purchased from Merck or SIGMA. Commercial ^{125}I-hPRL was purchased from DPC, Santiago, Chile.

Cebus apella *specimens*

A crude cebus PRL extract was prepared from a pituitary gland obtained from an untreated adult *Cebus apella* female that died during a routine surgical procedure. The pituitary was removed within one hour of death and kept frozen at -20°C. To prepare the extract, the gland was thawed and homogenized by pressing with a glass rod against the walls of a plastic capsule with a minimal volume of 10 mM phosphate-buffered saline (PBS) pH 7.5 and 0.6 mM PMSF (proteins inhibitor). The volume was completed to 1 ml with PBS, and the mixture was centrifuged for 10 min at 1000 x g. The supernatant was separated and stored in aliquots at -20°C until used.

Individual measurements of PRL were made in plasma samples from 15 cycling females in the follicular phase of the menstrual cycle, 6 non-nursing and 10 nursing females at 15 days postpartum, 10 newborns at 4 days of age and 4 prepuberal and 4 adult males. Blood samples were obtained by venipuncture under ketamine anesthesia (Ketostop, 10 mg/kg) (DragPharma Invetec, Chile) and collected in heparinized tubes. Blood was centrifuged and plasma stored at -20°C until assay. Aliquots of these samples were used to prepare representatives pools of each physiological condition. These pools were used to determine parallelism in the RIA. Additionally, plasma PRL concentration was measured in two nursing females treated with a single dose (1 or 10 mg) of intravenous bolus of thyrotropin releasing hormone (TRH) (Farmanuclear S.A.). Blood samples were collected at -15, 0, 15, 30, 45 and 60 min after TRH administration. All procedures were made in accordance with the Guiding Principles in the Care and Use of Laboratory Animals, endorsed by the American Physiological Society.

hPRL iodination

Iodination was performed using the method of Greenwood *et al* (1963), following the procedure recommended by NIDDK-NIH. In brief, 3.3 µg of hPRL dissolved in 15 µl of 10 mM $NaHCO_3$ were incubated with 700 µCi of Na[^{125}I], 15 µl of buffer phosphate 0.5 M, pH 7.5, and 12 mg of chloramine-T dissolved in 20 µl of PBS at 4°C for 45 s in a final volume of 60 µl. The reaction was stopped by adding 30 mg of L-Cysteine in 75 µl of PBS. The mixture was allowed to stand at 4°C for 15 s and then diluted with 200 µl of PBS. Iodinated hormone was separated in a 0.9 x 40 cm column filled with Sephadex G-100, previously equilibrated with PBS-BSA 0.5% and eluted with the same buffer, collecting fractions of 1 ml. Two main peaks of radioactivity were obtained, the first one corresponded to iodinated protein and the other to free ^{125}I. The radioactive fractions belonging to the protein peak were assayed for binding to the anti-m5PRL. From each fraction, approximately 15,000 cpm were diluted to 100 µl with 1% normal rabbit serum in PBS containing 10 mM EDTA, 0.5 % BSA and 15 mM NaN_3 (assay buffer) and incubated with 500 µl of anti-m5PRL (1:80,000) for 18 h at 4°C, in a final volume of 900 µl. The complex hormone-antibody was precipitated with the 2nd antibody diluted 1:400 in assay buffer containing 4% Polyethylene Glycol (PEG, SIGMA), and separated by centrifugation. Additional aliquots of each fraction were incubated in absence of antibody to determine non specific binding, *i.e.*, radioactivity attached to the tube walls, or carried down by the pellet. Specific binding was calculated by subtracting the non specific binding from the radioactivity precipitated in each fraction with the antibody and expressed as per cent of total radioactivity added. The fractions with the highest specific binding and the lowest non specific binding were utilized in the assay.

hPRL radioimmunoassay

Five hundred µl of anti-m5PRL (dilutions 1:80,000; 1:160,000; 1:200,000; 1:270,000) were pre-incubated with 100 µl (1.95-250 µIU/tube) of WHO hPRL (IRP-75/504) in assay buffer, 800 µl final volume, for 18 h at 4°C, in 12 x 75 polypropylene tubes. Then, 100 µl ^{125}I-hPRL (15000 cpm in assay buffer-1% normal rabbit serum) were added and incubated for 18 h at 4°C. An aliquot of ^{125}I-hPRL was incubated in absence of antibody (non specific binding). The complex hormone-antibody was precipitated by incubating with 100 µl of 2nd antibody (1:400) in PEG 4% final concentration, for 2 h at 4°C and separated by centrifugation at 4°C for 45 min at 1500 x g. When volume of the assay was reduced, 250 µl of anti-m5PRL were pre-incubated with 100 µl of PRL standard in a final volume of 400 µl, and precipitation was done with 500 µl of 2nd antibody in PEG, at the same final concentration than described above. The supernatants were decanted and the radioactivity in precipitates was counted and expressed as counts per minutes (cpm). The non specific binding was subtracted from the values of the other tubes of

the assay. The cpm of the pellet in the absence of unlabeled PRL represents total binding (Bo). The specific binding (B) of standard tubes was expressed as percentage of the total binding (B/Bo x 100) and plotted against log of standard concentration.

Recognition of cebus PRL by anti-m5PRL

To determine if anti-m5PRL cross reacts with cebus PRL, several volumes of the *Cebus apella* pituitary extract (0.025-5 µl) were assayed with 250 µl of anti-m5PRL diluted 1:160,000 following the procedure described in the preceding paragraph.

Calibration of the ***Cebus apella*** *pituitary extract*

To assess the amount of PRL present in the *Cebus apella* pituitary extract, dilutions of the pituitary extract were assayed in 9 RIAs using hPRL as standard. The dilutions in which the concentration of PRL fell within the first 3 points of the hPRL standard curve showed parallelism, *i.e.*, once corrected by the dilution factor, the calculated cebus PRL concentration was similar. This value was used to obtain an equivalence of the extract in terms of WHO hPRL, IRP-75/504.

Chromatofocusing

We mixed 0.3 ml of the pituitary extract (27 mIU of cebus PRL approximately) with 2.9 ml of a pool of human plasma containing low amounts of PRL (less than 1.95 µIU) used as a carrier. The mixture was applied to a 1.5 x 15 cm column filled with Polybuffer Exchanger gel (PBE 94; Pharmacia Biotech; N° 17.0712 Uppsala, Sweden) equilibrated with 15 bed volumes of 25 mM ethanol amine, pH 9.9. The elution started with Polybuffer 96 (PB 96; Pharmacia Biotech; N° 17.0714) diluted 1:8 at a flow rate of 12 ml/h, collecting 3 ml fractions. We measured the pH of each fraction with a digital pH meter (sensitivity 0.01 pH units). When the pH in the fractions reached 6.0 we changed the eluant to Polybuffer 74 (PB 74; Pharmacia Biotech; N° 17.0713) diluted 1:10. When the pH in the fractions reached 4.0 the eluant was replaced by 1 M NaCl, collecting 20 additional fractions. We added 0.5 ml BSA 1% as a carrier to each fraction and precipitated the proteins with 90 % ammonium sulfate, then centrifuged at 4000 x g for 3 h. Pellets were separated and reconstituted in 1 ml of PBS. PRL-immunoreactivity (IR-PRL) of the reconstituted precipitate was assayed using the RIA for cebus PRL described above. To asses whether the fractions with immunoreactivity had PRL bioactivity, they were dialyzed in Spectra/Por membrane (MWCO, 6-8000; Spectrum Medical Industries) against 10 mM PBS and measured by the Nb2 rat lymphoma assay. The biological activity (BIO-PRL) of fractions was compared with the immunoreactivity measured after dialysis. Both BIO- and IR-PRL were expressed as mIU/fraction.

The chromatofocusing column was previously calibrated with ^{125}I-hPRL diluted in 10 ml of amniotic fluid, following the procedure described above. We measured the pH and the amount of radioactivity present in each fraction and determined the isoelectric point for ^{125}I-hPRL.

Nb2 rat lymphoma cell assay

This assay is based on the ability of the Nb2 cells to proliferate in response to low concentrations of PRL or GH. The assay was performed following the procedure described by Campino *et al* (1994). In brief, Nb2 cells (200,000 cells/ml) were incubated at 37°C for 48 h in Fisher's assay medium (Gibco 430-1100 E) (Gibco, Grand Island, NY) supplemented with 10 % horse serum, 10^{-4} M 2-mercaptoethanol, 50 IU/ml penicillin and 0.05 mg/ml streptomycin, with hPRL (WHO, IRP 75/504) as standard (2.03-16.25 µIU/well) or with samples in a final volume of 1.1 ml, under an atmosphere of 5% CO_2-95% air. All points of the standard curve and the samples were assayed in duplicate. In addition, each sample was tested in two different aliquots. After 48 h incubation, Nb2 cells were treated with Trypan blue and the number of living Nb2 cells was determined in a hemocytometer by counting the cells that excluded the dye. All cells counts were done at least twice by the same person.

RIA for plasma cebus PRL using cebus pituitary extract as standard

We tested two variables: the effect of pre incubation times of 18 h and of 72 h and of different dilutions of anti-m5PRL (1:120,000, 1:160,000, 1:200,000, 1:270,000) with cebus PRL on Bo and sensitivity. The other conditions of the assay (amount of ^{125}I-hPRL, precipitation with 2nd antibody) were the same described above for the hPRL RIA.

Commercial ^{125}I-hPRL obtained from DPC was tested under the same protocol conditions.

Data analysis

PRL concentrations (± SEM) in *Cebus apella* from different groups were compared by Student's *t*-test using Bonferroni modification.

RESULTS

Human-PRL iodination

The radio iodination of hPRL produced a large peak of iodinated proteins. In different iodinations we found that the fractions with the best binding (31-45 %) to anti-m5PRL and the lowest non-specific binding (3-9%) corresponded to the fractions with elution ratio near to 0.61, with respect to the elution of the free ^{125}I peak. The mean ± SE specific radioactivity was 58.1 ± 8.0 µCi/ng of PRL.

hPRL radioimmunoassay

Dose response curves showed better slope using 500 µl than 900 µl as final volume; therefore an incubation volume of 500 µl was used in all subsequent experiments. Figure 1 shows the effect of assaying different anti-m5PRL dilutions. As expected, the percentage of total binding decreased as function of the dilution of antibody. Dilutions between 1:160,000 and 1:270,000 gave curves with better sensitivity, determined as the lowest concentration of hPRL that significantly decreases B from Bo. The dilution of 1:160,000 was chosen to be used thereafter considering a combination of a good sensitivity with high Bo.

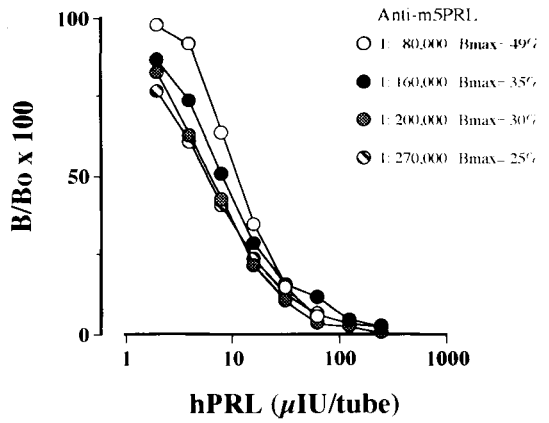

Fig 1. Effect of different anti-m5PRL dilutions on the displacement of ^{125}I-hPRL by 1.95 to 250 µIU of hPRL. Bmax: percentage of maximal binding with respect to total counts of ^{125}I-hPRL added. Bo: binding in absence of hPRL. B: binding in presence of different PRL concentrations. Conditions of assay described in Methods.

*Recognition of **Cebus apella** PRL by anti-m5PRL*

Using the hPRL RIA described above, the pituitary extract from *Cebus apella* displaced ^{125}I-hPRL from the anti-m5PRL antibody (Fig 2). However, it did not show parallelism with the hPRL standard. Moreover, a high amount of labeled ^{125}I-hPRL (approximately 30% of the Bo) was not displaced, in spite of using large amounts of extract. Only small volumes of extract approached parallelism to the hPRL standard at concentrations under 15.6 µIU/tube. Similar results were obtained when testing different aliquots of a plasma pool from nursing females (data not shown).

*Calibration of the **Cebus apella** pituitary extract to be used as reference preparation*

Using the dilutions of the extract that displayed parallelism to the human standard (Fig 2) we determined the concentration of PRL in the pituitary extract as hPRL(WHO IRP-75/504) equivalents. The value obtained was 90±8 µIU of cebus PRL per µl of extract.

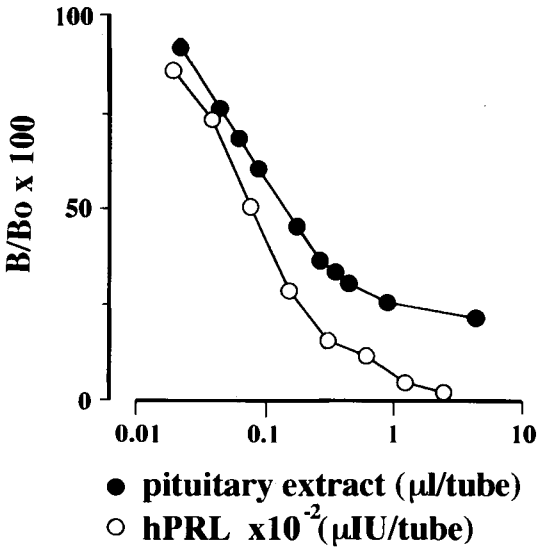

Fig 2. Displacement of ^{125}I-hPRL by dilutions of pituitary extract or 1.95 to 250 µIU of hPRL. Bo: binding in absence of hPRL. B: binding in presence of different PRL concentrations or pituitary extract. Conditions of assay described in Methods.

Chromatofocusing

To assess whether the cross reaction of the *Cebus apella* pituitary extract corresponds to PRL, we separated the proteins according to their isoelectrical points (pI) by chromatofocusing and measured the immunoactivity in the fractions. About 90% of immunoactivity eluted in the pH range 6.3-5.8, similar to ^{125}I-hPRL which eluted at pI 6.01-5.76. No immunoactivity was found at pH higher than 6.3 or lower than 5.2. The fractions that showed immunoactivity were assayed in the Nb2 assay. The large peak showed a good correlation between immuno- and bioactivity (Fig 3). The small acidic peaks did not show bioactivity in the Nb2 assay. These results suggest that the antibody recognizes cebus PRL and small amounts of some other non biologically active molecules.

RIA for cebus PRL using ***Cebus apella*** *pituitary extract as reference preparation*

To improve the sensitivity of the assay, so that small amounts of sample could be assayed (<10 µl of plasma), we tested the effect of increasing the pre incubation time of the standard preparation with anti-m5PRL. Anti-m5PRL (1:160,000) was pre-incubated with different amounts of pituitary extract (1.13-44.5 µIU) by 18 h or 72 h. Increasing the time of pre incubation to 72 h improved the sensitivity of the assay (data not shown). Using this condition we tested four dilutions of anti-m5PRL (1:120,000, 1:160,000, 1:200,000, 1: 270,000). As illustrated in Figure 4, the 1:120,000 dilution of anti-m5PRL gave the best Bo without

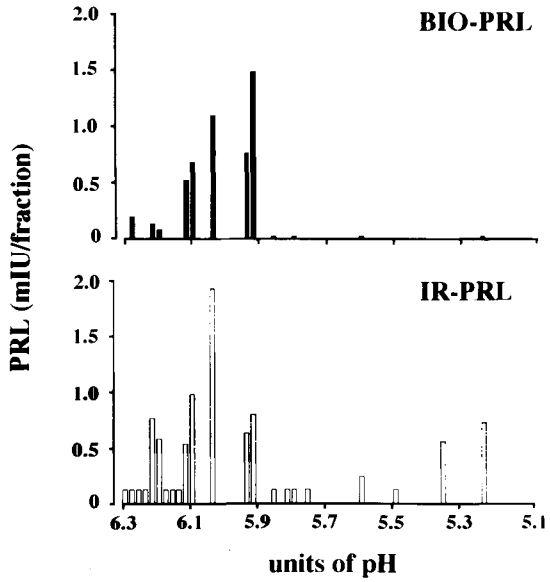

Fig 3. Distribution by isoelectric point of BIO-PRL (by Nb2 assay) and IR-PRL (by RIA) present in 300 µl of *Cebus apella* pituitary extract. See Methods.

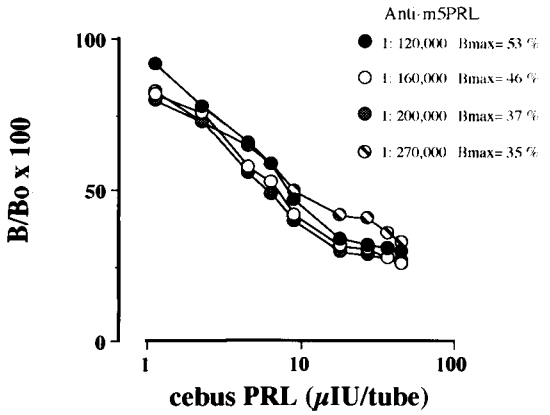

Fig 4. *Cebus apella* PRL assay. Effect of different anti-m5PRL dilutions on displacement of 125I-hPRL by 1.13 to 44.5 µIU of cebus PRL (pituitary extract). Bmax: percentage of maximal binding with respect to total counts of ^{125}I-hPRL added. Bo: binding in absence of cebus PRL. B: binding in presence of different cebus PRL concentrations. Conditions of assay described in Methods.

decreasing the sensitivity, that reached to 1.13 µIU of PRL.

Finally, the following assay conditions were selected: 250 µl of anti-m5PRL (1:120,000) were incubated with increasing amounts of *Cebus apella* pituitary extract (1.13 to 44.5 µIU), or 10 µl of *Cebus apella* plasma samples in a final volume of 400 µl with assay buffer for 72 h at 4°C. After the pre-incubation, 15,000 cpm of ^{125}I-hPRL diluted in 100 µl of assay buffer containing 1% normal rabbit serum were added and the tubes were incubated for additional 18 h at 4°C. The antigen-antibody complex was precipitated with 500 µl of goat anti-rabbit antibody at final dilution of 1:400 in 4% PEG in assay buffer. As internal control of the assay we used 10 µl of the plasma pool of nursing *Cebus apella* females. The inter- and intra-assay coefficients of variation (determined in 15 assays) were 14.3% and 6.5%, respectively. A standard curve with the same sensitivity was obtained when ^{125}I-hPRL from DPC was used, with the assay protocol described, except that the tracer was diminished to 9,000 cpm/tube.

Parallelism between ***Cebus apella*** *pituitary extract and plasma*

There was parallelism between *Cebus apella* pituitary extract and dilutions of pools of *Cebus apella* plasma from different physiological conditions (Fig 5). The parallelism between the extract and plasma, plus the evidence that the isoelectric point of the material recognized by the antibody falls within the expected for the PRL molecules and that it is bioactive in a PRL assay indicates that the *Cebus apella* pituitary extract can be used as a reference preparation to measure PRL in *Cebus apella* plasma samples.

Measurement of PRL in ***Cebus apella*** *plasma under different physiological conditions*

As shown in Table I, cycling females have lower PRL concentrations than non-nursing postpartum females. Nursing females have significantly higher concentrations than non-nursing females. Newborns have similar concentrations to those of nursing females. Figure 6 shows the response of two nursing females to 2 doses of TRH, a known secretagogue of PRL. In both cases, an elevation of PRL was observed 15 min after the injection of a bolus of TRH.

DISCUSSION

Results presented in this paper validate a reproducible, specific and sensitive heterologous radioimmunoassay to measure PRL in small volumes of *Cebus apella* plasma. Setting up homologous assays for protein hormones of species not currently used in experimental protocols is challenging since

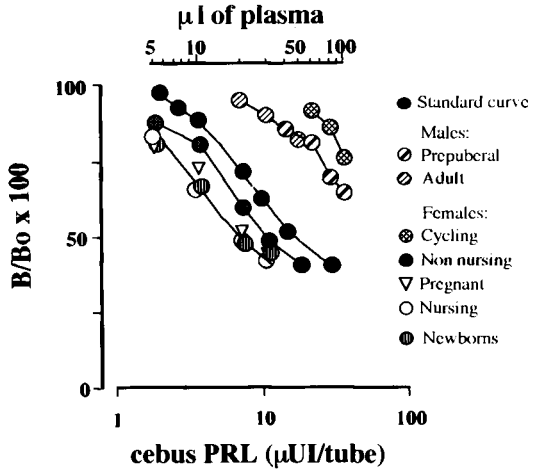

Fig 5. Parallelism of dilutions of plasma pools obtained from *Cebus apella* under different physiological conditions to PRL (pituitary extract) in the established *Cebus apella* PRL assay.

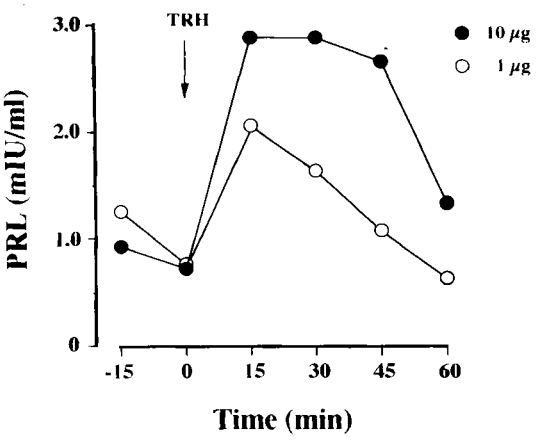

Fig 6. Plasma PRL response of 2 nursing *Cebus apella* females to the injection of 1 or 10 µg of TRH.

Table I.

Plasma PRL concentration (± SEM) in *Cebus apella* under different physiological conditions.

Group	n	PRL (μIU/ml)
Newborns	10	719.0 ± 49.2 *
Adult females		
Cycling	15	161.6 ± 15.0
Pregnant	4	677.8 ± 11.8 *
Nursing[a]	10	625.0 ± 47.0 *
Non-nursing[a]	6	369.6 ± 19.0 **
Males		
Prepuberal	4	101.2 ± 3.9
Adult	4	100.3 ± 7.6

[a] 15 days postpartum.
* $P < 0.05$ *vs* prepuberal and adult males, cycling and non-nursing females.
** $P < 0.05$ *vs* cycling females and prepuberal and adult males.
Comparisons by Student's *t* test, after Bonferroni modification.

purified hormones are not available to be used as standards, to prepare tracers or to produce antibodies. To purify cebus PRL would require access to a relatively large number of pituitaries. Alternatively, a few fresh pituitaries could be used to set up pituitary cell cultures to harvest a PRL enriched medium. Since neither of these possibilities were available to us, we undertook setting up an heterologous assay for cebus PRL.

The highly conserved sequence of PRL among closely related species, determines that some antibodies will cross-react against PRL of other species. We tested a polyclonal antibody (anti-m5PRL) developed against purified rhesus monkey PRL that shows strong cross-reacts with hPRL. Purified hPRL is readily available; therefore we established a RIA using this hormone as standard and iodinated tracer and the anti-m5PRL antibody. Using this assay we determined that a *Cebus apella* pituitary extract and a pool of plasma from nursing *Cebus apella* females compete with hPRL for the antibody. The chromatofocusing experiments provide convincing evidence that the competition found was due to recognition of cebus PRL by anti-m5PRL antibody. Immunoactivity eluted in the pH range expected for a PRL molecule. In addition, more than 90% of the total immunoactivity showed bioactivity in the Nb2 assay. The small amount of immunoactivity not accompanied by bioactivity may still correspond to PRL since molecules of PRL that are immunoactive but poorly bioactive have been detected in nursing and cycling women (Ampuero, 1994). Presently, we are unable to determine whether this is the case, or it represents degraded PRL molecules still recognized by the antibody. The crude pituitary extract ought to contain cebus GH, the most abundant hormone in mammalian pituitary. There is striking molecular homology between GH and PRL in most species (Neill & Nagy, 1994) and the Nb2 assay recognizes GH and PRL (Campino *et al*, 1992). In the whole extract and in one of the fractions obtained after chromatofocusing the concentration of PRL, was higher by bioassay than by RIA. The interpretation of these findings is that the bioassay was measuring GH in addition to PRL while the immunoassay was measuring only PRL. The lower bioactivity than immunoactivity in the remaining chromatofocusing fractions suggests that GH was not present in these fractions. The lack of cross-reaction of anti-m5PRL with cebus GH was not surprising, since this antibody in spite of showing strong cross-reaction with hPRL (the present paper and Bethea & Papkoff, 1986) does not cross react with hGH (Bethea & Papkoff, 1986).

The observation of lack of parallelism between dilutions of the *Cebus apella* pituitary extract and plasma with the hPRL standard indicates that the hPRL standard cannot be used to measure cebus PRL. The affinity of the antibody for cebus PRL was lower than that for hPRL. This conclusion is derived from the observation that the ability of the *Cebus apella* pituitary extract to displace ^{125}I-hPRL improved after increasing the preincubation time. A possible interpretation of this observation is that cebus PRL differs from hPRL in aminoacidic composition, in charge or in both. Slight differences in aminoacidic composition with human PRL have been described in the PRL molecules of two other primates (baboon and *cynomolgus* PRL) (Cole *et al*, 1991), so it is not unlikely that they are present in the cebus and human

PRL. A difference in charge between hPRL and cebus PRL is suggested by the slight differences in elution patterns after chromatofocusing the *Cebus apella* pituitary extract and ^{125}I-hPRL. Post translation modifications of PRL involving addition of carbohydrates have been described for human, baboon and cynomolgus (Cole *et al*, 1991). The observations of Bethea and Papkoff (1986) suggest differences between hPRL and RhPRL, since RhPRL could not completely displace the ^{125}I-hPRL tracer from anti-m5PRL.

The parallelism between the *Cebus apella* pituitary extract and the dilutions of *Cebus apella* plasma obtained from animals in a wide range of physiological conditions indicates that the antibody recognizes the same molecules in extract and plasma in all cases. The previous demonstration that most of the immunoactivity in the pituitary extract has biological activity of PRL supports the proposal that the molecule recognized is PRL. Since the hormonal patterns in these physiological conditions should be different, the parallelism observed implies that the antibody has no or minor cross-reaction with other pituitary hormones. These findings allow to use the pituitary extract as standard, overcoming the lack of purified cebus PRL. The practical problem of assigning a value in terms of cebus PRL to the *Cebus apella* pituitary extract was solved by determining its concentration relative to the hPRL standard from WHO. We followed this strategy to obtain values that could be compared to known physiological situations in humans and other primates. Varying the conditions of the assay, we improved the sensitivity in order to measure 5-50 µl of *Cebus apella* plasma. This is important considering that the *Cebus apella* is a small primate and therefore is not advisable to take large volumes of blood. The assay shows a good reproducibility, as shown by the low inter- and intra-assay coefficients of variation.

The assay allowed to distinguish differences in PRL concentration in plasma obtained from *Cebus apella* under several physiological conditions. PRL concentration in adult *Cebus apella* females that have regular menstrual cycles was similar to that found in adult and prepuberal males. PRL concentration was higher in pregnant and in nursing *Cebus apella* females than in post-partum non-nursing and cycling females. PRL concentration in *Cebus apella* newborns was similar to that found in pregnant and nursing females and higher than in the other females and males. These differences in PRL concentration in different physiological conditions are similar to those observed in humans and other primates (Gluckman, 1983; Yen, 1978). A PRL response to TRH was demonstrated in 2 nursing *Cebus apella* females, similar to the response found in nursing woman (Kletzky *et al*, 1980) and rhesus (Norman *et al*, 1980).

In summary, the data presented validate a RIA procedure that allows quantitative detection of PRL in small volumes of *Cebus apella* plasma. The method should provide a valuable new tool to further the study of the physiology of PRL in non human primates.

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