A HETEROLOGOUS RADIOIMMUNOASSAY FOR AVIAN PROLACTIN: APPLICATION TO THE MEASUREMENT OF PROLACTIN IN THE TURKEY

By
A. S. McNeilly1,2, R. J. Etches2 and H. G. Friesen1

ABSTRACT

A specific heterologous double-antibody radioimmunoassay has been developed to measure turkey prolactin (PRL) using a guinea pig anti-hPRL antiserum and 125I-labelled ovine PRL [125I]oPRL. Turkey pituitary prolactin and serum give parallel dose-response curves and no cross-reaction is seen with turkey growth hormone, LH or FSH, or mammalian LH, FSH, TSH, GH or placental lactogens. The RIA is accurate and precise and is sufficiently sensitive to measure PRL in all physiological situations investigated in the turkey. The RIA will measure PRL in several avian species including the chicken, duck, goose, pheasant, pheasant × chicken F1 hybrid, pigeon, quail and rook.

Plasma PRL concentrations in laying and broody turkey hens were not significantly different (46.5 ± 2.5 vs. 39.7 ± 3.8 ng/ml) but both were significantly higher (P < 0.001) than in non-laying turkey hens (4.6 ± 0.7 ng/ml).

Oestradiol injection into laying hens did not alter PRL levels while the same injection in non-laying hens caused a significant three-fold increase in plasma PRL levels.

Heterologous radioimmunoassays have been described in which antibodies raised against mammalian hormones cross-react with avian pituitary hor-
mones. Such radioimmunoassay systems have been described for avian GH (Hayashida 1970; Farmer et al. 1974) and avian FSH (Croix et al. 1974; Follett 1976).

Only one homologous radioimmunoassay for chicken prolactin has been described (Scanes et al. 1976) and this shows only limited cross-reaction with avian species other than the chicken. It was the purpose of this investigation to develop a heterologous radioimmunoassay for prolactin suitable for use in several avian species, in particular the turkey.

MATERIALS AND METHODS

Materials

Bovine serum albumin (Fraction V) was obtained from Miles Laboratory; lactoperoxidase from Calbiochem, La Jolla, California; Na[125I] (carrier free) from New England Nuclear, Boston, Mass., and H2O2 (30%, v. v.) from Fisher Scientific, Fair Lawn, N. J. All other reagents and chemicals were of reagent grade.

Purified hormones from several mammalian species were used to assess the initial specificity of the assay. These were hPRL (Friesen 73-7-28); oPRL (NIH P-S10); bPRL (NIH-P-B3); pPRL (NIH SP 162C); rPRL (NIAMDD P-I-1); dogPRL (Scherimg); hGH (NIH HS1146); mGH (NIH M945 A); pGH (NIH RIA 07313); bGH (NIH RIA 1003A); pGH (NIH P 526 B); rGH (NIAMDDGH-I-2); mouseGH (NIAMDD AFP 689-B); dogGH (Wilhelmi D100 A); hLH (NIH LER 960); oLH (NIH LH-S18); rLH (NIAMDD-LH-I-1); hFSH (NIH LER-1575 C); bFSH (CH-bF-1); rFSH (NIAMDD FSH-I-2). TurkeyGH (W 27CD) was kindly supplied by Drs. Farmer and Papkoff; turkeyFSH, turkeyLH and two turkeyPRL preparations (M SER-I and HS-I, II) were kindly supplied by Dr. K. W. Cheng, Winnipeg. ChickenLH (IRC-2) was supplied by Dr. F. J. Cunningham, Reading.

During the development of this RIA no turkeyPRL standard was available. A laboratory standard turkeyPRL (T2-76) was therefore prepared. Seventeen turkey pituitaries (343 mg) were extracted in 4 x 0.5 ml 0.2 M ammonium bicarbonate pH 7.8 for 3 min at 4°C and the extract was centrifuged at 10,000 g for 45 min at 4°C. The supernatant obtained was diluted 1:10 in 0.025 M TRIS-HCl buffer 7.4 containing 0.1% BSA, aliquoted and stored at −70°C until assayed. These conditions were optimal for the extraction of bio- and immunoassayable PRL (McNeilly & Etches, unpublished observation). The prolactin content was measured in the prolactin radio-receptor assay (RRA) using the mammary gland receptor as described in detail previously (Shiu et al. 1978). Ovine prolactin (oPRL, NIH P-S10, 26 IU/mg) was used as standard and the amount of prolactin in the pituitary extract (T2-76) was assessed and used as standard in all the RIA studies.

Antiserum and iodination of prolactin

During the development of heterologous radioimmunoassay systems for the measurement of prolactin in several mammalian species (McNeilly & Friesen 1978), it became apparent that one system was suitable for the measurement of prolactin in several avian species.
The antiserum used (AGP-4) was raised in a guinea-pig against human prolactin and the preparation has been described elsewhere (McNeilly & Friesen 1978).

Ovine prolactin (NIH P-S10, 26 IU/mg), generously provided by the NIH, Bethesda, Maryland, was used as labelled hormone. Iodination of oPRL was carried out by the lactoperoxidase method (Thorell & Johansson 1971) at room temperature exactly as described previously (McNeilly & Friesen 1978). $^{125}$I-labelled hormone were purified by gel filtration on Sephadex G-100 (2 x 50 cm) eluted with 0.025 M TRIS-HCl buffer pH 7.4 containing 0.1 % bovine serum albumin (BSA). The $^{125}$I)oPRL was stored at $-20\text{°C}$ until used for assay.

**Assay procedure**

Maximum binding of $^{125}$I)oPRL of around 94 % could be achieved with a final antibody dilution of 1:1000 and a working dilution (final dilution 1:30 000) estimated by antibody dilution curves, was chosen which would bind 30-40 % of the added tracer.

Assays were carried out using 0.01 M sodium phosphate buffer pH 7.4 containing 0.15 M NaCl, 2.0 % BSA and 0.1 % sodium azide (radioimmunoassay diluent, RIAD). The exact radioimmunoassay method has been described in detail elsewhere (McNeilly & Andrews 1974; McNeilly & Hagen 1974; McNeilly & Friesen 1978).

**Specificity, precision and accuracy of the assay**

The hormones used to assess the specificity of the RIA are listed in the Materials section above. Each hormone preparation was added at a starting concentration of at least 1 \(\mu\)g/ml. Cross-reactivity was calculated as the amount of hormone (w/w) giving 50 % ($B/Bo$) inhibition in the radioimmunoassay.

Extracts of several avian species were prepared as described above for the preparation of the turkey pituitary prolactin standard and stored in aliquots at $-20\text{°C}$ until assayed. The prolactin content in these pituitary extracts was assessed using both the RIA and the prolactin RRA (Shiu et al. 1973). Serum samples from these same species were also obtained and together with the pituitary extracts were double diluted and assayed to assess cross-reactivity and parallelism. The avian species assessed in this way were: domestic duck (Anas platyrhynchos), domestic pigeon (Columbia livia), domestic goose (Anser anser), ring-necked pheasant (Phasianus colchicus), domestic chicken (Gallus domesticus), pheasant x chicken F1 hybrid, Japanese quail (Coturnix coturnix), rook (Corvus frugilegus).

The parallelism between dose-response curves for turkey prolactin standard diluted in RIAD, serum from hypophysectomised chicken, quail and rat or human serum containing < 1 ng hPRL/ml by homologous RIA was assessed to determine whether any plasma or serum interference occurred in the RIA. At the same time sera from laying, non-laying and broody female turkeys were diluted and assayed against the turkey PRL standard diluted in RIAD.

The accuracy of the RIA was determined by the measurement of known amounts of turkeyPRL standard added to turkey serum containing low levels of PRL and to plasma from a hypophysectomized chicken. The precision of the assay expressed as the intra- and inter-assay co-efficients of variation (%) were assessed by the repeated assay of three pools of turkey sera containing PRL levels of 1, 15 and 36 ng/ml both within and between assays.
**Sephadex chromatography of turkey prolactin standard**

To assess the form of the immunologically active prolactin measured by the radio-immunoassay the turkey pituitary prolactin standard (T2-76) and turkey sera containing high or low levels of PRL were fractionated at 4°C by gel filtration on Sephadex G-100 (2 × 50 cm) eluted with 0.025 M TRIS HCl pH 7.6 containing 0.1% BSA. One ml fractions were collected and analysed for PRL by RIA and RRA (Shiu et al. 1973) and for GH by RRA (Tsushima & Friesen 1973). Protein concentrations were estimated by measuring absorbance at 280 nm.

**Plasma prolactin in mature turkey hens and the effects of oestrogen**

To determine the relationship between reproductive condition and plasma prolactin, blood samples were removed from 31 laying hens, 27 broody hens and 24 non-laying hens. These were Nicholas Large White hens which were provided by and housed at Cuddy Farms Ltd., Strathroy, Ontario.

To determine the effects of oestradiol on plasma prolactin, four laying and five non-laying hens were injected subcutaneously with 1.0 mg of oestradiol in ethanol: saline (1:1, v/v). Blood samples (5 ml) were removed 30 min before injection, immediately before injection and at 15, 30, 60 and 120 min after injection, centrifuged to remove plasma which was stored at −20°C until assay. Statistical analysis was by analysis of variance or Student’s t-test.

---

**Table 1.**

Cross-reaction of guinea-pig anti-human prolactin antiserum (AGP4-1) with [125I]oPRL with prolactin preparations from different species and available avian hormones.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sources</th>
<th>Cross-reaction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>turkeyPRL</td>
<td>a) McNeilly-Etches (T2-76)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>b) Cheng HS-1 G-100 II</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>c) Cheng M-SER G-100 I</td>
<td>17</td>
</tr>
<tr>
<td>dPRL</td>
<td>Schering</td>
<td>100</td>
</tr>
<tr>
<td>rPRL</td>
<td>NIAMDD P-I-1</td>
<td>27</td>
</tr>
<tr>
<td>hPRL</td>
<td>Friesen 75–7–28</td>
<td>126</td>
</tr>
<tr>
<td>pPRL</td>
<td>NIH SP162C</td>
<td>55</td>
</tr>
<tr>
<td>oPRL</td>
<td>NIH P-S-10</td>
<td>94</td>
</tr>
<tr>
<td>bPRL</td>
<td>NIH P-B-3</td>
<td>82</td>
</tr>
<tr>
<td>turkeyGH</td>
<td>W27CD Farmer &amp; Papkoff</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>turkeyLH</td>
<td>Cheng TLH</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>turkeyFSH</td>
<td>Cheng TFSH</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>chickenLH</td>
<td>LH IRC 2</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>
RESULTS

Heterologous assay for turkey PRL

The heterologous RIA developed was specific for prolactins and no cross-reaction (<0.1%) was seen with GH, LH, FSH, TSH or placental lactogens from those species, both mammalian and avian which were tested. The cross-reactions of the prolactins from different species is shown in Table 1 and for the turkey preparations in Fig. 1. All prolactin preparations tested showed parallel inhibition curves, while the most potent preparation was hPRL.

Standard curves of turkey PRL reference standard in RIAD, or hypophysectomized human or chicken serum were identical and these same sera failed to cause any inhibition in the RIA (Fig. 2). Dilutions of turkey plasma and serum samples were parallel to the turkey PRL (Fig. 2). The accuracy of the assay assessed by the recovery of turkey PRL standard (50 ng/ml to 1 ng/ml).

![Inhibition curves for turkey pituitary hormone preparations in the heterologous RIA for avian PRL. PRL reference standard T2-76 (○—○); PRL preparations HS-1 G100 II (Cheng; ●—●); PRL preparation M-SER G100 I (Cheng; ▲—▲) and turkeyGH (W27CD, Farmer & Papkoff), turkeyLH (Cheng TLH) and turkeyFSH (Cheng TFSH) (■—■).](#)
Inhibition curves for turkey PRL reference standard (Tg-76) in diluent (○) or plasma from a hypophysectomized chicken (▲) and dilutions of plasma from a laying (●) and non-laying (□) turkey hen and a hypophysectomized chicken (△) in the heterologous avian PRL RIA.

Correlation between RIA and RRA estimates of pituitary prolactin content in several avian species (duck, ○; pigeon, ●; goose, ⊗; pheasant, △; pheasant × chicken, □; quail, ■; turkey, ▲; chicken, ⊙).
added to turkey serum containing 1.7 ng/ml PRL was 98 ± 4 (SEM) % (n = 38). Intra- and inter-assay variation (expressed as the coefficient of variation) of results on replicate samples was 8.9 % (n = 38) and 13.7 % (n = 24), respectively.

Pituitary extracts from all eight avian species studied were parallel to the turkey PRL standard in the RIA. Comparison of the PRL levels obtained for these pituitary extracts by RIA and by the prolactin RRA showed a highly significant (P = < 0.001) linear correlation (Fig. 3). In all these species the assay was of sufficient sensitivity to measure PRL in the sera, and serum dilutions from each avian species were parallel to the standards.

Sephadex chromatography of turkey PRL

A single peak of radioimmuno- and radioreceptor assayable PRL was found after gel filtration on Sephadex G-100 of the turkey pituitary prolactin standard (Fig. 4). While no attempt was made to establish an accurate molecular weight for this activity the elution volume would suggest a molecular weight approximately 32 000.

The concentrations of prolactin in Nicholas Large White turkey hens varied according to the reproductive state of the bird. The plasma concentrations of prolactin in laying, broody and non-laying hens were 46.5 ± 2.5 ng/ml (mean

![Fig. 4](image_url)

Gel filtration on Sephadex G-100 (50 × 2.5 cm) of a turkeyPRL preparation. One ml fractions were collected and assayed for PRL by RIA (○) and RRA (△).
Plasma concentrations (ng/ml) of prolactin following injections of 1 mg of oestradiol into four laying hens (O—O) and five non-laying hens (●—●). Results are mean ± sd.

\[ 39.7 \pm 3.8 \text{ ng/ml and } 4.6 \pm 0.7 \text{ ng/ml, respectively.} \]

The difference between broody and laying hens was not significantly different \((P > 0.05)\) but both groups were significantly higher \((P < 0.001)\) than in the non-laying hens.

The injection of oestradiol (Fig. 5) into laying hens did not alter the concentration of prolactin \((t = 0.48, P > 0.05)\), whereas the same injection given to non-laying hens was followed by a three-fold increase \((t = 4.18, P < 0.01)\) in plasma prolactin.

**DISCUSSION**

The heterologous radioimmunoassay described in this paper is specific for prolactins in all species investigated, is precise, accurate and reproducible. No non-specific interference is seen in the RIA, and plasma or serum from hypo-
physectomised avian and mammalian donors do not cause any displacement in the assay. This is in marked contrast to the report of Nicoll (1975). The binding achieved during routine assay (30–40% B/T) is of the same order as that in homologous RIAs and the slope of the inhibition curve is sufficient to ensure accuracy and precision. This is further demonstrated by the recovery data (98 ± 4%) and the intra- and inter-assay variation (8.9 and 13.7%, respectively).

Evidence that the RIA is measuring prolactin in the avian species is gained from the gel filtration of the turkey prolactin standard. Both the immuno-assayable and bioassayable (RRA) activities coincided suggesting that both assays are detecting the same activity. The estimated molecular weight of approximately 32,000 is similar to that of purified turkeyPRL (Cheng, personal communication). The lower cross-reactivity of semipure preparations of turkeyPRL (Table 1; Fig. 1) compared to the house standard preparation of turkeyPRL (T2-76) merely reflects the fact that the house preparation is a crude pituitary homogenate which was assigned a potency per ml of extract in terms of RRA activity. The relative potencies of the two semipure turkeyPRL preparations supplied by Dr. K. W. Cheng (i.e. TPRL HS-1 G-100–II = 2 x potency of TPRL M–SER G-100 T) estimated by the heterologous PRL RIA described here (Table 1; Fig. 1) is in agreement with the relative biological potencies of these preparations as estimated by RRA (Cheng, personal communication).

The heterologous RIA may be used to estimate PRL in other avian species. This was demonstrated by the high correlation between the PRL activity in the avian pituitary extracts determined by the RIA and the RRA. That the correlation is with the PRL content of the pituitaries is emphasized by the lack of cross-reaction of GH in the RRA for PRL (Shiu et al. 1973) and the presence of a very low GH content in the pituitary extracts and total lack of correlation between the GH content and the PRL content as estimated by either RIA or RRA. Dilutions of serum in each of these avian species were parallel to the pituitary extracts dilutions and to the purified PRL preparations. These results indicate that the heterologous RIA described here is applicable to the measurement of PRL in other avian species. Before finally accepting this observation for other avian species however, it is suggested that a lack of non-specific interference of plasma or serum should be demonstrated for each avian species investigated.

Absence of non-specific serum interference in this RIA is further emphasized by the good agreement between serum levels of PRL samples from cow, sheep, rat and human as measured by this RIA and the relevant homologous RIAs (McNeilly, unpublished observations).

Injection of oestradiol into non-laying hens resulted in a highly significant increase in PRL levels which were maximal between 30 and 60 min after the
injection. By contrast, no changes were observed after the same injection into laying hens. Similar increases in prolactin following the administration of oestradiol have been observed in man (Frantz 1973), rats (Neill & Smith 1974) and cows (Schams et al. 1974). The increase in prolactin following oestradiol correlates well with plasma concentrations of prolactin in broody, laying and non-laying Nicholas Large White hens where large differences were observed between non-laying or broody hens.

ACKNOWLEDGMENTS

The authors wish to thank Cuddy Farms Ltd., Strathroy, Ontario and Hyrid Turkeys Ltd., Kitchener, Ontario for the gift of turkey hens. The technical assistance of Mrs. R. Derksen and Mrs. C. Duke was greatly appreciated. The work was supported by Agriculture Canada Grant No. 6054, the Ontario Ministry of Agriculture and Food, USPHS HD 0723-04 and MRC (Canada) Grant 1824. ASMcN was supported by a Canadian MRC Visiting Scientist Award (VS41). Our thanks for the supply of hormone preparations used in this study are due to Drs. K. W. Cheng, S. Farmer, H. Papkoff and F. J. Cunningham, Schering, and the National Institute of Health and NIAMDD, Bethesda, Maryland, USA.

REFERENCES


Received on March 15th, 1978.