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Heat-labile prolactin immunoreactivity in normal human tissues: artifacts of peptide hormone radioimmunoassay in human tissues

C. A. ADEJUWON
Department of Chemical Pathology, College of Medicine, University of Ibadan, Nigeria

Summary

We recently demonstrated that a substantial proportion of the previously reported widespread distribution of human chorionic gonadotropin-like material in nonplacental normal human tissues is due to non-specific activity of tissue proteases (Adejuwon & Segal, 1984). The study was extended to another polypetide hormone, measured by the same general method of radioimmunoassay. Normal tissue samples of colon (n = 1) and testes (n = 5) obtained at surgery were analysed by a highly specific and sensitive radioimmunoassay (RIA) for human prolactin (hPRL). All tissues investigated appeared to contain immunoreactive hPRL-like material (6.7 ± 5.3 ng/g weight, mean ± s.d.) Heat at 55°C for 15–20 min was found to have no effect on the immunoreactivity of purified hPRL. Exposure of normal tissues to these conditions was, however, found to completely eliminate the hPRL-like immunoreactivity. The factor present in these normal tissues giving positive radioimmunoassay results for hPRL is not identical to native hPRL. It may be due to tissue proteases interfering with the radioligand assay. True polypeptide hormone immunoreactivity must be distinguished from proteolytic activity by treatment with heat or protease inhibitor.

Résumé

Il a été recemment demontre que la large distribution d'une substance senblable a la gonadotropin chorionique humaine non d'origine placentaire est due a l'activite nonspecifique des proteases tissulaires (Adejuwon & Segal, 1984). Cette etude a ete elargie a une autre hormone, polypeptidique mesuree pa la meme methode generale de la radioimmunoassay. Des echantilons des tissues normaux en provenance du colon (n = 1) et des testicles (n = 5) ont ete amalyses a la recherche de prolactin humain (hPRL) par une radioimmunoassay specifique et tres Sensible. Tous les tissues amalyses senble contenir un materiel immunoreactif qui resemble au prolactin humain (6.7 ± 5.3 ng)g poid moyenne ± s.d.) La chaleur a 55°C pendant 15–20 min n'avait pas d'effelt sur l'immunoreactivite au l'hPRL purifie. La soumission des tissues normaux a ces conditions cependant avait completement elimine l'immunoreactivite. Le facteur conferant l'immunoreactivite aux tissues normaux n'est pas identique avec la hPRL naturel. Il pent etre du aux proteases tissulaires que interferent avec l'assai radioligand. L'immunoreactivite d'un vrai hormone polypeptidique doit etre distinguée de l'activite proteolutique par traitment avec la charleur ou l'inhibiteur proteique.

Introduction

The presence of the complex glycoprotein, human chorionic gonadotropin, hCG, has been reported in various normal non-placental human tissues (Yoshimoto, Wolfsen & Odell, 1977; Braunstein, Rason & Wade, 1975; Chen *et al.*, 1976; Robertson *et al.*, 1978; Braunstein *et al.*, 1978). On the contrary, however, we have recently demonstrated that the apparent hCG activity in normal non-placental human tissues is due almost entirely to an artifact caused by tissue proteases (Adejuwon & Segal, 1984). As a follow-up of these observations, we have extended the study to another polypeptide

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hormone which, although is not a glycoprotein, is measured by the same basic type of radioimmunological technique as hCG.

Materials and methods

Normal human tissue specimens obtained at surgery were frozen at −20°C until use. The complete radioimmunoassay kit for human prolactin was purchased from Serono Laboratories Incoporated, Massachusetts. TLCK was purchased from the Sigma Chemical Company (TLCK = N-d-Tosyl-Lysine Chromethyl Ketone hydrochloride).

Tissue preparation

Tissue samples were weighed and homogenized into 10% solutions with double distilled water using the polytrone tissue homogenizer model PCU −2− 110. Homogenates were centrifuged at 12,000 *g* for 30 min at 4°C. The supernatant volume was measured, frozen and lyophilysed and the resulting powder was weighed, crushed and reconstituted into a small volume (1–4 ml) with 0.1 M phosphate buffered saline (PBS), pH 7.4 and containing 0.1% sodium azide. The clear supernatant was used for radioimmunoassay before and after treatment with 2 mg TLCK or heat 55°C for varying periods of time. Acid extraction initially used gave extremely low results due to tissue damage. Hence water extracts were used.

RIA

The Serono kit utilized RIA technique based on the double antibody method utilizing rapid sheep anti-rabbit gamma globulin as a second antibody. The specificity of the antiserum used in this assay was pre-determined by the Serono Company by comparing the amount of human follicle-stimulating hormone (hFSH), human growth hormone (hGH), human chorionic gonadotrophin (hCG), thyroid-stimulating hormone (TSH), and human albumin required to reduce the binding of ^{125}I-hPRL by 50% with the amount of hPRL (unlabelled) required to do the same.

Cross-reaction data obtained by Serono are as follows: (i) hFSH: absent up to 19,500 μU/ml; (second IRP — HMG); (ii) hGH: absent up to 780 μU/ml (WHO 66/217); (iii) hTSH: absent up to 781 μU/ml (WHO 68/38); (iv) hCG: absent up to 81 μU/ml (2nd IRP — hCG); (v) Human albumin: absent up to 100 mg/ml; (vi) hPRL: 100% by definition.

In our hands, no cross-reaction was observed with up to 8000 ng hCG (CR119 of NIH) per ml and with up to 4000 ng of highly purified hLH (LER960 of NIH) per ml. The impure hLH preparation LER 907 (NIH) shows some displacement only after 2000 ng/ml.

Treatment with protease inhibitor

Two milligrams TLCK was added to each radioimmunoassay tube containing each tissue extract (100 μl homogenate in 500 μl of 0.1% BSA). Control tubes contained tissue and buffer instead of TLCK. The effect of this 2.0 mg dose of TLCK per assay tube was separately studied on the slope of the hPRL standard curve.

Heat treatments

One hundred μl aliquots of tissue extracts or purified hPRL were added to 500 μl of 0.1% bovine serum albumin (BSA) and 100 μl of 0.1 M EDTA. The mixture was heated at either 90°C for 20 min or 55°C for 15, 30, 60 and 120 min. The remaining activity was determined, in each case, by RIA. Corresponding control samples were not heated.

Results

Positive values for hPRL were obtained in all six tissues specimens analysed, including five specimens of testes and one specimen of colon (Table 1). The mean hPRL-like immunoreactivity in all tissues was 6.7 ± 5.3 ng/g wet weight ($\bar{x}$ ± s.d.) (Table 1). Treatment with 2.0 mg TLCK eliminated both the tissue hPRL-like immunoreactivity and the activity of purified hPRL. Similarly, heat at 90°C for 20 min eliminated both the immunoreactivity of purified hPRL and the tissue hPRL-like material. However, treatment with milder heat (55°C for varying periods of time) eliminated only the hPRL-like immunoreactivity of normal

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Table 1. Apparent hPRL immunoreactivity in non-pituitary human tissues

Specimen	Type	Weight of tissue (g)	hPRL level (ng/g wet tissue)
1	Testis	12.1	12.7
2	Testis	10.9	2.5
3	Testis	19.5	13.0
4	Testis	15.4	8.0
5	Testis	13.0	1.8
6	Colon	5.1	2.0

$\bar{x} \pm$ s.d. of testis samples = 6.68 ± 5.29 ng/g wet weight.

Table 2. Effect of heat (55°C for varying periods of time) on apparent hPRL immunoreactivity in human tissues

Tissue No	Tissue type	Apparent hPRL immunoreactivity ng/ml extract or solution				
		0 min	15 min	30 min	60 min	120 min
1	Testis	51.4	1.6	1.6	1.6	1.6
3	Testis	64.3	1.6	1.6	1.6	1.6
4	Testis	21.9	1.6	1.6	1.6	1.6
6	Colon	6.2	3.9	1.6	1.6	1.6
25 ng pure hPRL (Serono)		23.8	27.7	21.8	23.2	29.2

The lowest concentration of the standard used is 1.6 ng

human testis within 15 min and of colon samples within 30 min. Exposure of purified hPRL to these conditions resulted in no appreciable loss of immunoreactivity (Table 2). The tissue hPRL-like material demonstrates a dose response curve parallel to the slope of purified hPRL (Fig. 1).

Discussion

Unlike hCG, purified hPRL proved to be labile to TLCK (2 mg/assay tube) and heat at 90°C for 20 min. These procedures, therefore, cannot be used to discriminate between apparent and real prolactin immunoreactivity. However, milder heat at 55°C for 15–30 min which does not reduce the immunoreactivity of native hPRL completely destroys the apparent hPRL activity in normal human tissues. The TLCK interference with the purified hPRL standard curve is probably due to its overloading effect on the RIA system. Serine proteases (trypsin, chymotrypsin and substilisin) interfere with peptide hormone RIA by digesting the radioligands (Richert & Ryan, 1977). TLCK prevents this digestive hydrolytic process by inhibiting the proteases (Segal *et al.*, 1978). This is the mechanism of action of TLCK in the RIA system.

These various observations clearly demonstrate that the presence of apparent polypeptide hormone immunoreactivity in normal human tissues probably represents a non-specific phenomenon which is due almost entirely to tissue enzymes.

Positive identification of polypeptide hor-

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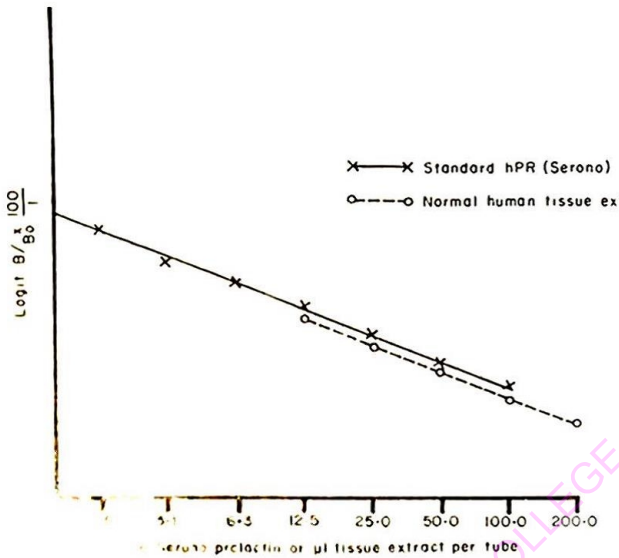

Fig. 1. Inhibition curves for human prolactin and unheated normal human tissue extracts.

reports based on radioligand assays (RIA or IRMA) with or without a demonstration of dose-response curves showing parallelism with purified parent hormone can no longer be considered valid in the light of the findings of this study and our previous study (Adejuwon & Segal, 1984). I claim that prolactin has proved undetectable in the tissues so far examined.

The relative ease with which heat destroys human prolactin (results of this study) compared to hCG (results of our previous study) (Adejuwon & Segal, 1984) can be explained on the basis of the absence of carbohydrate moiety in prolactin, unlike hCG. This carbohydrate moiety in hCG confers some degree of stability on the molecule. This theory is based on the following lines of evidence (i) Mori (1970) had shown that removal of carbohydrate moiety from hCG by either treatment with neuraminidase or acid hydrolysis causes a decrease in the *in vivo* biological activity of the hormone. (ii) The extent of this de-silation is related to the degree of decrease in biological activity. (iii) Intact hCG molecule is not affected immunologically by heat (Adejuwon & Segal, 1984) whereas prolactin with no carbohydrate content is labile to heat (present study results). Spiking of tissue samples with purified hormone prior to hormone extraction or use of acid extraction of the tissue hormone yielded extremely low and inconsistent results hence they were abandoned. The method reported in this study had been previously used for hCG (Adejuwon & Segal, 1984; Segal *et al.*, 1978).

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