THE SEPARATION OF MONKEY PROLACTIN FROM MONKEY GROWTH HORMONE BY AFFINITY CHROMATOGRAPHY

H. J. Guyda* and H. G. Friesen
McGill University, Royal Victoria Hospital
Montreal, Canada

Received January 27, 1971

SUMMARY

Sepharose-coupled antibodies to human placental lactogen (HPL) were able to remove more than 99% of monkey growth hormone (MGH) present in incubation media or tissue homogenates of monkey pituitaries. However the potency of prolactin in these fractions remained unaltered or even increased after the immunoadsorption of monkey growth hormone.

The inability to separate primate pituitary growth hormone activity from prolactin activity has led to the view that, in primates, the pituitary gland secretes only one hormone, growth hormone, which mediates both hormonal activities. In our studies in which monkey and human pituitaries were incubated in vitro with ^{3}H-leucine (1-3), we found that the principal radioactive protein released into the media was virtually identical to human growth hormone (HGH) in molecular weight and electrophoretic mobility. However, immunologically most of the radioactive proteins in the media cross-reacted with antisera to ovine prolactin (OPr) and not with antisera to human growth hormone (4). This finding led us to conclude that the radioactive protein which was immunologically related to ovine prolactin was primate prolactin. These results also suggested that it should be possible to separate growth hormone from prolactin

*Medical Research Council (Canada) Research Fellow

by affinity chromatography, an immunologic method which has proven successful for the purification of enzymes and hormones (5-9). We have coupled specific antibodies to human placental lactogen to Sepharose and have used this immunoadsorbant to remove monkey growth hormone from pituitary fractions, without significant loss of prolactin activity. This procedure for the first time provides a reliable method for separating primate prolactin and growth hormone and offers unequivocal evidence for the separate existence of the two hormones.

METHODS AND MATERIALS

Preliminary studies indicated that antibodies to human placental lactogen could effectively bind monkey growth hormone. Sheep were immunized with purified human placental lactogen (HPL) (10) and specific antibodies to HPL were isolated by immunoadsorption as outlined below.

Preparation of Immunoadsorbants: (a) HPL-Sepharose - 100 ml of Sepharose 4 B (Pharmacia) were activated with cyanogen bromide (Eastman) as outlined by Cuatrecasas et al (11) and coupled to 1000 mg of HPL in 100 ml 0.1 M NH_4HCO_3 (pH 8.5) by rotation at 4°C for 24 hrs. The immunoadsorbant was extensively washed until no further proteins were eluted. Sheep antisera to HPL, diluted 1:7 in .05 M Tris-HCl (pH 7.4) buffer, was passed through this Sepharose coupled HPL column at a rate of 30 ml/hr at 4°C until significant antibody binding activity appeared in the effluent. The column was extensively washed with buffer to remove non-specifically adsorbed serum proteins. Elution of the adsorbed antibody was performed with 4 M KSCN in phosphate buffered saline (pH 7.0) as reported by Dandliker

et al (12). The specific high affinity antibodies to HPL which were eluted were immediately diluted to 0.5 M SCN, dialyzed and concentrated (Amicon, UM-10 Membrane) until 1 ml of anti-HPL antibody concentrate was able to bind 4 to 5 mg of HGH. The binding capacity was determined using the method outlined by Suwa and Friesen (13).

(b) Anti-HPL Sepharose - Concentrated anti-HPL antibody (3 ml), containing 205 mg of protein with a total HGH binding capacity of 15 mg, was coupled to 10 ml activated Sepharose at pH 8.5 with an efficiency of coupling of 96%. The anti-HPL Sepharose was washed extensively as described for the HPL-Sepharose. Separate 1 ml aliquots of this immunoadsorbant were utilized to remove monkey growth hormone (MGH) from monkey pituitary incubation media and tissue homogenates.

Monkey Pituitary Incubation Media and Tissue Homogenates:

Incubation media and tissue homogenates from male, pregnant or lactating Rhesus monkey pituitary glands which had been fractionated by gel filtration on Sephadex G-100 were used in these experiments (1,3,4). As shown in Figure 1, after gel filtration one major radioactive protein peak was obtained that had the same elution volume as MGH. Aliquots of the fractions which contained the radioactive protein peak were passed through small (1.0 cm) columns of anti-HPL Sepharose. The following determinations were made prior to and after immunoadsorption:

1) ^{3}H-proteins were counted in Bray's solution (14) in a Packard tricarb liquid scintillation counter, 2) total proteins were measured by fluorescence using an Aminco-Bowman Spectrophotofluorometer with excitation at 278 mμ

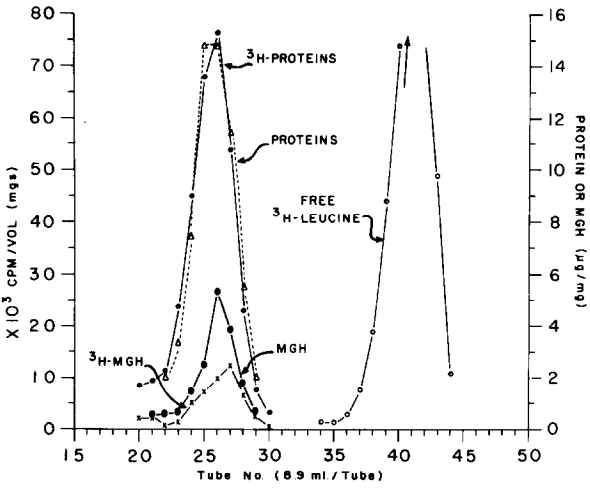

Fig. 1. Gel filtration on Sephadex G-100 of the medium obtained after 24 hr. incubation of pregnant monkey pituitary glands using 0.1 M NH_4HCO_3 as buffer. The distribution of proteins, MGH, ^{3}H-proteins and ^{3}H-MGH is shown. The latter was determined by immunoprecipitation of aliquots of each fraction with antibodies to HPL. A single large radioactive protein peak (tubes 22-29) encompasses the tubes containing MGH.

and emission at 350 mµ using HGH or HPL as standards, 3) monkey growth hormone and prolactin were determined by radioimmunoassay (15) using HGH[1] and ovine prolactin[1] as standards respectively. Prolactin activity was assayed by pigeon crop sac assay (16) using ovine prolactin N.I.H. P-S7 (24.3 µ/mg) as standard.

RESULTS AND DISCUSSION

Table I shows the results obtained when fractions of monkey pituitary incubation media and tissue homogenates after gel filtration on Sephadex G-100 were passed through 1 ml aliquots of anti-HPL Sepharose. These columns were capable of removing more than 99% of monkey growth hormone

[1] - Gifts of Endocrine Study Section, NIH, with potencies of 1.45 µ/mg for NIH-HGH C-1216 and 30.3 µ/mg for NIH P-S9.

TABLE I - MONKEY PITUITARY INCUBATION MEDIA AND TISSUE PASSED THROUGH ANTI-HPL SEPHAROSE COLUMNS

EXPT. NO.	NO. OF PITUITARIES	SOURCE PITUITARY PROTEIN	PERCENT UNADSORBED BY SEPHAROSE			POTENCY OF MONKEY PROLACTIN	
			PROTEIN	^{3}H-PROTEIN	MGH	RADIOIMMUNOASSAY IU/mg	BIOASSAY IU/mg (95% C.L.)
1	3	MALE MEDIA	40%	41%	.06%	A[a] 0.02 U[b] N.D.[c]	N.D. N.D.
2	5	PREGNANT MEDIA	70%	90%	.42%	A 0.43 U 1.33	N.D. 13.1 (11.0 - 15.6)
3	4	PREGNANT MEDIA	65%	63%	.91%	A 0.86 U N.D.	3.7 (3.2 - 4.2) 4.0 (——————[d])
4	3	LACTATING MEDIA	45%	24%	.02%	A 0.46 U 0.51	N.D. N.D.
5	11	PREGNANT TISSUE	57%	——[e]	.03%	A 1.84 U 1.83	3.5 (3.0 - 4.2) 4.1 (3.6 - 4.6)
6	2	PREGNANT MEDIA	61%	43%	.38%	A 1.80 U 2.89	N.D. N.D.
7	10	PREGNANT MEDIA	65%	63%	.00%	A 3.78 U 5.01	N.D. N.D.

a. A - Applied to Sepharose Columns

b. U - Unadsorbed by Sepharose Columns

c. ND- Not Determined

d. Not calculated due to non-parallel slope of response

e. Not incubated with ^{3}H-leucine

in all experiments. Despite this loss of growth hormone, the potency of prolactin in the unadsorbed fractions actually increased by both bioassay and radioimmunoassay. Figure 2 shows that the dose response curve of the monkey pituitary fractions paralleled the ovine prolactin standard curve in the bioassay. In the virtual absence of monkey growth hormone prolactin activity as high as 13.1 IU/mg (experiment 2) was obtained although the usual biopotency attained was approximately 4 IU/mg. Immunologic potencies varied from 0.5 IU/mg to 5 IU/mg in pregnant media to almost negligible in male pituitary media.

Affinity chromatography with Sepharose coupled antibody

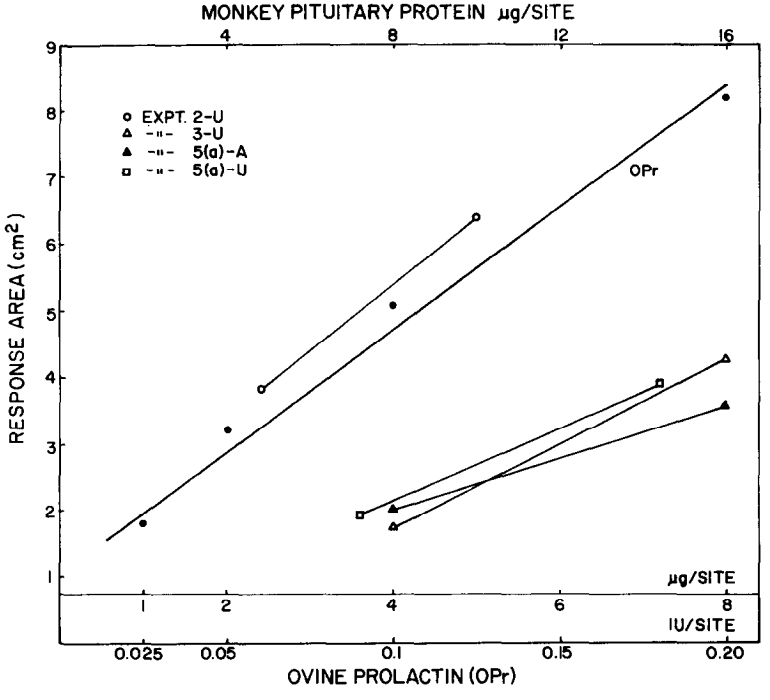

Fig. 2. Increase in pigeon crop-sac area (CM^2) in response to ovine prolactin (OPr) standard at four dose levels and four monkey pituitary preparations at 2 doses each. Each point is the mean of 5 hemicrop area responses at two sites. The monkey preparations are identified according to Table I.

to HPL is the first reliable method which has been described for obtaining primate pituitary protein preparations free of growth hormone, but with significant prolactin activity by bioassay and immunoassay. In preliminary experiments we have observed that this method is also satisfactory for isolation of human prolactin. Using these fractions, antisera have been obtained which are capable of distinguishing primate prolactin from growth hormone in a radioimmunoassay for prolactin (15). We have demonstrated that it is possible to separate monkey growth hormone from prolactin by taking advantage of the immunologic specificity of each hormone. It will now be feasible to obtain sufficient amounts of primate prolactin, both monkey and human, free of growth hormone for more complete chemical and biological characterization.

ACKNOWLEDGEMENT

We are indebted to Mrs. Doris Yung, Miss Jean Henderson and Mrs. Judy Joba for technical assistance and to Miss Francine Dupuis for typing the manuscript. This research was supported by MRC MA-1862 and NIH-01727-06.

REFERENCES

1) Friesen, H., and Guyda, H. 52nd Meeting of the Endocrine Society, p. 110, St. Louis (1970).

2) Friesen, H., Guyda, H., and Hardy, J., J. Clin. Endocrin. & Metab. 31:611 (1970).

3) Friesen, H., Guyda, H., Endocrinology (Manuscript accepted).

4) Friesen, H., Guyda, H., and Hwang, P., Nature (accepted).

5) Chidlow, J.W., Stephen, J., and Smith, H., Biochem. J., 117:49 (1970).

6) Weintraub, B.D., Biochem. & Biophys. Res. Comm. 39:83 (1970).

7) Cuatrecacas, P., Biochem. & Biophys. Res. Comm. 35:531 (1969).

8) Yip, C.C., and Logothetopoulos, J., Proc. Nat. Acad. Sci. (U.S.A.) 62:415 (1969).

9) Omenn, G.S., Ontjes, D.A., and Anfinsen, C.B., Nature 225:189 (1970)

10) Friesen, H.G., Nature (London) 208:1214 (1965)

11) Cuatrecacas, P., Wilchek, M., and Anfinsen, C.B., Proc. Nat. Acad. Sci. (U.S.A.) 61:636 (1958).

12) Dandliker, W.B., and deSaussure, V.A., Immunochemistry 5:357 (1968).

13) Suwa, S., and Friesen, H., Endocrinology 85:1028 (1969).

14) Bray, G.A., Anal. Biochem. 1:279 (1964).

15) Guyda, H., Hwang, P., and Friesen, H., J. Clin. Endocrin. & Metab. (In press).

16) Grosvenor, C.E., and Turner, C.W. Endocrinology 63:530 (1958).