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Canine Pituitary Prolactin: Isolation and Partial Characterization (39577)

HAROLD PAPKOFF

Hormone Research Laboratory and Reproductive Endocrinology Center, University of California, San Francisco, California 94143

For many years the only readily available prolactin preparations have been of ovine or bovine origin (1). Consequently, the bulk of chemical and physiological studies has been performed with these preparations. In recent years, isolation procedures have been described for obtaining prolactin from several other species: rat (2), pig (3, 4), and the human (5). The complete amino acid sequences are now known for ovine (6), bovine (7), and porcine (8) prolactin, and in a preliminary communication, the sequence of rat prolactin was reported (9). The availability of the above-mentioned species of prolactin have materially advanced structure-function as well as physiological studies of prolactin action by allowing, for instance, the development of radioimmunoassays. There is still a need, however, for other species of prolactin. One of these is canine prolactin, insofar as the dog is an extensively employed experimental animal. I wish to report here a simple procedure for the isolation of highly purified prolactin from a limited number of dog pituitaries. In addition, some of the properties of the canine prolactin were determined.

Purification. In this study, 300 (approximately 13.8 g) frozen dog pituitaries obtained commercially from Pel-Freez Co., Little Rock, Ark., were employed. The presence of prolactin in various fractions was detected by a mixed heterologous radioimmunoassay (porcine prolactin tracer; anti-ovine prolactin serum) which cross-reacted with canine prolactin (10) and by disc electrophoresis at pH 8.3 in 7.5% polyacrylamide gels. The pituitaries were homogenized in a Waring Blendor with 600 ml of H_2O, adjusted to pH 9.5 with CaO, and stirred overnight at 4°. Following centrifugation, the extract was adjusted to 0.15 *M* $(NH_4)_2SO_4$ and the pH to 4.0 by addition of freshly prepared 0.2 *M* HPO_3. The precipitate which formed was dialyzed against water, lyophilized (yielding 2.1 g), and extracted with pH 5.1–0.45 *M* $(NH_4)_2SO_4$ buffer. The residue was suspended in 50 ml of H_2O, dialyzed against water, and adjusted to pH 10.0 with *N* NaOH. Insoluble material was centrifuged off and cold (−20°) EtOH was added to a concentration of 50% (v/v). The resultant precipitate was centrifuged off, the supernatant fluid was adjusted to pH 5.0 with *N* HCl, and 4 vol of cold EtOH was added. The ethanolic precipitate was then extracted with 0.05 *M* NH_4HCO_3 and the extract was applied to a column of Sephadex G-100 in 0.05 *M* NH_4HCO_3 for final purification. The major symmetrical peak which eluted with a *Ve/Vo* of 2.2 was lyophilized and used for characterization studies. A yield of approximately 6 mg was obtained from the 13.8-g wet weight pituitaries.

Biological characterization. The purified canine prolactin was assayed several times in pigeons by the local crop-sac test (11). The results showed that the canine prolactin preparation had a dose-dependent response which was nonparallel (a flatter slope) when compared with ovine prolactin. Its potency relative to ovine prolactin (taken as 30 units/mg) was between 11–27 units/mg. Contamination with growth hormone was estimated to be 0.87% as measured by radioimmunoassay (12).

Amino acid and NH_2-terminal group analyses. The amino acid content of the canine prolactin was determined by the method of Spackman *et al.* (13), following hydrolysis in 5.7 *N* HCl at 105° for 20 hr in sealed evacuated tubes. The results are shown in Table I and compared to several other species of prolactin. Noteworthy is the general similarity between the prolactins, particularly with respect to the half-cystine content (six in each), and the high content of leucine, aspartic acid, and glutamic acid. The methionine content of canine prolactin, however, is

TABLE I. AMINO ACID COMPOSITION OF CANINE PROLACTIN COMPARED TO OVINE, PORCINE, AND MURINE PROLACTIN.

Amino acid	Canine[a]	Canine[b]	Ovine[c]	Porcine[d]	Murine[e]
Lysine	7.0	9	9	9	13
Histidine	5.9	7	8	9	6
Arginine	12.5	13	11	13	8
Aspartic	24.2	22	22	22	23
Threonine	6.1	6	9	5	6
Serine	15.0	15	15	16	12
Glutamic	26.5	24	22	24	28
Proline	9.1	8	11	7	12
Glucine	11.6	11	11	8	7
Alanine	13.3	13	9	10	10
Cystine (one-half)	6.0	N.D.	6	6	6
Valine	9.6	11	10	11	12
Methionine	3.3	1	7	4	2
Isoleucine	10.3	11	11	15	14
Leucine	25.2	22	23	26	23
Tyrosine	5.4	6	7	7	7
Phenylalanine	6.8	6	6	6	7
Tryptophan	1.6[f]	N.D.	2	2	2

[a] Average of five determinations.
[b] Data of Knight *et al.* (17).
[c] Structural data of Li *et al.* (6).
[d] Structural data of Li *et al.* (8).
[e] Structural data of Parlow and Shome (9).
[f] Determined spectrophotometrically.

somewhat lower than that found in ovine prolactin (three to four in canine vs seven in ovine) as are several other residues (lysine, histidine, threonine). Tryptophan content was determined spectrophotometrically (14) and the results suggest the presence of two residues as are present in ovine, porcine, and rat prolactin. Estimates of the molecular weight of the canine prolactin from amino acid analyses and gel filtration behavior on Sephadex G-100 suggest a value of about 22,000 which is consistent with that of ovine prolactin (1). Amino terminal group analysis by the dansyl-Edman procedure (15, 16) showed leucine to be the major terminal amino acid with a trace of phenylalanine.

Recently, Knight *et al.* (17) reported on the preparation of canine prolactin by means of the technique of isotachophoresis. Their preparation had a similar biological potency to that reported here. Their amino acid analysis (Table I) was incomplete (no values for half-cystine and tryptophan), but in general was similar to that reported here except for a much lower content of methionine (one vs three).

Summary. Highly purified canine prolactin has been prepared and partially characterized. The material is comparable in potency to ovine prolactin, and similar in amino acid composition as well.

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1. Li, C. H., *in* "Handbook of Physiology" (E. Knobil and W. H. Sawyer, eds.) Vol. IV, Sec. 7, Part 2, p. 103. Amer. Physiol. Soc., Washington, D.C. (1974).
2. Ellis, S., Grindeland, R. E., Nuenke, J. M., and Callahan, P. X., Endocrinology **85,** 886 (1969).
3. Eppstein, S. H., Nature (London) **202,** 899 (1964).
4. Bewley, T. A., and Li, C. H., Arch. Biochem. Biophys. **167,** 80 (1975).
5. Lewis, U. J., Singh, R. N. P., and Seavey, B. K., Biochem. Biophys. Res. Commun. **44,** 1169 (1971).
6. Li, C. H., Dixon, J. S., Lo, T. B., Schmidt, K. D.,

and Pankov, Y. A., Arch. Biochem. Biophys. **141,** 705 (1970).
7. Wallis, M., FEBS Lett. **44,** 205 (1974).
8. Li, C. H., Int. J. Peptide Protein Res. **8,** 205 (1976).
9. Parlow, A. F., and Shome, B., Fed. Proc. **35,** 219 (1976).
10. Lovinger, R. D., Kaplan, S. L., Ganong, W. F., and Grumbach, M. M., *in* "Advances in Human Growth Hormone Research" (S. Raiti, ed.), p. 256. National Pituitary Agency Symposium, Baltimore (1973).
11. Nicoll, C. S., Endocrinology **80,** 641 (1967).
12. Lovinger, R. D., Connors, M. H., Kaplan, S. L., Ganong, W. F., and Grumbach, M. M., Endocrinology **95,** 1317 (1974).
13. Spackman, D. H., Stein, W. H., and Moore, S., Anal. Chem. **30,** 1190 (1958).
14. Beaven, G. H., and Holiday, C. R., Adv. Protein Chem. **7,** 319 (1952).
15. Gray, W. R., and Hartley, B. S., Biohem. J. **89,** 59 (1963).
16. Woods, K. R., and Wang, K. T., Biochim. Biophys. Acta **133,** 369 (1967).
17. Knight, P. J., Gronow, M., and Hamilton, J. M., J. Endocrinol. **69,** 127 (1976).

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