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Purification of canine prolactin and growth hormone by fast protein liquid chromatography

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ABSTRACT

Prolactin and growth hormone are two peptide hormone with a very similar structure. A simple method is described for the simultaneous purification of these two peptides from canine pituitary extract by fast protein liquid chromatography. After extraction at pH 5.0 and 9.6 and anion-exchange chromatography on a MonoQ column, prolactin and growth hormone are then separated by gel filtration chromatography on two Superose columns coupled in series. The different fractions of the purification scheme are checked for the presence of the peptide hormones by sodium dodecyl sulphate gel electrophoresis in the Pharmacia PhastSystem. Each hormone is also characterized by its behaviour in a Western Blotting Detection System.

INTRODUCTION

Since the dog is a very useful model in medical research, canine prolactin (cPRL) and canine growth hormone (cGH) have been purified by several authors in order to prepare the components of radioimmunoassays used in the study of plasma levels of these hormones in various physiological states [1–6]. The development of fast protein liquid chromatography (FPLC) has allowed the rapid separation of complex mixtures of biological molecules [7–8]. We have applied this technique to purifiy cPRL and cGH from canine pituitary glands simultaneously, in only three steps.

EXPERIMENTAL

Equipment

The FPLC system (Pharmacia, Uppsala, Sweden) consisted of an LCC-500 liquid chromatography controller, two P-500 pumps, an MV-7 injection valve and a FRAC 100 fraction collector. The protein content of the eluent was analysed on a UV-M monitor (280 nm) and recorded with a two-channel REC-482 recorder. The anion-exchange column was a Mono Q HR 5/5 prepacked column. A Superose 6 HR 10/30 column was coupled in series with a Superose 12 HR 10/20 column for the gel filtration chromatography.

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Polyacrylamide gel electrophoresis and Western blotting

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) was carried out on PhastGel gradient 10–15 (Pharmacia). Samples treated with SDS and β-mercapthoehanol were processed in the Pharmacia PhastSystem and the gels were developed with the Coomassie staining procedure [9].

After electrophoresis, the proteins were transferred over 30 min on a nitrocellulose membrane (LKB) with the PhastTransfer semi-dry transfer kit (Pharmacia). Bound proteins were detected by incubation with an antiserum against ovine PRL (BioScience) or an antiserum against bovine GH (Ventrex) and a secondary antibody conjugated to alkaline phosphatase (Immum-Blot kit, Bio-Rad).

Purification procedure

A total of sixteen pituitaries were removed from Beagle dogs within 2 h of death and kept frozen until required. The glands were sonicated in 20 ml of 50 m*M* ammonium acetate buffer, pH 5.5 at 4°C in a Soniprep 150 (MSE). The homogenate was first extracted in buffer pH 5.5. After centrifugation, the pellet was resuspended in 20 m*M* piperazine, pH 9.6 and extracted overnight. After centrifugation at 27 000 *g* at 4°C for 30 min, the supernatant was removed to be processed by anion-exchange chromatography.

A total of 10 ml of supernatant were injected on a Mono Q HR 5/5 column. The separation was carried out with an elution gradient from 20 m*M* piperazine, pH 9.6 to

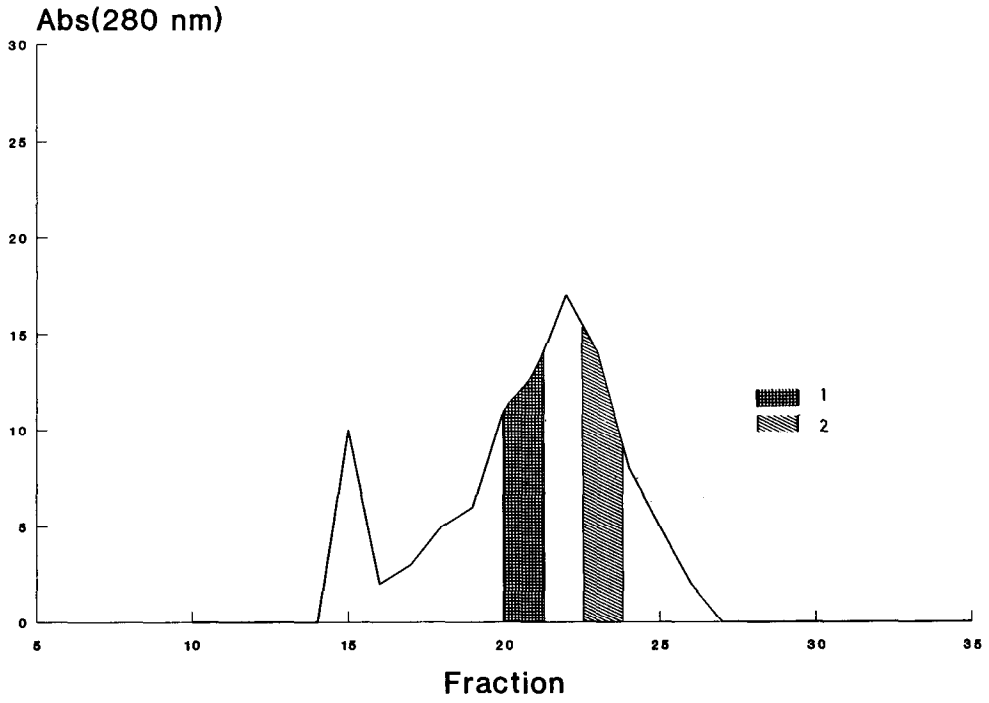

Fig. 1. Gel filtration chromatography on a series of Superose 12 HR 10/30 and Superose 6 HR 10/30 columns. (-) Absorbance at 280 nm; 1 = cPRL fractions; 2 = cGH fractions.

20 m*M* piperazine–1 *M* sodium chloride pH 9.6. The buffer was chosen according to the methods of Stanton *et al.* [10] on ovine prolactin. The segmented gradient started 5 min after injection with a flow-rate of 1 ml/min: 0–5 min, 0% NaCl; 5–25 min, 0–20% NaCl (linear); 25–30 min, 20% NaCl; 30–40 min, 20–40% NaCl (linear); 40–45 min, 40% NaCl; 45–55 min, 40–100% NaCl (linear); 55–60 min, 100% NaCl with a flow-rate of 1 ml/min. The 1-ml fractions were analysed for the presence of cPRL and cGH by SDS-PAGE.

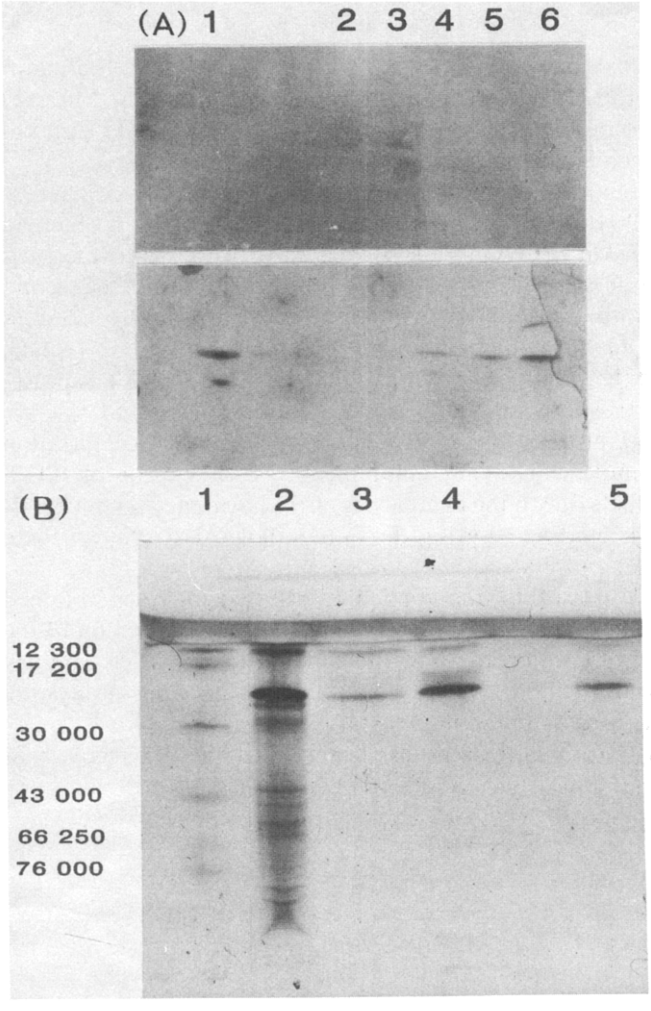

Fig. 2. (A) Western blotting of the fractions obtained after gel filtration chromatography. The membrane on top was incubated with an antiovine PRL serum while the membrane below was incubated with an antibovine GH serum. Lanes: 1 = pool of the fractions obtained after anion-exchange chromatography (not applied on the first membrane); 2–6 = fractions 20–24 respectively. (B) SDS-PAGE of the fraction obtained during the purification process. Lanes: 1 = molecular weight marker (LKB); 2 = extract of canine pituitary glands S2; 3 = cGH obtained after Superose gel filtration; 4 = pool of the fraction obtained after Mono Q anion-exchange chromatography; 5 = cPRL obtained after Superose gel filtration.

The fractions from the Mono Q column containing cPRL and cGH were pooled and concentrated overnight on Bio-Gel concentrator resin (Bio-Rad). The concentrated fraction (1 ml) was then injected on Superose 12 HR 10/30 and Superose 6 HR 10/30 coupled in series and eluted with a solution of 20 m*M* piperazine, pH 9.6 at a flow-rate of 0.5 ml/min. The 35 fractions of 1 ml obtained were checked by SDS-PAGE and Western blotting. The pure fractions obtained after gel filtration chromatography were dialysed overnight and lyophilised.

RESULTS AND DISCUSSION

The advantage of the extraction procedure used is the removal of the major blood proteins [5] and therefore the improvement of the resolution on the Mono Q HR 5/5 column. During anion-exchange chromatography, cPRL and cGH elute together as a large peak between 140 and 200 m*M* NaCl.

The resolution of the fractions from the Mono Q on the two columns, Superose 6 HR 10/30 and Superose 12 HR 10/30 is shown in Fig. 1. A large peak is obtained between fractions 17 and 27. On the membrane incubated with the antiovine PRL serum, one band is seen for fractions 20 and 21 (lanes 2 and 3) (fig. 2A). On the second membrane, incubated with antibovine GH serum, only fractions 22 to 24 react (lanes 4–6). According to these results, fractions 20 and 21 containing cPRL are pooled, fraction 22 is kept apart and the cGH-rich fractions 23 and 24 are pooled. The purity of the two hormone batches is shown by SDS-PAGE (Fig. 2B, lanes 3 and 5).

Only one band for cPRL and one band for cGH are observed after gel filtration chromatography. The low-molecular-weight components which appear on SDS-PAGE are degradation products due to the proteolysis of the hormones. These bands are also detected by Western blotting showing the immunological activity of these components (not shown here).

The technique of the Western blotting applied on selected fractions obtained after each chromatographic step can be very useful in checking the resolution of the pituitary hormones. The use of the PhastSystem and of the PhastTransfer semi-dry transfer kit allows the analysis of the fractions in less than 2 days while 5 days are necessary to have the results of the radioimmunoassay.

This procedure of purification of the pituitary hormones allows the recovery of an average of 258 μg of cPRL and of 735 μg of cGH (Table I).

TABLE I

PURIFICATION TABLE

Stage	Concentration proteins (mg/ml)	Total proteins (mg)	Yield (%)
Crude extract	3.34	53.5	
Extraction	0.43	5.2	9
Ion-exchange	0.42	2.5	48
Gel filtration			
PRL fraction	0.13	0.3	10
GH fraction	0.24	0.7	29

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