MULTIPLE FORMS OF SOMATOSTATIN-LIKE IMMUNOREACTIVITY IN CANINE PANCREAS

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1. Introduction

Incompletely characterized polypeptides displaying somatostatin-like immunoreactivity (SLI) [1–4] and somatostatin-like biological activity [2], have been described in extracts of hypothalamus, extrahypothalamic brain, stomach, and pancreas. Gel filtration studies have shown that the SLI exists in multiple molecular forms. Similarly, pulse-chase incubation experiments have shown incorporation of labeled amino acids into somatostatin-like polypeptides in the 7000–13 000 mol. wt range in anglerfish islets [5] and in the > 150 000 and 3500 mol. wt range in slices of rat hypothalamic and pancreatic tissue [6]. Immunoaffinity chromatography using immobilized antibodies directed against different regions of the somatostatin molecule has been employed to isolate and differentiate between the molecular forms of somatostatin-like immunoreactivity in canine plasma [7]. In this study we employ these methods to characterize the SLI present in extracts of canine pancreas with respect to molecular size, charge and immunometric properties.

2. Materials and methods

Trypsin insolubilized on polyacrylamide (250 U/g insoluble enzyme), horse cytochrome *c*, and DL-dithiothreitol were purchased from Sigma Chemical Company, St Louis. Antiserum S-27, directed against residues 5–9 and 11 [8], and antiserum S-81, directed against residues 1–4 of somatostatin [8], were provided by Dr W. Vale, Salk Institute, La Jolla. Antibodies from these antisera were immobilized on cyanogen bromide activated Sepharose-4B as in [9].

The pancreases of normal dogs were removed immediately after sacrifice and frozen on solid CO_2. Tissue extracts were prepared by a modification of the method in [10]. Minced pancreatic tissue was heated to 90°C for 5 min in 2 M acetic acid, rapidly cooled and homogenized in a Waring blender. The crude tissue extracts were centrifuged at 2500 rev./min for 15 min and the supernatant adjusted to pH 7.4 with 10 M sodium hydroxide solution. After further centrifugation at 2500 rev./min for 15 min, the supernatant was chromatographed on columns of immobilized antibodies S-27 and S-81 as in [9]. After elution of non-biospecifically adsorbed material by irrigation with 1 M NaCl adjusted to pH 10.4 with aqueous ammonia (spec. grav. 0.88) [11], the SLI bound to antibody was recovered by irrigation with 1 M formic acid. Fractions containing SLI were lyophilized and subjected to gel filtration on columns (60 × 2.5 cm) of Biogel P-10 using 0.1 M NH_4HCO_3, pH 8.8, for elution. Fractions containing SLI were again lyophilized and subjected to isoelectric focusing at 4°C using an LKB Model 8100 isoelectric focusing column (110 ml) [12]. Linear pH gradients in the pH 3.5–10 and pH 9–11 ranges were generated in sorbitol density gradients containing 2%, v/v, LKB ampholines. pH measurements were made at 4°C using a Beckman Model 3550 pH meter. Horse cytochrome *c* (pI 10.04 ± 0.04 at

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20°C) [13], was included in the pH 9–11 gradients to monitor the reproducibility of different isoelectric focusing runs. Tryptic digestion of SLI fractions was carried out in 0.2 M NH_4HCO_3 buffer (pH 8.2) at 25°C using insoluble trypsin at 2×10^{-2} units/nmol SLI. The reaction was terminated by centrifugation and removal of the supernatant. Fractions of SLI in 6 M guanidinium hydrochloride (recrystallized) were treated with 100-fold molar excess of dithiothreitol at 50°C for 3 h in an atmosphere of N_2. The reaction mixture was chromatographed on columns of Biogel P-10, at pH 8.8. SLI was measured by the radioimmunoassay procedure in [14] as modified [9] using Arimura antiserum R101 which is directed toward the same region of the somatostatin molecule as antiserum S-27.

3. Results and discussion

Over 95% SLI was removed from extracts of canine pancreas by passage through columns of immobilized antibodies specific for the central residues of somatostatin (antibody S-27), but only 10–20% SLI was removed by passage through the column of antibodies specific for the N-terminal region of somatostatin (antibody S-81). In contrast, > 95% immunoreactivity associated with synthetic somatostatin in protein-free buffer (at the same concentration of immunoreactivity as in the pancreatic extracts) was bound by both antibody conjugates. The elution profiles of the immunoreactive material recovered from immunoaffinity columns are shown in fig.1. SLI isolated with the antibody S-27 column was eluted as 3 peaks in the regions of mol. wt 12 000, 3500 and 1600 (approx. relative abundance 10:5:200) (fig.1A), but SLI recovered from the antibody S-81 column was eluted as a single peak in the 1600 mol. wt region (fig.1B). The fact that the N-terminally directed antibody conjugate S-81 shows reduced binding of pancreatic SLI relative to synthetic somatostatin may indicate that

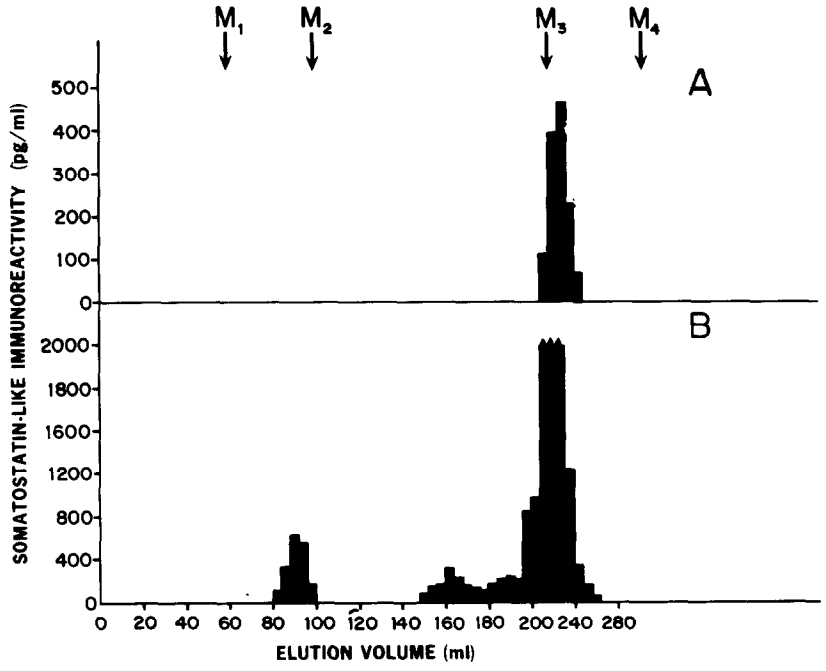

Fig.1. Elution profile of SLI from canine pancreatic extracts on Biogel P-10 columns of pH 8.8: (A) After recovery from columns of immobilized antibodies specific for the N-terminal region of somatostatin (residues 1–4); (B) after recovery from columns of immobilized antibodies specific for the central region of somatostatin (residues 5–9 and 11). The serrated peak in B indicates that the levels of immunoreactivity could not be accurately measured without further dilution of the fractions. The arrows indicate the elution volumes of blue dextran (M_1), cytochrome *c* (M_2), synthetic somatostatin (M_3) and potassium chromate (M_4).

the N-terminal residues of SLI are rendered inaccessible to antibody by interaction with proteins in the pancreatic extract. The presence of proteins that bind SLI has been demonstrated in tissues [15] and plasma [7,9]. The elution volume of the 12 000 mol. wt and 3500 mol. wt SLI components on Biogel P-10 at pH 8.8 were unaffected by incubation of the SLI with 8 M urea or with 6 M guanidinium hydrochloride, indicating that these forms of SLI were not aggregates of the 1600 mol. wt SLI or non-covalently bound complexes of the 1600 mol. wt polypeptide with nonimmunoreactive material. The elution profile of the 3500 mol. wt component was unchanged after treatment with dithiothreitol under conditions which are known to cleave both intra- and intermolecular disulfide linkages [16] but after similar treatment of the 12 000 mol. wt component all the immunoreactivity was eluted from Biogel P-10 columns in the 1600 mol. wt zone (fig.2).

The molecular charges and immunometric properties of the pancreatic SLI fractions were compared with those of synthetic somatostatin. Both the 3500 mol. wt and 1600 mol. wt fractions were isoelectrically focused to single immunoreactive bands in the same pH region as synthetic somatostatin (pH 10.5), whereas the 12 000 mol. wt polypeptide was focused into two bands at pH 4.6 and pH 5.7. Serial dilutions of the 12 000, 3500 and 1600 mol. wt SLI fractions from the pancreas were proportional and the dilution slopes were parallel to that of synthetic somatostatin in a radioimmunoassay using Arimura antiserum R101 (fig.3). Thus, the predominant molecular species of SLI in the pancreas resembles the tetradecapeptide, somatostatin, with respect to its molecular size, charge and immunometric properties.

The effect of incubation of the 3500 mol. wt SLI with immobilized trypsin for a period of 10 min is shown in fig.4. After 10 min, immunoreactivity was eluted from Biogel P-10 columns in the 1600 mol. wt as well as in the 3500 mol. wt zone. Trypsin-like enzymes have been implicated in the conversion of high molecular weight biologically inactive precursor molecules to the biologically-active hormone in a wide variety of systems [17–21] and these results are consistent with a possible role of the 3500 mol. wt SLI as a precursor of pancreatic somatostatin. The conversion of the 12 000 mol. wt SLI component into the 1600 mol. wt component by dithiothreitol suggests that this large molecular weight component may represent a post-translational modification of the 1600 mol. wt component by binding to a nonimmunoreactive polypeptide via a disulfide linkage. While this

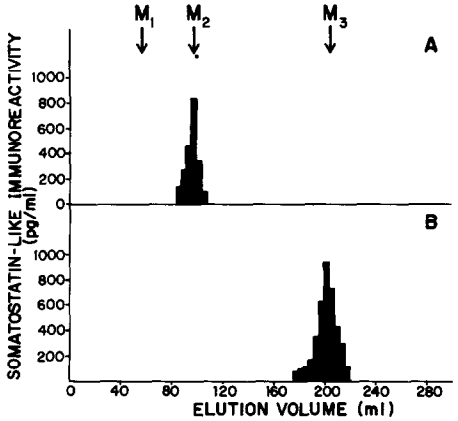

Fig.2. Elution profile of the 12 000 mol. wt pancreatic SLI component on Biogel P-10 columns at pH 8.8: (A) After incubation with 6 M guanidinium hydrochloride; (B) after incubation with 6 M guanidinium hydrochloride containing dithiothreitol. The arrows indicate the elution volumes of blue dextran (M_1), cytochrome *c* (M_2) and synthetic somatostatin (M_3).

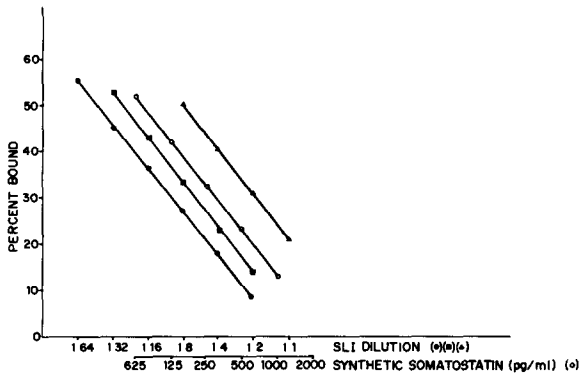

Fig.3. Dilution slopes of pancreatic SLI components of 12 000 mol. wt (■–■–■), 3500 mol. wt (▲–▲–▲) and 1600 mol. wt (●–●–●–) in comparison to serial dilutions of synthetic somatostatin (○–○–○). The pancreatic SLI samples were purified by affinity chromatography and gel filtration.

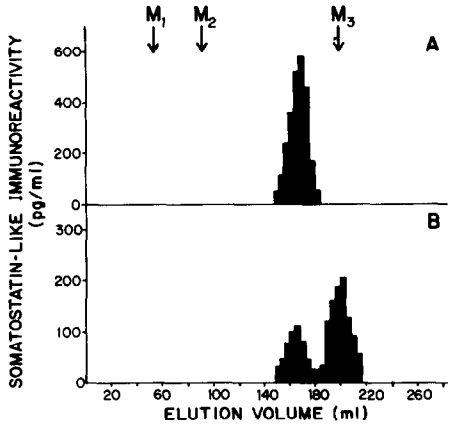

Fig.4. Elution profile of the 3500 mol. wt pancreatic SLI component on Biogel P-10 columns at pH 8.8: (A) After incubation with 6 M guanidinium hydrochloride containing dithiothreitol; (B) after incubation with insolubilized trypsin. Experimental details are given in the text. The arrows indicate the elution volumes of blue dextran (M_1), cytochrome *c* (M_2) and synthetic somatostatin (M_3).

investigation was in progress, a 12 000 mol. wt polypeptide, displaying somatostatin-like immunoreactivity and with similar physicochemical properties to the component described in this study, has been isolated from extracts of pigeon pancreas [22]. It has been shown that only the 1600 mol. wt component is released from the isolated perfused canine pancreas in response to an arginine stimulus [7] and the physiological significance of the 12 000 mol. wt component is not understood.

Acknowledgements

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