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Heterogeneity of Somatostatin-Like Immunoreactivities Extracted from Rat Small Intestine

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ABSTRACT. The small intestines of 5 rats were successfully divided into the epithelial cells and the muscle layer, and substances with somatostatin-like immunoreactivity (SLI) were extracted to investigate the heterogeneity of rat intestinal SLI.

Separation of SLI by gel chromatography revealed that there were large differences in the relative amounts of 3.5K SLI and 1.6K SLI between the epithelial cells and the muscle layer. The ratio of 3.5K SLI / (3.5K SLI + 1.6K SLI) was 67.3±4.0% (Mean±SD, 54.2−81.0%) in the epithelial cells and 14.2±2.6% (Mean±SD, 2.8−18.8%) in the muscle layer. By High Performance Liquid Chromatography (HPLC), 3.5K SLI and 1.6K SLI were shown to correspond to somatostatin-28 (SS-28) and somatostatin-14 (SS-14), respectively. Thus it was demonstrated that SS-28 is predominantly distributed in the epithelial cells, and the SS-14 is mainly located in the muscle layer of rat small intestine.

Key words : SLI — heterogeneity — rat small intestine

Somatostatin (SRIF, SS-14) is a tetradecapeptide hormone originally isolated from the ovine hypothalamus by Brazeau et al. in 1973. Subsequent studies have shown that, in addition to SRIF, high molecular weight substances with somatostatin-like immunoreactivity (SLI) were present in most of the somatostatin-containing tissues examined.

Since the discovery of somatostatin-28 (SS-28) by Pradyrol et al. in 1980, the relative amounts of SS-14 and SS-28 in various somatostatin-containing tissues were reported, and the significance of the distribution of the hormones has been discussed.

In this study, the heterogeneity of rat small intestinal SLI was examined by gel chromatography followed by High Performance Liquid Chromatography (HPLC).

MATERIALS AND METHODS

Five male Wistar rats (300-350g) were killed by decapitation, and their

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proximal small intestines extending 15–20 cm from the ligament of Treitz were removed rapidly. The samples (about 1.0 g each) were frozen and stored at -80°C until use.

Tissue preparation and extraction

Epithelial cells were isolated by a modification of a previously reported vibration technique.[1,2] The proximal small intestine was everted over the rounded end of a glass rod and tied in place with silk thread. The sample was vibrated for 30 min at 0°C in 30 ml of medium (0.01M PBS, 0.01M EDTA, pH 7.8) with a modified vibromixer. Epithelial cells which were shed into the medium were harvested by centrifugation at 1000 rpm for 10 min. To obtain the muscle layer, the remaining tissue was thoroughly scraped with the edge of a glass microscope slide in order to remove the submucosal layer. The adequacy of tissue separation was examined histologically.

The epithelial cells and the muscle layer were homogenized in 2 ml of 2 N acetic acid, and heated to 100°C for 5 min. The homogenates were then centrifuged for 15 min at 1000 rpm, and the supernatants were lyophilized.

Chemicals

Synthetic SS-14 (Protein Research Foundation, Osaka, Japan) and SS-28[3] (a gift of Dr. Tomokuni Kokubu, Research Institute for Polymers and Textiles) were used as standards for gel chromatography, high performance liquid chromatography and radioimmunoassay.

Gel Chromatography

Tissue extracts were dissolved in 1.1 ml of 1 N acetic acid, and filtered through a 1.0×47 cm Bio-Gel P-6 column equilibrated with 1 N acetic acid at a flow rate of 0.2 ml/min. Aliquots of each fraction were assayed for SLI after lyophilization, and the fractions of SLI which eluted at the same volumes as SS-28 (3.5K SLI) and SS-14 (1.6K SLI) were respectively pooled and lyophilized.

High Performance Liquid Chromatography (HPLC)

3.5K SLI and 1.6K SLI obtained from gel-filtration were partially characterized by reverse phase HPLC under previously described conditions.[4]

Each sample was dissolved with 100μl of the mobile phase and filtered through a nitrocellulose membrane (pore size 0.45 μ) before injection. The stationary phase was a 3.9×30 cm μ Bondapac C-18 column, and the isocratic mobile phase was 24.6% acetonitrile, 75.4% 0.1M triethylamine phosphate, pH 3.0; the flow rate was 1.5 ml/min. Fractions (0.75 ml each) were neutrized with 67 μl of 1 N NaOH and lyophilized.

Radioimmunoassay

The radioimmunoassay for somatostatin was performed as previously

described.[5] On a weight basis, SS-28 was half as reactive with our anti-SRIF antiserum (#1-5) as SS-14, but it reacted equally with SS-14 on a molar basis (data not shown).

RESULTS

Histological examination showed that the epithelial cells and the muscle layer were successfully separated (data not shown).

Gel filtration of the extract from the epithelial cells revealed that the predominant SLI peak was in the same region as SS-28 (3.5K SLI), although there was a small SLI peak in the region of SS-14 (1.6K SLI) (Fig. 1, upper pannel).

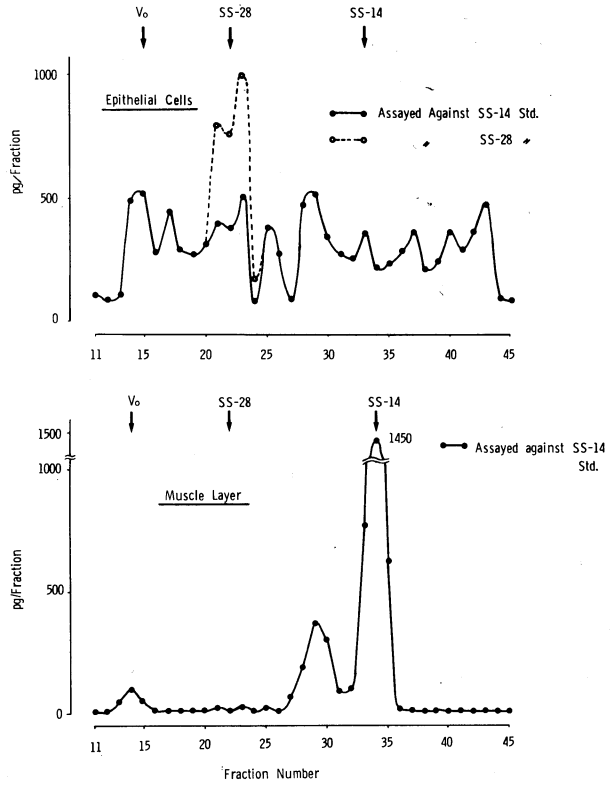

Fig. 1. Typical gel chromatograms of SLI extracted from the epithelial cells and the muscle layer of rat small intestine.
SLI extracted from the epithelial cells (upper pannel) and the muscle layer (lower pannel) of rat small intestine were filtered through a Bio-Gel P-6 column (1×47cm, 1 N acetic acid). Each fraction was assayed for SLI. Standards of SS-14 and SS-28 were subjected to gel chromatography under identical conditions, separately. The void volume (Vo) and elution volumes of SS-28 and SS-14 are indicated by arrows.

However, when the muscle layer extract was chromatographed, almost all of the SLI was eluted at the region corresponding to SS-14 (1.6K SLI), while very little SLI coeluted with SS-28 (3.5K SLI) (Fig. 1, lower pannel). To assess the molecular heterogeneity of SLI in both tissues, the 3.5K SLI/(3.5K SLI+1.6K SLI) ratio was calculated (Table 1), and it was found to be 67.3±4.0% (Mean±SD, 54.2−81.0%) in the epithelial cells and 14.2±2.6% (Mean±SD, 2.8−18.8%) in the muscle layer.

TABLE 1. Relative proportions of 3.5K SLI and 1.6K SLI in the epithelial cells and the muscle layer of rat small intestine.
The content of SLI were calculated from the gel-chromatograms.

	Epithelial Cells		Muscle layer	
Subject	3.5K SLI / (3.5K SLI + 1.6K SLI) (%)	Total SLI content (pg/mg)	3.5K SLI / (3.5K SLI + 1.6K SLI) (%)	Total SLI content (pg/mg)
1	62.7	0.80	2.8	8.8
2	81.0	0.69	14.6	4.5
3	70.9	0.59	17.9	10.0
4	67.9	0.77	18.8	10.9
5	54.2	0.36	17.1	7.0
Mean±SD	67.3±4.0	0.64±0.07	14.2±2.6	8.2±1.02

Total SLI contents of the epithelial cells and the muscle layer were 0.64 ±0.07 pg/mg wet weight and 8.2±1.02 pg/mg wet weight, respectively.

The SLI fractions obtained from gel chromatography at the 3.5K and 1.6K regions were analysed separately by HPLC (Fig. 2). HPLC of the 3.5K SLI obtained from the epithelial cells revealed three main peaks. The largest peak corresponded to that of SS-28 and the smaller peak to SS-14, but the exact nature of the remaining SLI peak is still uncertain (upper pannel). On the other hand, most of the 1.6K SLI of the muscle layer corresponded to SS-14, although some SLI substances were not absorbed by the reverse phase packing and were detected as early peaks (lower pannel).

DISCUSSION

The whole small intestine has been shown to contain endocrine D-cells in its epithelium and autonomic innervations of the submucosa and muscle layer which both contain SLI. The small intestine was divided into the epithelial cells and the muscle layer, and substances with SLI were extracted from each individually in this study. By the gel chromatography of SLI extracts from the epithelial cells and the muscle layer, it was shown that 3.5K SLI was predominant in the epithelial cells, while 1.6K SLI was predominant in the muscle layer. These results agree well with the reports of other investigators,[6−8] even though previous techniques for separating epithelial cells from other tissues

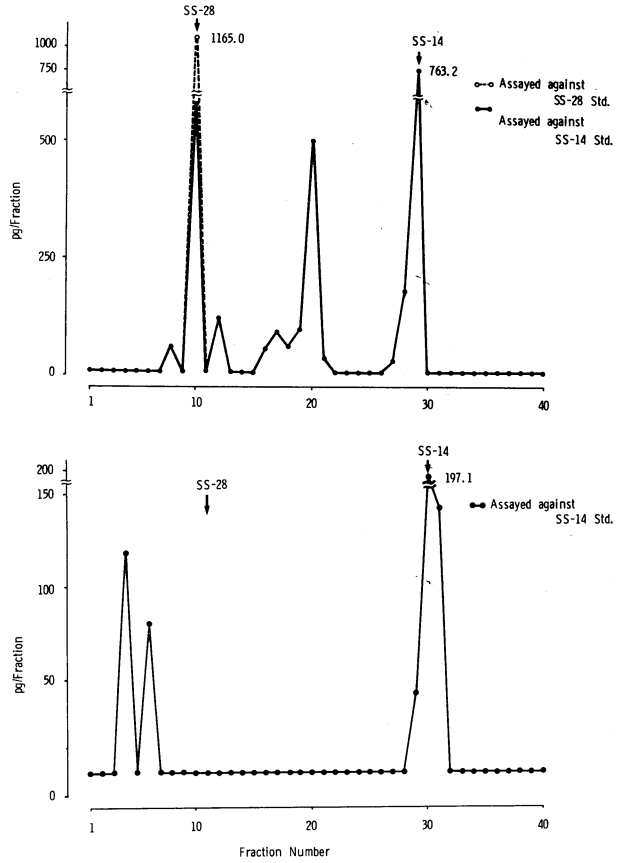

Fig. 2. HPLC analysis of 3.5K SLI and 1.6K SLI.
SLI fractions obtained by gel-filtration at the 3.5K and 1.6K regions were subjected to reverse phase HPLC (μ Bondapac C-18, 0.39×30 cm, 24.6 % acetonitrile, 75.4 % triethylamine phosphate, pH 3.0, 1.5 ml/min). Each fraction was assayed for SLI. Standards of SS-14 and SS-28 were subjected to HPLC under identical conditions, separately, and their elution volumes are indicated by arrows.

seemed inadequate,[6,7] and the fractions of SLI corresponding to SS-14 and SS-28 have not been identified by HPLC.[6—8] In this study, 1.6K SLI and 3.5K SLI were verified to be SS-14 and SS-28 by reverse phase HPLC analysis.

Recent Studies have shown that hypothalamic and pancreatic large molecular weight substances with SLI (mol. wt. 8000-15000) were converted to smaller molecular weight SLI substances.[9—12] Considering such data, the clear difference in the relative amounts of SS-28 and SS-14 in the epithelial cells and the muscle layer of rat small intestine could be due to differences in somatostatin biosynthesis in each tissue.

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