Hypersomatostatinemia in Chronic Renal Failure

TARO WASADA† KAORU INOUE, SATORU FUJIMI*, YASUHIRO SAKO, AKITAKA HISATOMI AND HIROSHI IBAYASHI

The Third Department of Internal Medicine, Faculty of Medicine, Kyushu University, Fukuoka 812 Japan

*The Kidney Center, Fukuoka Red Cross Hospital, Fukuoka 815, Japan

Abstract

Plasma concentrations of somatostatin-like immunoreactivity (SLI) were determined in uremic patients on maintenance hemodialysis. Plasma SLI levels were significantly (p<0.001) elevated in 26 diabetic uremic patients (67.1±6.8 pg/ml, mean±SE) and in 24 non-diabetic uremic patients (43.5±7.2 pg/ml), when compared with 60 healthy subjects (5.0±0.7 pg/ml).

Paired pooled plasma from uremic patients before and after hemodialysis was subjected to a reverse-phase octadecasilyl-silica (C-18) cartridge and then the extract was gel filtered on a Sephadex G-25 column (1.6×90 cm). Both elution profiles showed two peaks of SLI which coeluted with synthetic somatostatin (SS)-28 and SS-14 markers, respectively. The SS-28-like immunoreactivity (LI) peak, which was estimated by using SS-14 as a reference standard, was 3-fold larger than that for SS-14 LI. On the basis of immunoequivalency of the two components in the present assay, SS-28 LI constitutes approximately 75% of circulating somatostatin.

In conclusion, plasma SLI is substantially high in uremic patients of both diabetic and non-diabetic etiology and the SS-28 is a predominant form of circulating SLI in these patients, probably, in part, for a lower clearance of this molecule.

Somatostatin-like immunoreactivity (SLI) is found in the portal and peripheral blood of animals (Pimstone et al., 1978; Schusdziarra et al., 1978; Wasada et al., 1980; Patel et al., 1981) and humans (Pimstone et al., 1978; Kronheim et al., 1978; Saito, 1980; Wass et al., 1980; Mackes et al., 1981; Vinik et al., 1981), while its physiologic function is poorly understood. It is probably released from the pancreas and gastrointestinal tract and is cleared from circulation by a wide variety of tissues such as kidney and liver and also by plasma itself. However, the relative importance of organs as a site of degradation of circulating somatostatin is yet unknown in man. To clarify a role of kidney in the metabolism of somatostatin the present study attempted to determine the plasma levels of circulating SLI and their molecular forms in patients with chronic renal failure.
Materials and Methods

Subjects
Sixty healthy subjects (age 49.3 ± 6.3 yr, mean ± S.E.) and fifty uremic patients (26 from diabetic nephropathy, 58.7 ± 2.3 yr and 24 from chronic glomerulonephritis, 57.9 ± 2.7 yr) participated in the present study. The clinical features of the patients are presented in Table 1. Blood samples were drawn from a cannula of A-V shunt in the uremic patients at the beginning of hemodialysis and collected in a tube containing 6 mg EDTA and 500 KIU Trasylol/ml of blood, then immediately centrifuged at 4°C and plasma was stored at −20°C until assay.

Radioimmunoassay of plasma SLI
Synthetic cyclic SS-14 (Peptide Institute Co., Osaka), synthetic SS-28, an NH2-terminally extended form of SS-14 (Peninsula International plc., Buckinghamshire, UK), 125I-Tyr SS-14 (Amersham International plc., Buckinghamshire, UK) and anti-somatostatin-14 serum 80C from R. H. Unger (University of Texas) were used. The anti-serum 80C (final concentration 1:150000) detects SS-28 equally well on a molar basis. Plasma SLI was measured by a method identical to that previously described and validated by Conlon et al. (1983). In brief, plasma was treated with 30% (W/V) polyethylene glycol 6000 (final concentration 13.3%) and the mixture was centrifuged at 3000 rpm for 60 min at 4°C, then an aliquot of supernatant was used for radioimmunoassay. Standard curves were set up in assay buffer containing 13.3% PEG, similarly to the sample tubes. All SLI concentrations refer to the concentrations in plasma or buffer before the addition of PEG and were determined by using the reference to SS-14 standard. The mean recovery of synthetic somatostatin added to plasma and assayed in the PEG-treated supernatant was 92% (range 79–107%). To validate the assay with regard to the void volume SLI which constitutes most of the circulating SLI (Mackes et al., 1981) and reportedly represents an artifact of measurement (Conlon et al., 1982), a plasma specimen containing a high level of injected synthetic SS-14 was gel chromatographed on a Sephadex G-25 column (1.6 × 90 cm) and the eluates were measured for SLI. The void volume peak was totally eliminated when the eluates were treated with PEG before assay (Data were shown in the separate paper; Wasada et al., 1986). Thus, our present assay excludes the void volume component of SLI. The minimal sensitivity of this assay is 7.8 pg/ml for plasma. The values under these levels were read off automatically from the computer-fitted standard curve. The intra-assay and inter-assay coefficients of variation at the level of 30–40 pg/ml were 9.6% and 15.2%, respectively. The paired samples for plasma and eluates before and after hemodialysis were always run in the same assay. Statistical significance was calculated using Student’s t-test for paired or unpaired data.

Concentration of plasma for gel filtration chromatography
Twenty-four ml specimens of plasma from eight uremic patients were obtained before and after hemodialysis. Extraction and concentration

Table 1. Clinical parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>N(M/F)</th>
<th>Age (yr)</th>
<th>BW(kg) [B]</th>
<th>[A]</th>
<th>BUN(mg/dl) [B]</th>
<th>[A]</th>
<th>Cr(mg/dl) [B]</th>
<th>[A]</th>
<th>Ca*(mEq/L) [B]</th>
<th>[A]</th>
<th>Ht(%)</th>
<th>Glc (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRF</td>
<td>26(14/12)</td>
<td>± 2.3</td>
<td>1.6</td>
<td>1.6</td>
<td>3.0</td>
<td>1.5</td>
<td>0.4</td>
<td>0.2</td>
<td>0.07</td>
<td>0.07</td>
<td>1.2</td>
<td>± 18.7</td>
</tr>
<tr>
<td>Diabetic</td>
<td>26(14/12)</td>
<td>57.9</td>
<td>51.7* 50.6</td>
<td>60.7*</td>
<td>20.9</td>
<td>11.6*</td>
<td>4.6</td>
<td>4.4*</td>
<td>5.4</td>
<td>26.1</td>
<td>105.7</td>
<td></td>
</tr>
<tr>
<td>Non-diabetic</td>
<td>24(12/12)</td>
<td>± 2.7</td>
<td>1.5</td>
<td>1.5</td>
<td>4.2</td>
<td>3.2</td>
<td>0.5</td>
<td>0.3</td>
<td>0.07</td>
<td>0.07</td>
<td>1.1</td>
<td>± 6.3</td>
</tr>
<tr>
<td>Control</td>
<td>60(38/22)</td>
<td>± 49.3</td>
<td>6.3</td>
<td>6.3</td>
<td>2.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Mean ± SE, [B]: before hemodialysis, [A]: after hemodialysis, cr: serum creatinine [B] vs [A]: * p<0.001, Diabetic vs Non-diabetic: † p<0.05
of plasma SLI were performed as follows: A Sep-Pak C-18 cartridge (Waters Assoc., Milford, MA) was first rinsed with 2-ml acetonitrile (Wako Pure Chemical Ind., Ltd., Osaka), followed by 5-ml distilled water. Three ml plasma was passed through the cartridge to which SLI adsorbed. The cartridge was then rinsed with 5-ml of 0.1% trifluoroacetic acid (Wako Pure Chemical), and this portion was saved. After lyophilization and reconstitution in 2M acetic acid, the pooled samples, before and after hemodialysis, were then chromatographed on a Sephadex G-25 (fine) column (1.6 × 90 cm) equilibrated with 2M acetic acid containing 0.1% BSA (pH 2.5).

The extraction rate with the Sep-Pak cartridge of standard SS-14 and SS-28, separately added to the charcoal-treated somatostatin free plasma and determined by an identical radioimmunoassay, was almost comparable (78±9.3% vs 80±7.8%).

Results

Plasma SLI concentrations in control subjects and uremic patients

As shown in Figure 1, plasma SLI levels in 60 healthy subjects were 5.0±0.7 pg/ml (mean±SE). Uremic patients showed marked elevation of plasma SLI before hemodialysis: 67.1±6.8 pg/ml for 26 diabetic uremic patients and 43.5±7.2 pg/ml for 24 non-diabetic uremic patients. These values were significantly (p<0.001) high as compared with controls, but no difference was
noted between two groups of uremic patients. Unexpectedly, the mean plasma SLI increased significantly (p<0.05) to 83.3±6.8 pg/ml in the diabetic and 77.6±10.1 pg/ml in non-diabetic groups after hemodialysis.

Gel filtration chromatography

Figure 2 depicts the gel chromatographic profiles of SLI in paired plasma extracts from uremic patients before and after hemodialysis. Two peaks, coeluting with SS-28 and SS-14 markers, respectively, were present in both samples. The major peak (SS-28 LI) was not affected by treatment with PEG, indicating that this peak is independent of non-specific interference substances. The area under the curve of the SS-28 LI peak was 3-fold larger than that for SS-14 LI, when estimated by the reference to the SS-14 standard. Consequently, SS-28 LI constitutes approximately 75% of the circulating somatostatin, on a molar basis. The ratio of SS-28 LI/SS-14 LI remained unchanged by hemodialysis.

Discussion

The present study clearly demonstrates that hypersomatostatinemia of a considerable degree exists in patients with chronic renal failure. In the literature, however, Lundqvist et al. (1979) showed no elevation of plasma SLI in patients with chronic renal failure. Sheppard et al. (1979) reported that the metabolic clearance rate (MCR) of exogenous SS-14 was markedly lowered in uremic subjects, but basal plasma SLI levels did not differ from the controls. On the other hand, Conlon et al. (1984) found a slight but significant increase in plasma SLI (29±5 vs 10±2 pg/ml), while the MCR remained unimpaired in uremic patients on hemodialysis. The reason for the discrepancy
between these studies and ours is not obvious at the present time. It may depend on the subject group studied, or, even more, the assay method employed.

The most intriguing observation of this study is that plasma SLI in uremic patients consists mainly (75%) of the SS-28 LI component. This finding is unique if consideration is given to the fact that the molar ratio of plasma SS-14 vs SS-28 in healthy subjects is 69% : 31% (Polonsky et al., 1983) and 71% : 29% (Miyazaki et al., 1986), respectively.

It has been reported that the extraction rate of SS-28 by both kidney and liver is significantly lower than that of SS-14 in dogs (Polonsky et al., 1982). Thus, we can suppose that such is the case in uremic patients, and somatostatin, especially SS-28 component, may easily accumulate in their plasma, even under maintenance hemodialysis.

The reason why plasma SLI levels were raised after hemodialysis is unclear. Probably the hemoconcentration may be one explanation of this finding, because there was no change in the plasma concentration ratio of SS-14 vs SS-28 before and after hemodialysis.

The physiologic implication of these observations remains to be elucidated. In view of the findings that somatostatin has a broad spectrum of inhibitory actions on the central and peripheral nervous system, endocrine and digestive organs and that SS-28 is more active than SS-14 in some biological systems (Polonsky et al., 1982), elevated plasma levels of somatostatin may be partly responsible for the uremic symptoms and pathophysiology of chronic renal failure.

References


