Response of plasma somatostatin-like immunoreactivity (SLI) to a 75 g oral glucose tolerance test in normal subjects and patients with impaired glucose tolerance

Mitsuyasu Itoh¹, Yoshifumi Hirooka and Noriyuki Nihei

The Third Department of Internal Medicine¹,
Hamamatsu University, School of Medicine, Handa-cho, Hamamatsu 431-31, Japan

Abstract. To study the role of somatostatin in the pathophysiology of glucose intolerance in man, plasma somatostatin-like immunoreactivity (SLI) was measured in 8 normal subjects, 6 patients with insulin dependent diabetes mellitus (IDDM), 13 with non-insulin dependent diabetes mellitus (NIDDM), and 9 with hyperthyroidism, by extraction of plasma SLI and radioimmunoassay. The extraction method gave a recovery rate for synthetic somatostatin-14 and somatostatin-28 of 72 ± 6 and 55 ± 7%, respectively. No SLI corresponding to somatostatin-28 in human peripheral blood was observed. Incubation of somatostatin-28 in plasma gave a rapid decrease of immunoreactivity, and no conversion to somatostatin-14 was observed. It is speculated that SLI extracted with acid-acetone mainly represents a molecular weight similar to somatostatin-14. After oral administration of glucose (75 g), a clear and sustained rise in plasma SLI was seen in normal subjects from an initial value (± SEM) of 29.9 ± 5.4 pg/ml to a peak value, at 60 min of 93.4 ± 15.5 pg/ml. The increase of plasma SLI after 75 g glucose was also observed in IDDM and NIDDM. The peak level of SLI was significantly less than that for normal subjects. The extraction of plasma SLI with acetic acid and acetone gave reproducible results and showed a fluctuation of SLI with glucose concentration.

Somatostatin, which is distributed in several organs, has several functions, as hormone, neuroregulator, neurohormone, and cybennin (local regulatory factor, Gerich 1981). Its regulatory role in insulin and glucagon secretion (Itoh et al. 1980) suggests an important function in glucose homeostasis. Somatostatin is also located in the gastrointestinal tract and has a suppressive function on motility and absorption (Gerich 1981). Therefore, its pathophysiological importance in impaired glucose tolerance may be related to the endocrine pancreas or to the islet-gut interrelationship. Many reports indicate a possible role for somatostatin in diabetic animals in vivo (Schusdziarra et al. 1977) as well as in vitro (Hara et al. 1979), but there are no studies in human diabetics. The measurement of somatostatin in human blood has several difficulties, related to degradation in blood (Bennuck & Marks 1976), size heterogeneity (Mackes et al. 1981), and a non-specific plasma inhibition of the radioimmunoassay (Arimura et al. 1978; Mackes et al. 1981). A few reports from human studies suggest that somatostatin-like immunoreactivity (SLI) in plasma is increased by several stimuli such as arginine (Saito & Saito 1982), glucose (Wass et al. 1980), hypoglycaemia (Vinik et al. 1981), and meals (Wass et al. 1980; Vinik et al. 1981). In vitro studies clearly indicate that glucose stimulates the release of SLI from pancreas (Schauder et al. 1976; Ipp et al. 1977) and gastro-intestinal tract (Schusdziarra et al. 1978), but the influence on SLI in peripheral blood has not been determined. The present studies were undertaken to establish a reliable method for the measurement of SLI, and to use this method for observations on plasma SLI in response to oral glucose load (75 g) in some endocrinopathies with impaired glucose tolerance. Our results demonstrate that oral glucose loads increase plasma SLI in normal subjects and in patients with impaired glucose tolerance.
Materials and Methods

The study subjects consisted of 8 healthy non-obese controls (7 men and 1 woman, aged 27–46 years), 6 patients with insulin dependent diabetes mellitus (IDDM; 6 men, aged 40–55 years), 13 patients with non-insulin dependent diabetes mellitus (NIDDM; 9 men and 4 women, aged 23–75 years), and 9 patients with hyperthyroidism of Graves’ disease (2 men and 7 women, aged 29–68 years). The patients with IDDM had been diabetic for 2–10 years, and those with NIDDM for 0.1–20 years. All 9 patients with newly diagnosed hyperthyroidism were studied before treatment. The procedure for the 75 g OGTT followed the standard of NIH (National Diabetes Data Group 1979). The blood was drawn from the antecubital vein after an overnight fast and collected in pre-chilled tubes containing EDTA-2Na (7.4 mg), heparin (25 USP), and sodium fluorinate (2.5 mg). The tubes were immediately centrifuged at 4°C and plasma samples were collected in Nunc Minisorp® tubes. The plasma SLI was extracted with acetic acid and acetone according to a modified method of Arimura et al. (1978). Four ml of an ice-cooled mixture of 2 N acetic acid and acetone (1:4, vol/vol) was added by drop to 0.5 ml of plasma, and the mixture was centrifuged at 4°C for 15 min at 3000 r.p.m. The supernatant was subsequently transferred to a new Nunc Minisorp® tube and another 4 ml of the mixture was added to the precipitate. The precipitate was sonicated with Sonifier B-12 (Branson Sonic Power Co., Danbury, Conn.). The tube was centrifuged again, and the supernatant was added to the previous tube. The combined supernatants were concentrated to 1–1.5 ml under N₂ gas and were subsequently washed with 3 ml of organic solvent, which consisted of 3 parts ethylacetate and 1 part anhydrous ether. The organic phase was carefully removed, and the aqueous layer was evaporated under N₂ gas. The dried residue in the tubes was kept at −20°C until assay, in which phosphate-buffered saline (PBS) was added to tubes. SLI was measured by a radioimmunoassay as previously de-
Reverse-phase high performance liquid chromatography (HPLC) of somatostatin-28 incubated with normal human plasma. Synthetic somatostatin-28 was incubated with plasma for 0, 5 and 15 min at 37°C. SLI was extracted with acetic acid and acetone as described in the text, and was subsequently subjected to HPLC. For chromatography, isocratic conditions were used with a mobile phase containing 27% acetonitrile in a 0.1 M phosphate buffer, pH 3.0. The flow rate was 1.5 ml/min. Fractions of 1.5 ml were collected and aliquots used for radioimmunoassay. AUFS = Absorbance Unit Full Scale. Hatched bar indicates SLI.

Results

Gel filtration of plasma SLI (Fig. 1)
The elution profiles of plasma SLI following gel filtration of extracted plasma are given in Fig. 1. Somatostatin-28 and -14 extracted with normal plasma were eluted in the tube of the same fraction as the standard somatostatin-14 and -28 used as markers. The extracted SLI from normal human plasma (5 ml) showed a single peak at the position of somatostatin-14.

Synthetic somatostatin-28 was incubated with human plasma of normal subjects for 5 and 15 min at 37°C. Plasma SLI was extracted with acid-acetone and was subjected to reverse phase HPLC using µ-Bondapac C-18. Acetonitrile (27%) in a 0.1 M phosphate buffer, pH 3.0 was used as a mobile phase. Each fraction of 1.5 ml was eva-
Response of plasma SLI to 75 g OGTT in normal subjects (Fig. 3)

Plasma SLI was significantly increased from 29.9 ± 5.4 pg/ml to 93.4 ± 15.5 pg/ml 60 min after administration of 75 g glucose ($P < 0.005$). The level of SLI slowly decreased to the baseline level at 180 min. Plasma IRI and plasma glucose showed peak levels of $74.1 \pm 10.8 \mu$U/ml and $7.34 \pm 0.21$ mmole/l, respectively, at 30 min.

Response of SLI to 75 g OGTT in IDDM and NIDDM (Fig. 4)

The increase of plasma SLI in response to 75 g OGTT was observed at 120 min only in IDDM, and at 60, 90, 120 and 180 min in NIDDM. The peak of somatostatin-28 was gradually decreased after incubation with plasma in vitro (Fig. 2). No increase of the peak at 1600 dalton was observed. Therefore, it is speculated that SLI extracted with acid-acetone mainly represented a molecular weight similar to somatostatin-14 and was not converted from somatostatin-28, which is known to exist in the pancreas and gastro-intestinal tract.

Response of SLI to 75 g OGTT in normal subjects (Fig. 3)

Plasma SLI was significantly increased from 29.9 ± 5.4 pg/ml to 93.4 ± 15.5 pg/ml 60 min after admin-
Response of plasma SLI to 75 g OGTT in hyperthyroidism. Hatched area indicates mean ± SEM of normal subjects.

Discussion

Extraction of human plasma with acetic acid and acetone has been used to omit degradation and non-specific interference, and it gave a consistent and satisfactory recovery rate for clinical use. A significant increase of plasma SLI in response to an oral glucose load was observed in normal subjects. Glucose is known to be a potent stimulant of SLI from studies in animal pancreas and duodenum in vitro (Schauder et al. 1976; Ipp et al. 1977) as well as in vivo (Schusdziarra et al. 1977; Utsumi et al. 1979). The present study confirms the report that glucose administration increases plasma SLI measured in man using a different extraction method (Wass et al. 1980). The contribution of SLI released from the pancreas and gastro-intestinal tract to venous blood has been underestimated because of its rapid degradation in blood. In response to stimuli such as a glucose load, peripheral SLI levels seem to reflect the wholebody dynamics of SLI to some extent. Plasma SLI has not been extensively studied in man. The present studies indicate that oral administration of glucose increased SLI in patients with diabetes and hyperthyroidism. In animal models of diabetes, such as the alloxan-induced diabetic rat (Hara et al. 1979) and dog (Schusdziarra et al. 1977), the release of SLI from the pancreas and gastro-intestinal tract was increased in vitro. In the present studies, the response of plasma SLI to an oral glucose load in both IDDM and NIDDM was not augmented but rather blunted. The reason for these discrepancies remains uncertain, but they may be explained by difference of species and experimental conditions (in vitro vs in vivo). A lower response of SLI to insulin-induced hypoglycaemia has also been reported in NIDDM (Vinik et al. 1981). The pathogenesis of impaired glucose tolerance in hyperthyroidism is a matter of dispute. A rapid increase of glucose absorption (Holdsworth & Besser 1968), antagonistic action of thyroid hormone to insulin (Doar et al. 1968), increased glucose turnover (Perez et al. 1980) have all been proposed. No significant difference between the patients with hyperthyroidism and normal subjects was observed as far as SLI was concerned. The increase of SLI might be secondary to other events produced by glucose, since it appears late after the administration of glucose. However, this increase of SLI seems to be independent of an increase in insulin (Fig. 4). The gastro-intestinal hormones and cate-
cholamines may also influence plasma SLI, but further investigations are awaited.

Recently, a 28-amino acid N-terminally extended somatostatin has been isolated from gut (Pradayrol et al. 1980), hypothalamus (Esch et al. 1980) and pancreas (Benoit et al. 1980). Its biological role remains to be determined, although this peptide has been reported to be more potent on a molar basis than somatostatin-14 in inhibiting growth hormone (Brazeau et al. 1980) and insulin (Mandarino et al. 1981). Neither the existence nor the metabolism of somatostatin-28 has yet been clearly demonstrated. In the present studies, synthetic somatostatin-28 was found to be recovered by 55% and SLI that eluted on Sephadex G-25 corresponded to synthetic somatostatin-28. The elution profile of plasma SLI extracted with acid-acetone showed only one peak co-eluted with synthetic somatostatin-14. Possible reasons why SLI with larger molecular weight was not detected are the small quantity, more rapid degradation of somatostatin-28 than -14, or the conversion of somatostatin-28 to -14. However, the last possibility is unlikely since somatostatin-28 was not converted in vitro, and in addition, direct conversion of 15,000-molecular weight SLI into somatostatin-14 has also been reported (Zingg & Patel 1982).

In conclusion, the extraction of plasma SLI with acetic acid and acetone gave consistent results and partly at least reflects a fluctuation of SLI in response to certain stimuli, such as glucose. It would thus appears to be a useful tool to study the roles of somatostatin in human pathophysiology. In this method, somatostatin-28 was also extracted, but no SLI in relation to somatostatin-28 was observed in human plasma.

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References


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