Neurotensin and Substance P and Dumping Syndrome

SEIKI ITO, YOICHI IWASAKI, TAKESHI MOMOTSU, KATSUMI TAKAI, AKIRA SHIBATA, YOICHI MATSUBARA* and TERUKAZU MUTO*

The First Department of Internal Medicine and *the First Department of Surgery, Niigata University School of Medicine, Niigata 951

ITO, S., IWASAKI, Y., MOMOTSU, T., TAKAI, K., SHIBATA, A., MATSUBARA, Y. and MUTO, T. Neurotensin and Substance P and Dumping Syndrome. Tohoku J. exp. Med., 1981, 135 (1), 11-21 — To investigate the pathophysiological relation between releases of gut hormones and dumping syndrome, plasma radioimmunoassayable neurotensin, substance P, glucagon-like immunoreactivity (GLI), insulin and blood sugar were measured in both gastrectomized patients and control subjects after 50 g oral glucose tolerance tests. Remarkable rises of radioimmunoassayable neurotensin and GLI were found in all gastrectomized patients, but not in control subjects. In contrast, plasma radioimmunoassayable substance P responses were not detected in either gastrectomized patients or control subjects. There were three patients with symptoms of dumping syndrome in the early stage of the test. Plasma radioimmunoassayable neurotensin responses in two out of these three were higher than those in other patients, though the other patient with symptoms had the same degree of neurotensin elevation as patients with no symptoms. In view of the pharmacological effects of neurotensin, it could not be ruled out that a part of the early symptoms of dumping syndrome may result from the remarkably enhanced plasma neurotensin release in some patients, although the enhanced neurotensin responses did not always accompany symptoms of dumping syndrome. —— neurotensin; substance P; pathogenesis of dumping syndrome

Neurotensin and substance P, originally isolated from bovine hypothalamus (Carraway and Leeman 1973) and from equine hypothalamus and intestine (Euler 1936) respectively, have recently been added to a family of gut hormones, since their presence was immunohistochemically demonstrated in the epithelial cells (Orci et al. 1976) and nerve of the gut (Polak 1976) and by far the largest amount of these peptides is found in the gut when their distribution in the body was checked by radioimmunoassay (Carraway and Leeman 1976a; Nilsson and Brodin 1977). In spite of high localization of these peptides in the gut, the physiological role of the peptides in the gut remains still obscure. However, in view of the findings that these peptides have a wide range of pharmacological effects including hypotension, gut contraction and increased vascular permeability (Carraway and Leeman 1975; Hallberg and Pernow 1975), it seems reasonable to postulate that the peptides in
the gut may be secreted after meals and, thus secreted, may play a role in the pathogenesis of gut disease, especially dumping syndrome.

In order to examine this possibility, changes of plasma levels of these two peptides were determined in gastrectomized patients after 50 g oral glucose tolerance tests (OGTT). As it is widely accepted that glucagon-like immunoreactivity (GLI) in the gut is released after oral administration of glucose in gastrectomized patients (Marco et al. 1972; Shima et al. 1976), plasma levels of GLI in the patients were also measured in the present study, in addition to the measurement of plasma neurotensin and substance P levels. Further, to examine which peptides among the three kinds of peptides mentioned above are related to symptoms of dumping syndrome, rises of plasma levels of these peptides in patients with symptoms of dumping syndrome were compared with those in patients with no symptoms and with those in control subjects after 50 g OGTT.

**Materials and Methods**

The present study was performed on 20 patients (16 males and 4 females) aged from 30 to 65 affected by gastric cancer and on 8 outpatients who did not suffer from either gut disease or diabetes mellitus. The out-patients, ranging in age from 40 to 66, were examined as control subjects. The gastric cancer patients had been either totally or subtotally gastrectomized from 2 months to 14 years before. After an overnight fast, 50 g of glucose was administered to patients and to control subjects. Blood samples were obtained by an indwelling catheter before and at 15, 30, 60, 90 and 120 min after oral administration of glucose. The blood was collected either in iced test tubes containing 1 mg of 2Na-EDTA/ml of blood or in iced test tubes containing 1 mg of 2Na-EDTA and 500 units of trasylol/ml of blood. They were then centrifuged at 4°C for 25 min at 3000 rpm. Plasma was stored at -25°C until assay. Plasma collected in the former tubes was used to measure plasma substance P and insulin, and that in the latter to determine plasma neurotensin and GLI.

**Preparation of anti-neurotensin and anti-substance P antisera.** Anti-neurotensin and anti-substance P antisera were raised in rabbits by either injections of synthetic neurotensin-bovine serum albumin (BSA) conjugates or substance P-BSA conjugates, respectively. Conjugation of the peptides with BSA was carried out as follows: 5 mg of either synthetic neurotensin or substance P (Protein Research Foundation, Osaka), 3 mg of BSA and 0.1 ml of 1% glutaraldehyde were dissolved, in that order, into 2 ml of distilled water and allowed to react at room temperature for 2 hr. After the reaction, the entire mixture was subjected to dialysis against distilled water at 4°C overnight. The dialyzate containing either neurotensin-BSA or substance P-BSA conjugate was emulsified with Freund's complete adjuvant and injected into leg muscles of New Zealand white rabbits at an interval of 40 days. Antiserum were collected 10 days after each booster. The antisera used in the present study were collected after 8 boosters.

Anti-glucagon antisera cross-reactive to GLI were prepared in rabbits. Preparation and immunological characterization of the antisera have been reported previously (Ito and Kobayashi 1976).

**Radioimmunoassay for neurotensin and substance P.** To examine the specificity of anti-neurotensin and anti-substance P antisera used in the present study, the cross-reactivity of these antisera to other hormones, including somatostatin, met-enkephalin, leu-enkephalin, bombesin, phyhsalaemin, edeoidin, bradykinin (Protein Research Foundation, Osaka), glucagon (Lilly Co., Ltd.), insulin (Novo Institute), ACTH (Shionogi Pharmaceutical Co., Ltd.) and β-endorphin (Peninsula Laboratory) was studied by radioimmunoassay. Labeled neurotensin and substance P were prepared by the modified Chloramine T method (Shima...
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Fig. 1. Standard curve of neurotensin in the radioimmunoassay system. No interference of other peptide hormones including somatostatin, glucagon, substance P, bradykinin, ACTH, β-endorphin, etc. to the assay system was shown by horizontal bars.

Fig. 2. Standard curve of substance P in the radioimmunoassay system. Antisubstance P antiserum used in the present study did not have any cross-reactivity to other peptide hormones except eledoisin and physalaemin as mentioned in Materials and Methods. It has 15 and 30% cross-reactivities to eledoisin and physalaemin, respectively, as indicated by ×—× (eledoisin) and ○—○ (physalaemin).
et al. 1975). Labeled neurotensin and substance P were purified by using affinity chromatography (Carraway and Leeman 1976b) and QUSO G-25 (Rosselin et al. 1966), respectively. Incubation was performed in 3 ml plastic tubes. The labeled peptides, diluted antisera (anti-neurotensin antisera diluted to 1:125,000 and anti-substance P antisera diluted to 1:250,000) which bind 45 to 60% of labeled peptides, standard peptides or other peptides were diluted up to 0.9 ml by using 0.2 M glycine-NaOH buffer pH 8.4 or by using 0.05 M phosphate buffer pH 7.4, containing 0.025 M $2\text{Na-EDTA}$ 0.25% of BSA, and 0.1% NaN$_3$. The former buffer was used in the neurotensin assay and the latter buffer in the substance P assay. Further in the assay of neurotensin, 500 units of trasylol were added to the incubation buffer. The mixture was incubated at 4°C for 3 days. After the 3 days incubation, dextran-coated charcoal was added to the tubes in order to separate the antisera bound peptides from free peptides. The tubes were centrifuged at 4°C for 20 min, at 3000 rpm. The supernatant and the precipitates were then separated and the latter were counted for 5 min in a deep-well automatic gamma counter (Aroka ARC-251). Figs. 1 and 2 show typical standard curves of neurotensin and substance P and the cross-reactivity of these two kinds of antisera to other peptide hormones mentioned in Materials and Method. Although anti-neurotensin antisera had a negligible cross-reactivity to other gut hormones, anti-substance P antisera possessed 15% and 35% cross-reactivity to physalaemin and edeoidin, respectively. The character of the anti-neurotensin antisera was, further, examined by radioimmunoassay using extracts of rat antrum and ileum, since it had been reported (Carraway and Leeman 1976a) that anti-neurotensin antiserum against the N-terminal portion of amino acid sequence in neurotensin reacts only with the extract of ileum while the antiserum against C-terminal portion of neurotensin reacts with extracts of both ileum and antrum. As the anti-neurotensin antiserum used in the present study reacted only with extracts of ileum, it seems likely that the antiserum was against the N-terminal portion of neurotensin. The least detectable levels of neurotensin and substance P were 30 pg/ml and 25 pg/ml, respectively. All blood samples were measured within assay. Within assay coefficients of neurotensin and substance P were 5% and 7%, respectively.

Radioimmunoassay for GLI and insulin. Plasma GLI levels were measured by radioimmunoassay using anti-glucagon antiserum cross-reactive to GLI (AE-1). The assay buffer and separation technique described by Falloona and Unger (1974) were used in the present assay. Plasma levels of insulin were determined by using Dainabot IRI kit. Blood glucose was measured in whole blood in the Autoanalyzer by Hoffman’s ferricyanide method. All data were analyzed by using the Student $t$ test.

RESULTS

Mean ($\pm$ S.E.) plasma radioimmunoassayable neurotensin (RNT) responses of the 8 control subjects after 50 g OGTT were compared with those of the 20 gastrectomized patients. The results shown in Fig. 3 indicated that plasma RNT responses at 15, 30 and 60 min were significantly higher in gastrectomized patients than in control subjects.

Changes of mean ($\pm$ S.E.) plasma radioimmunoassayable substance P (RSP) levels after the test are shown in Fig. 4. Neither control subjects nor gastrectomized patients had any significant elevation of plasma RSP levels during the test.

Mean ($\pm$ S.E.) plasma GLI and insulin responses of the 8 control subjects were compared with those of the 20 patients. As seen in Fig. 5, it was apparent that plasma GLI response at 15 and 30, 60, 90 and 120 min was stronger in the gastrectomized patients than in control subjects and that plasma insulin response in the patients was higher at 15, 30 and 60 min than in control subjects.

Likewise, blood sugar levels at 15, 30 and 60 min after the test were higher in
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Fig. 3. Mean plasma levels of radioimmunoassayable neurotensin (RNT) in 20 gastrectomized patients (○—○) and 8 control subjects (●—●) after 50 g OGTT. RNT levels at 15, 30 and 60 min in patients were significantly higher than in healthy subjects. *p<0.05, †p<0.02, ‡p<0.01.

Fig. 4. Mean plasma radioimmunoassayable substance P levels in patients (○—○) and control subjects (●—●). RSP levels in the two groups after the test were not significantly different.
the gastrectomized patients than in the control subjects.

There were three patients with symptoms of dumping syndrome in the early stage of the test. The symptoms appeared until 30 min and included nausea, vomiting, cold sweating, diarrhea, slight abdominal pain, etc. The degree of plasma RNT, GLI and insulin responses of the three patients was compared with that of patients with no symptoms. As indicated in Fig. 6, plasma RNT levels were higher in the two patients than in other patients with no symptoms though the remaining one patient had no significant elevation of RNT in comparison with the RNT response in the patients with no symptoms. The degree of plasma GLI response of patients with and without symptoms of dumping syndrome is shown in Fig. 7. Patient K, who had moderately enhanced RNT release, also had a remarkable increase of GLI, though GLI releases in the other two patients with symptoms were not different from those in the patients with no symptoms. Changes of plasma insulin levels in patients with symptoms were almost the same as those in patients with no symptoms. Further, no difference in blood glucose response was noted between the two groups. Although it has been reported that intravenous administration of neurotensin inhibited insulin release (Brown and Vale 1976), as indicated in Fig. 8, an inverse correlation between insulin release and RNT response was not found in the gastrectomized patients.

Fig. 5. Changes of plasma GLI in patients (○—○) and control subjects (●—●). During the test, GLI levels in patients were higher than those in control subjects. *p<0.05, †p<0.02, ‡p<0.01.
DISCUSSION

The present study shows that orally administered glucose causes enhanced releases of both RNT and GLI in all gastrectomized patients, though it has not any significant influences on the release of the two peptides in control subjects. In view of the report that intravenous administration of neurotensin induces hypotension, gut contraction and vascular permeability (Carraway and Leeman 1975), it seems probable that the enhanced RNT release in the gastrectomized patients after 50 g OGTT may play an important role in the pathogenesis of dumping syndrome. In the early stage of the test, only three out of 20 patients complained of nausea, abdominal pain, diarrhea, cold sweating and palpitation, in spite of elevation of plasma RNT in all patients examined. The finding, therefore, suggests that the enhanced RNT response does not always accompany symptoms of dumping syndrome. However, considering that the degree of rises of plasma RNT in two out of three patients with symptoms was remarkably higher than that in the patients with no symptoms, it cannot be ruled out that remarkably enhanced RNT release may be related to a part of the pathogenesis of dumping syndrome in the early stage of the test. Furthermore, as to the pathogenetic relation between

![Graph showing RNT levels after the test in three patients with symptoms of dumping syndrome compared to those in patients with no symptoms. Shadow area represents mean (± S.E.) RNT responses in patients with no symptoms. RNT levels at early stages of the test in two patients (Patients K and M) were higher than those in other patients, though one patient did not have a remarkable elevation of RNT as compared with the RNT in patients with no symptoms.](image-url)
Fig. 7. Changes of plasma GLI responses in three patients with symptoms were compared with those in other patients with no symptoms. Only Patient K, who also had remarkable rises of RNT, possessed dramatic elevation of GLI response in comparison with GLI levels in patients with no symptoms.

Fig. 8. The relationship between insulin responses and neurotensin releases at 15 min in all gastrectomized patients studied. No inverse relation between two peptide hormones was found.
symptoms of the other one patient and the slightly elevated RNT, there remained two possibilities: One is that the biochemical structure of RNT in patients with symptoms may be different from that in patients with no symptoms. The other is that interaction between neurotensin receptors and neurotensin may be stronger in patients with symptoms than in patients with no symptoms. Bloom et al. (1978) recently reported that neurotensin responses in patients with symptoms of dumping syndrome are significantly higher than in those with no symptoms. Thus, they proposed the hypothesis that neurotensin release may play an important role in symptoms of dumping syndrome. Their findings differ from the present result that all patients examined had the elevated neurotensin responses after 50 g OGTT, irrespective of symptoms of dumping syndrome. Although the exact reasons of the discrepancy between the present result and the finding of Bloom et al. still remain obscure, it may be due to different operative procedures. That is, patients in our study were either totally or subtotally gastrectomized for gastric cancer and those in the study of Bloom et al. may be partially gastrectomized for either gastric or duodenal ulcer.

Like RNT elevation in all gastrectomized patients examined, plasma GLI levels in all patients increased after 50 g OGTT. Although the degree of rises of plasma GLI in one out of three patients with symptoms was higher than that in patients with no symptoms, a possible significance of GLI in pathogenesis of dumping syndrome seems to be ignored, since all patients with no symptoms had enhanced GLI responses and the physiological effects of GLI still remains unclear. The present finding that there were rises of plasma GLI in gastrectomized patients after 50 g OGTT agreed with the reports issued by Marco et al. (1972) and Shima et al. (1976).

In contrast to the enhanced releases of the two peptides in gastrectomized patients after 50 g OGTT, rises of the plasma RSP were not found in either gastrectomized patients or control subjects. The finding suggested that substance P was not secreted following orally administered glucose, even in the pathological condition and, therefore, substance P was not related to the pathogenesis of dumping syndrome. However, considering that substance P is inactivated in the liver and substance P secreted from the gut to blood stream may be carried to the liver before entering the peripheral blood, it was not completely ruled out that substance P may play some role in the pathogenesis of dumping syndrome if substance P has local effects. The present findings in the control subjects were identical to report described by Skrabanek et al. (1977) in which plasma substance P levels did not increase after oral administration of glucose.

Although hyperinsulinemia was seen in gastrectomized patients in early stages of the test, hypoglycemia was not detected until 120 min. Therefore, it was concluded that hyperinsulinemia did not influence the pathogenesis of dumping syndrome in the early stage of the test.

It was reported that intravenous administration of neurotensin inhibited insulin release (Brown and Vale 1976). Does the enhanced neurotensin release in
gastrectomized patients inhibit insulin release? To answer this question, correlation between plasma insulin response and RNT release was examined in gastrectomized patients. An inverse relation between plasma insulin and RNT release was, however, not detected. The finding may be accounted for by three possibilities: One is that neurotensin in the gut does not inhibit insulin release in pathological conditions. The second is that B-cells in the pancreas may respond to hyperglycemia stronger than to hyperneurotensinemia. The third is that other gut hormones possessing effects on insulin release may be secreted after 50 g OGTT and the effects of the hormones may be stronger than the inhibitory effect of neurotensin.

References


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