대한생리학회지 : 제19권 제 2 호

# Plasma Secretin Concentrations in Fasting and Postprandial States of Normal Korean Subjects

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=국문초록=

## 정상 한국 성인의 공복시 및 식후 혈장 Secretin 농도

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**심여림 · 조양혁 · 심상수 · 남상채 · 김명석**

한국의 정상 성인 20 명에서 공복시 및 음식물 섭취후의 혈장 secretin 농도를 방사면역 측정법(radioimmunoassay)으로 다음과 같이 측정하였다.

20 명중 12 명은 단백질 음식물인 햄버거와 쌀밥을 일주일 간격으로 섭취하였다. 햄버거 섭취 후 평균 혈장 secretin 농도(12~16 pg/ml)는 공복시의 평균 혈장 secretin 농도(10 pg/ml 이하) 보다 유의하게 증가하였으며 그 증가가 오랫동안 지속하였다. 쌀밥의 섭취 후에는 혈장 secretin 농도(9~13 pg/ml)가 공복시의 값보다 증가하는 경향이었으며, 단지 식후 30분의 값에서만 유의하게 증가하였다. 식후 혈장 secretin 농도의 증가폭은 햄버거에서의 증가 값이 쌀밥에서의 값보다 더 컸다. 나머지 8 명은 자당 용액과 생리 식염수를 마셨다. 자당 용액의 섭취후 평균 혈장 secretin 농도(10~14 pg/ml)는 공복시의 값보다 유의하게 증가하였으나 쌀밥의 값과 마찬가지로 일시적이었다. 그러나 생리 식염수를 섭취한 후에는 혈장 secretin 농도에 이렇다할 변화가 없었다.

이상의 결과로 미루어 정상 한국 성인에서는 단백질 음식물은 물론 탄수화물 음식물의 섭취로도 혈장 secretin 농도가 증가한다고 사료된다.

## INTRODUCTION

The history of pancreas physiology and secretin goes back to the last century. Stimulation of the exocrine pancreas by intraduodenal acetic acid was reported as early as 1825 by Lauret and Lassaigne. In 1902, Bayliss and Starling showed that infusion of hydrochloric acid into a denervated jejunal loop of an anaesthetized dog stimulated secretion of juice from the panceas. The stimulatory factor was named secretin which was the first hormone to be discovered. With this discovery of secretin the entire study of endocrinology began, in a sense, although half a century passed before the implications of gastrointestinal (GI) hormones in health and disease were realized.

In 1961, Jorpes and Mutt successfully purified secretin from porcine small intestine as

This study was supported by the Research Grant from the Ministry of Education (1984 year), and in part by the Catholic Medical Center Research Fund (1985 year).

a 27 amino acid polypeptide and soon determined its amino acid sequence (Mutt et al., 1965). Synthesis of the hormone was achieved by Bodanszky and collaborators(1965).

Although an early attempt at a radioimmunoassay for determination of secretin in the blood was reported (Young et al., 1968), it was only 1973 when a couple of groups started measuring secretin in body fluids with any success(Bloom & Ogawa, 1973; Boden & Chey, 1973). In recent years the sensitivity of the assay has improved to such a level that the very low amounts of secretin present in the systemic circulation can be detected(Fahrenkrug et al., 1976; Tai & Chey, 1978). Plasma immunoreactive secretin concentration was well known to increase in response to duodenal acidification in man(Boden & Chey, 1973; Bloom & Ward, 1975; Chey et al., 1975; Rayford et al., 1976; Fahrenkrug & Schaffalitzky de Muckadell, 1977: Hanssen & Torjesen, 1977) and in dogs (Boden et al., 1974; Lee et al., 1976; Rayford et al., 1976; Chey et al., 1979). On the other hand, it has only recently been observed that a significant increase of secretin level occurs in the circulating blood after ingestion of a meal in man (Chey et al., 1978; Pelletier et al.,1978; Schaffalitzky de Muckadell & Fahrenkrug, 1978; Rominger et al., 1981) and dogs(Kim et al., 1979; Gyr et al., 1984). However, circulating secretin value has not been yet documented by means of sensitive radioimmunoassay in fasting and postprandial states in Korean subjects.

The study therefore that we report here was designed to investigate plasma secretin concentration before and after ingestion of various test meals in normal Korean subjects.

## MATERIALS AND METHODS

**Subjects:** The subjects in which we studied included 20 normal healthy adults(17 males and 3 females) with ages ranging from 20 to 44 years and with a mean age of 26. Twelve subjects(10 males and 2 females) of them ingested a hamburger and a rice meal at an interval of a week to investigate effect of protein meal on plasma secretin concentration. In the remaining 8 subjects a sucrose solution and a physiological saline were ingested at an interval of a week.

**Blood Sampling:** After 15 hours of fasting each subject ingested test meals. One group (N=12) ingested a hamburger meal consisting of 150 g of cooked ground beef, 2 slices of white bread, 180 ml of milk and 50 g vegetables(protein 44.1 g, fat 12 g, carbohydrate 33.8 g), and the rice meal consisting of 250 g of boiled rice, 50 g of vegetables and 180 ml of barley tea(protein 6.8 g, fat 0.5 g, carbohydrate 80.5 g). The other group (N=8) ingested a 300 ml of 10% sucrose solution and a 300 ml of physiological saline. Serial blood samples were obtained for 30 min in the fasting state and 90 min after ingestion of the test meal at 15-min or 30-min intervals. Blood samples were drawn from the forearm through a needle for pediatric scalp vein placed in a vein and were kept in heparinized tubes in ice. Plasma was separated by refrigerated centrifugation(4°C) for 15 min at 1,500 *g*, and Trasylol(FBA Pharmaceuticals, N.Y.) was added to the plasma in a concentration of 500 KIU/ml of plasma. The plasma was kept frozen at −20°C for future assay.

**Radioimmunoassay(RIA) of Plasma Secretin:** Plasma secretin concentration were determined by the radioimmunoassay method

(Chang & Chey, 1980) with light modification. The method was as follows.

Prior to RIA, secretin was purified and concentrated from plasma by ethanol extraction (Schaffalitzky de Muckadell & Fahrenkrug, 1977). The extraction with ethanol was carried out by mixing thoroughly 2 ml of the plamsa with 2 ml absolute ethanol and placing it in an ice bath for 20 min before centrifugation at 1,500 $g$ for 30 min to pellet precipitate. An additional 6 ml of ethanol was then added into the same tube and mixed gently with supernatant without disturbing the pellet, and the mixture is allowed to stay in the ice bath for another 20 min and centrifuged again for 30 min at 1,500 $g$. The final clear supernatant is then decantated into another test tube and dried at 40°C under a stream of air, and stored at −20°C before assay. Extract of hormone-free plasma samples in 2 ml alone or containing known amounts of authentic secretin (10, 15, 25, 50 and 100 pg/ml), and a control plasma sample should be included for construction of standard curve, determination of recorvery factor, intraassay and interassay variations, respectively. Immediately before assay the extract was reconstructed in 1 ml 0.05 M sodium phosphate buffer, pH 7.0 containing 0.5% bovine serum albumin (BSA) and 0.02% $NaN_3$.

Synthetic porcine secretin was iodinated with $^{125}I$ (Amersham, England) by means of a modified chloramine T method of Tai and Chey (1975). Synthetic secretin (5 $\mu$g) was dissolved in 10 $\mu$l of 0.4 M sodium acetate buffer, pH 5.6 and stored in a aliquot at −60°C. To the secretin aliquot (5 $\mu$g/10 $\mu$l), 30 $\mu$l of 0.5 M sodium borate buffer at pH 8.0 and 20 $\mu$l of $Na^{125}I$ (2 mCi) were added and immediately followed with 5 $\mu$l of 5 mg/ml chloramine T (in 0.05 M borate buffer, pH 8.5). After 2 min at room temperature (23° C), the reaction was terminated by adding 20 $\mu$l of sodium metabisulfite (0.5 mg) in the 0.05 M borate buffer, pH 8.0. The final mixture was diluted 1 min later with 100 $\mu$l of 0.01 M sodium phosphate, pH 7.2 containing 0.5 M sucrose, 0.05 M KI, 1.0% BSA, 0.02% $NaN_3$ and 50 $\mu$g/ml of protamine, and applied onto a 10 ml column of a mixture of Sephadex G15/G50, fine (7/3, w/w) packed in a 10 ml disposable pipet and equilibrated in 0.05 M sodium borate, pH 8.5 containing 0.08 M NaCl, 0.5% BSA and 0.02% $NaN_3$. The column was eluted with the same buffer and fractions of 0.3 ml were collected. Radioactivity was monitored by counting 10 $\mu$l for 0.2 min. The $^{125}I$-secretin fractions with counts higher than $2\times10^5$ (per 10 $\mu$l/0.2 min) were pooled and diluted with 5 volumes of 0.02 M sodium phosphate, pH 5.5 containing 0.5% BSA and 0.02% $NaN_3$, and chromatographed on a column of SP-Sephadex C25 (10 ml bed) previously equilibrated with 50 ml of the same phosphate buffer containing 0.02 M NaCl (starting buffer). The column was washed with 50 ml of the starting buffer and then eluted with a gradient of 90 ml each of the starting buffer in the mixing reservoir and the diluting buffer containing 0.2 M NaCl in the other. Fractions of 3.5 ml were collected from the beginning of the sample application. The fractions with peak immunoreactivity were stored frozen at −20°C. Since the tracer is stable for 6 weeks when stored at −20°C, secretin was routinely iodinated every 6 weeks and rechromatography was biweekly carried out.

Rabbit anti-(synthetic) secretin serum (R-1-5) was kindly donated by Prof. Chey (Rochester, N.Y.) (Boden & Chey, 1973).

Hormone-free plasma was prepared by tre-

ating pooled plasma from healthy subjects with dextran-coated charcoal as below. Charcoal (90 mg/ml) was first suspended in a solution of 0.05 M Tris-HCl, pH 7.8 containing 0.1% BSA and 9 mg/ml dextran (M.W. = 79,400) stirred gently for 2 hr at room temperature. For each volume of plasma, 1/2 volume of dextran-coated charcoal was filtered (Whatman No. 42 filter paper) to obtain solid charcoal. The solid charcoal was mixed with pooled plasma and then stirred gently for 2 hr at room temperature. The hormone-free plasma was then separated from the bulk of charcoal by filtering three times through Whatman No. 42 filter paper, followed by filtration through 5 $\mu$m and 0.2 $\mu$m Milipore membranes to remove fine charcoal particles. The hormone-free plasma was dialyzed against saline (five volumes, 6 changes over 48 hr) after adding Trasylol to final concentration of 500 KIU/ml. The hormone-free plasma was kept at −20°C.

Standard secretin was prepared from synthetic secretin at 5, 10, 15, 25, 50, 100, 150, 250, 500 and 1,000 pg/ml in 0.05 M sodium phosphate buffer, pH 7.0 (diluting buffer) containing 0.5% BSA, 0.02% $NaN_3$, 50 $\mu$g/ml protamine-free base and 500 KIU/ml Trasylol, and stored in 0.5 ml aliquots at −60°C.

During assay, the unlabeled secretin (i.e., standards or unknown sample) was incubated first with antibody in a final volume of 1.5 ml consisting of 0.4 ml of reconstituted ethanol extract of hormone-free plasma (for standards), recovery standards or unknown samples; 0.2 ml of standard solution or diluting buffer (for unknowns), 0.9 ml diluted antiserum ($1 : 10^{-6}$), in 0.05 M sodium phosphate buffer, pH 7.0 containing 0.1% BSA, 0.02% $NaN_3$, and 875 KIU/ml Trasylol. After 48 hr at 4°C, 0.2 ml of $^{125}$I-secretin (approximately 5,000 cpm) diluted in the reconstituting buffer is then added and incubated for another 48 hr. All samples were assayed in duplicate including blanks from each individual subjects. Separation of bound and free counts was achieved by adding 0.4 ml dextran- and plasma-coated charcoal suspensions and centrifugation, and both bound (supernatant) and free (pellet) tracers are counted. The charcoal suspension used was prepared by mixing 1 volume of dialyzed hormone-free human plasma, 1 volume of saline and 2 volumes of dextran-coated charcoal suspension (90 mg/ml).

All data were calculated via computer programs loaded to a HP-97 calculator (Hewlett-Packard). All bound to total ratio of tracer counts were corrected for corresponding blank values. A straight-line standard curve was fitted by the method of unweighted least squares to the logit transform function of B/Bo plotted against the logarithm of the concentration of added standard secretin (Campfield, 1983). The logit transform function was defined as follows:

$$\text{logit}(B/Bo) = \log\left(\frac{B/Bo}{1-B/Bo}\right)$$

where B is the bound to total ratio of each sample and Bo is the same ratio obtained in the absence of standard secretin. The regression correlation coefficient of the standard curve was usually greater than 0.99. A correction factor for recovery of secretin from samples was determined in each radioimmunoassay by measuring the recovery of synthetic porcine secretin added to hormone-free plasma at four different concentration, 30, 50, 100 and 200 pg/ml. The average recovery was 59.5±10.6% (M±S.D., n=13) with a range of 45~74%. The intraassay precision was expressed by the coefficient of variation of duplicate determinations and was found to

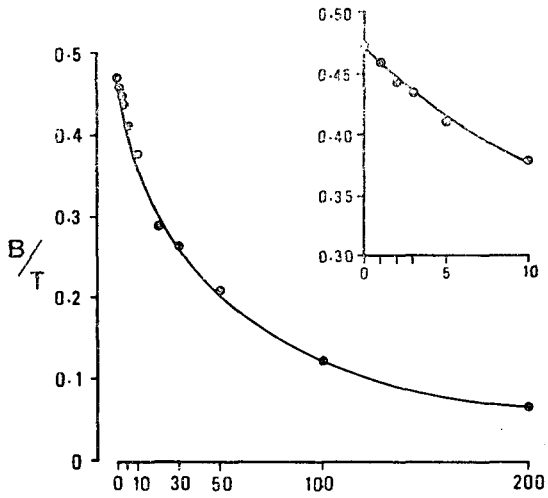


Fig. 1. A standard curve of secretin RIA conducted in ethanol extract of hormone-free plasma. Ratio(B/T) of antibody-bound (B) to total (T) labeled secretin is plotted against the concentration of unlabeled synthetic secretin. Right upper figure shows a expanded part of the standard curve.

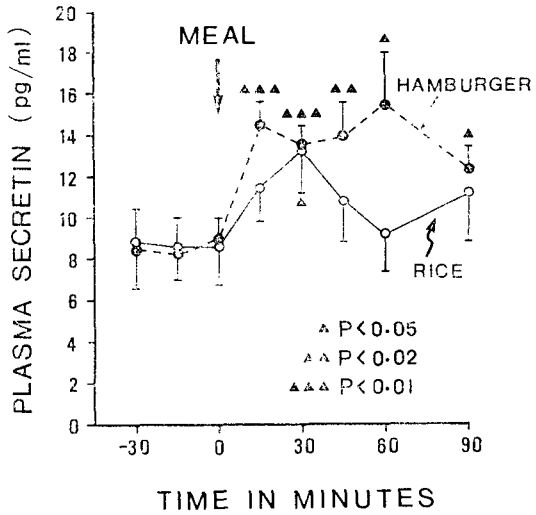


Fig. 2. Mean plasma secretin concentration before and after ingestion of a hamburger meal or a rice meal in 12 healthy subejcts. Vertical graduations represent 1 S.E.
▲: Significantly different from fasting value (t test).

be 3.6% at 30 pg/ml (n=10). The reproducibility, or the interassay precision was calculated as the coefficient of variation of mean of duplicate determinations of a pooled recorvery standard, extracted and assayed on different days. The interassay precision was 14.6% at 30 pg/ml in 7 consecutive assays.

**Analysis of Data**: The results were expressed as a mean±1 S.E. and analyzed statistically by Studient's t test. P values equal to or less than 0.05 were considered statistically significant.

## RESULTS

Standard curves for secretin RIA were conducted in buffer containing ethanol extract of charcoal-treated hormone-free human plasma. A typical standard curve was shown in Fig. 1.

In 20 healthy subjects, fasting plasma samples were obtained at 15-min intervals for 30 min before the subjects were fed. The mean fasting plasma secretin concentrations at each period in subjects were less than 10 pg/ml.

The postprandial plasma secretin concentrations at 15, 30, 45, 60, and 90 min after the ingestion of a hamburger meal were 14.5± 1.3(M±S.E.) 13.4±1.0, 13.9±1.5, 15.5± 2.6, and 12.2±1.1 pg/ml, respectively, in 12 subjects(Fig. 2). These all postprandial secretin concentrations were significantly higher than the mean fasting value, 8.7±0.9 pg/ml ($p<0.05-0.01$). The postprandial plasma secretin concentrations after ingestion of a rice meal tended to increase compared with the mean fasting concentration in the 12 subjects(Fig. 2). The postprandial value, 13.4± 1.8 pg/ml, at 30 min after the rice meal was significantly higher than the fasting value,

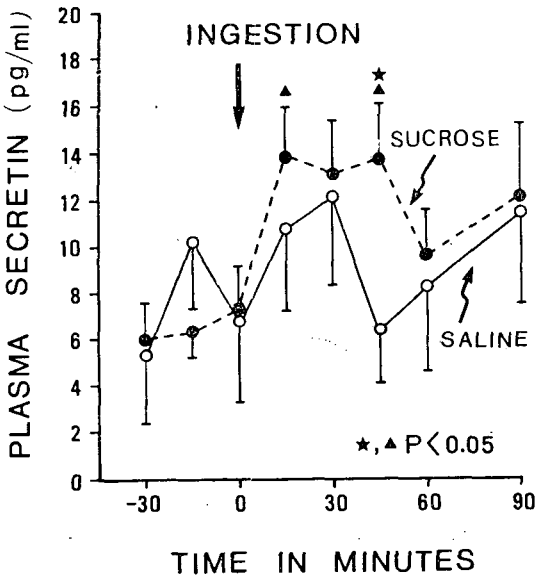


Fig. 3. Mean plasma secretin concentration in response to ingestion of a sucrose solution or a physiological saline in 8 healthy subejcts. Vertical graduations represent 1 S.E.
▲: Significantly different from fasting value (t test).
★: Significantly different from saline value (t test).

8.4±1.6 pg/ml ($p<0.05$). As shown in Fig. 2, the mean postprandial secretin level after the ingestion of the hamburger was greater than the level in response to the rice meal at every time point of sampling. At 60 min after the ingestion of the hamburger, the secretin level was almost significantly higher than the level of the rice meal ($p=0.053$). After 60 min the postprandial secretin level in response to the rice meal returned to the fasting secretin level, Whereas the secretin level did not return to the fasting level until 90 min after the ingestion of the hamburger meal.

Plasma secretin concentrations before and after the ingestion of a sucrose solution or a physiological saline in the remaining 8 subjects were depicted in Fig. 3. The plasma secretin levels increased from 7.5±1.8 pg/ml in the fasting state to 13.8±2.0, 13.1±2.2, 13.8±2.3, 9.6±2.0, and 12.2±3.0 pg/ml at 15, 30, 45, 60, and 90 min ater the ingestion of the sucrose solution, respectively. After 15 min and 45 min the increases in the secretin levels were statistically significant ($p<0.05$). After 60 min the postprandial secretin level almost returned to the fasting level. On the other hand, after the ingestion of the physiological saline the mean postprandial secretin levels were not significantly changed from the fasting levels. The mean postprandial secretin concentration 60 min after the ingestion of the sucrose solution was significantly higher than that of the physiological saline ($p<0.05$).

## DISCUSSION

In the present study, fasting plasma secretin concentrations were generally less than 10 pg/ml in normal Korean subjects. This low value was obtained by the elimination of an interfering substance in plasma and comparable to the fasting plasma secretin levels obtained from the Caucasian race (Fahrenkrug & Schaffalitzky de Muckadell, 1977; Chey et al., 1978; Hanssen et al., 1978; Rominger et al., 1981). The elimination of the interfering substance was achieved by the ethanol extraction of the plasma before secretin RIA procedure (Hanssen & Torjesen, 1977; Schaffalitzky de Muckadell & Fahrenkrug, 1977). It therefore appears from the results of this investigation that there is not any significant difference between the Caucasian race and the Korean race in the release of secretin in the fasting state.

After ingestion of a protein meal, a ham-

Table 1. Comparison of published mean(or M±S.E.) plasma(or serum) secretin concentrations(pg/ml) in fasting state or postprandial state after ingestion of a standard protein meal in various subjects

| Authors | Subjects | Fasting | | Postprandial | |
|---|---|---|---|---|---|
| Lee et al.(1976) | dog | 124~127 | (n=10) | Not changed | |
| Rhodes et al.(1976) | human | 38.6 | (n=9) | Not changed | |
| Fahrenkrug et al.(1977) | pig | 16.8±8.6 | (n=4) | Not changed | |
| Fahrenkrug & Schaffalitzky de Muckadell(1977) | human | 4.0±1.2 | (n=7) | Not changed | |
| Thompson et al.(1978) | dog | 80±12 | (n=7) | 129±13 | ↑ |
| | rat | 205±19 | (n=12) | 265±20(n=12) | ↑ |
| Pelletier et al.(1978) | human | 10.5±1.43 | (n=10) | 13.7±1.82 | |
| Häcki et al.(1978) | human | 7.64±1.04 | (n=9) | 15.6±2.05 | ↑ |
| Chey et al.(1978) | human | 4.4±0.38 | (n=13) | >6 | ↑ |
| Schaffalitzky de Muckadell & Fahrenkrug(1978) | human | 5.5(2.4~9.2) | (n=7) | 11.0(7.9~20.8) | ↑ |
| Kim et al.(1979) | dog | 5.6±0.9 | (n=8) | 55(peak) | ↑ |
| Rominger et al.(1981) | human | 6.7±0.5 | (n=26) | >10 | ↑ |
| Gyr et al.(1984) | dog | 4.0±1.5 | (n=15) | ca. 9~30 | ↑ |

↑ : Significant increase from fasting value

burger, mean plasma secretin concentration significantly increased compared with mean plasma secretin concentration in the fasting state. Similar observations in man were already accomplished by Chey and his colleagues (1978), Häcki et al. (1978) and Schaffalitzky de Muckadell & Fahrenkrug(1978) during the postprandial period after the ingestion of a protein meal(Table 1). As shown in Table 1, the postprandial increases in plasma secretin concentration were also approved in dogs and rats. Meat meal is the staple food of the Caucasian race, while rice meal is that of the Korean race. In spite of this difference of dietary life, it seems that there is not any significant difference between the two races in the endogenous release of secretin during the postprandial period after the ingestion of a protein meal as well as in the fasting state.

In our investigation, plasma secretin concentration also increased during the postprandial period after ingestion of a carbohydrate meal, a rice meal, or a sucrose solution. The magnitude of the increase in the postprandial plasma secretin concentrations was somewhat lesser than that after the hamburger meal. These results indicate that the ingestion of the carbohydrate meal or the sucrose solution might have a facilitatory effect on the endogenous of release secretin in the Korean subjects. However, the effect seems to be weaker than that of the protein meal. Although the increase in the release of endogenous secretin after the ingestion of the rice meal has not been yet reported in the Caucasian race, some observations in response to a glucose solution were reported. Even if the method of secretin RIA was somewhat less sensitive, Young et al. (1968) and Chisholm et al. (1969) each of them observed the increase in serum secretin level in man after an oral ingestion of a glucose solution. While the

secretin levels were reported not to bechanged by an intraduodenal infusion of the glucose in man (Fahrenkrug & Schaffalitzky de Muckadell, 1977) and in pigs(Fahrenkrug et al., 1977) or a sucrose in dogs(Boden et al., 1975). It is likely that differences in the administration route of the test meal resulting from the oral ingestion and the intraduodenal infusion may have produced the discrepancy. The reason why the difference produced the discrepancy was that the oral ingestion added the cephalic phase and the gastric phase to the mechanism of gastric acid secretion in the intraduodenal infusion. Therefore, it is inferred from our results and others that the ingestion of the carbohydrate tends to facilitate the endogenous release of secretin during the postprandial period but its intraduodenal infusion may not contribute to the release.

As described above, we observed in the Korean subjects through this study that ingestion of a protein meal resulted in a significant increase in the plasma secretin concentration, indicative of an increased release of endogenous secretin. The magnitude of increase in the plasma secretin concentrations during the postprandial period was greater after the hamburger meal than after the rice meal or after drinking of the sucrose solution. After ingestion of a meat meal, Malagelada et al. (1977), Chey et al. (1978) and Kim et al. (1979) already observed that pH in the proximal duodenum which remained above 6.0 in the fasting state decreased below 4.5 intermittently, and concluded that more acid was indeed delivered to the duodenum during the postprandial period. It has been well established that the endogenous release of secretin from the duodenum occurs specifically to hydrogen ions delivered to the duodenum (Boden et al., 1974; Chey et al., 1974; Boden et al., 1975; Chey et al., 1975). It therefore could be concluded that the acid delivered to the duodenum was very attributable to the endogenous release of secretin during the postprandial period after the ingestion of the protein meal or the carbohydrate meal. Moreover, it looks like that the endogenous release of secretin after the ingestion of glucose or sucrose may be attributable to the acid delivered to the duodenum rather than the glucose itself in the duodenum.

In our laboratory, the release of gastrin was recently reported to increase during the postprandial period after the ingestion of the rice meal as well as the hamburger meal (Kim et al., 1981 & 1982). These observations suggest that the ingestion of the rice meal as well as the hamburger meal enhance the secretion of gastric acid. However, further experiments are needed because acidity or pH of the duodenal content has not been measured during the postprandial period after the meals in the Korean subjects.

## SUMMARY

This study was conducted to investigate fasting plasma secretin and postprandial secretin concentrations after ingestion of a protein meal or a sucrose solution in 20 healthy Korean subjects.

In 12 subjects, ingestion of a protein meal, hamburger resulted in a significant and sustained increase in the mean plasma secretin concentrations, from mean fasting levels of less than 10 pg/ml to 12~16 pg/ml, and the mean plasma secretin concentrations, 9~13 pg/ml, after a rice meal increased significantly but transiently compared with mean fasting levels. The magnitude of postprandial

increase in the plasma secretin concentration after the hamburger was greater than that of the rice meal. In the remaining 8 subjects, drinking of a sucrose solution resulted also in a significant but transient increase in the mean plasma secretin concentrations, from mean fasting levels of less than 10 pg/ml to 10~14 pg/ml which were significantly greater than that after a physiological saline. Significant increase in the plasma secretin concentration was not observed during the postprandial period after the physiological saline.

It is inferred from the above results that the plasma secretin levels increase significantly after ingestions of a carbohydrate meal as well as a protein meal in the Korean race.

## ACKNOWLEDGEMENTS

The authors greatly appreciate Prof. W.Y. Chey (Director, Isaac Gordon Center for Digestive Diseases and Nutrition and The University of Rochester School of Medicine and Dentistry, Rochester, N.Y., U.S.A.) for the donation of synthetic secretin and antisecretin serum, and his generous advice for the preparation of this manuscript.

## REFERENCES

Bayliss, W. and E.H. Starling: *Mechanisms of pancreatic secretion. J. Physiol. (Lond.), 28: 325-353, 1902.*

Bloom, S.R. and O. Ogawa: *Radioimmunoassay of human peripheral plasma secretin. J. Endocrinol., 58:24-25, 1973.*

Bloom, S.R. and A.S. Ward: *Failure of secretin release in patients with duodenal ulcer. Br. Med. J., 1:126-127, 1975.*

Bodanszky, M., M.A. Ondetti, S.D. Levine, V.L. Narayanan, M. van Saltza, J.T. Sheehan, N.J. Williams and E.F. Sabo: *Synthesis of a heptadecapeptide amide with the hormonal activity of secretin. Chem. Indust., 42:1757-1758, 1966.*

Boden, G. and W.Y. Chey: *Preparation and specificity of antiserum to synthetic secretin and its use in a radioimmunoassay (RIA). Endocrinology, 92:1617-1624, 1973.*

Boden, G.,N. Essa, O.E. Owen, F. Reichle and W. Saraga: *The effect of intraduodenal administration of HCl and glucose on circulating immunoreactive secretin and insulin concentration. J. Clin. Invest., 53:1185-1193, 1974.*

Boden, G., N. Essa and O.E. Owen: *Effects of intraduodenal amino acids, fatty acids, and sugars on secretin concentration. Gastroenterology, 68:722-727, 1975.*

Campfield, L.A.: *Mathematical analysis of competitive protein binding assays. In: Principles of Competitive Protein-Binding Assays (2nd Ed.), edited by W.D. Odell and P. Franchimont. New York: John Wiley & Sons, 1983, p.125-148.*

Chang, T.-M. and W.Y. Chey: *Radioimmunoassay of secretin: A critical review and current status. Dig. Dis. Sci., 25:529-552, 1980.*

Chey, W.Y., A. Oliai and M. Boehm: *Radioimmunoassay (RIA) of secretin II. Studies on correlation between RIA secretin levels and biological investigations. In: Endocrinology of the Gut, edited by W.Y. Chey and F.P. Brooks. Thorofare: Charles B. Slack, Inc., 1974, p. 320-326.*

Chey, W.Y., R.A. Rhodes, K.Y. Lee, H.-H. Tai and J.G. Hendricks: *Radioimmunoassay of secretin: Further studies. In: Gastrointestinal Hormones, edited by J.C. Thompson. Austin: University of Texas Fress, 1975, p.269-281.*

Chey, W.Y., Y.H. Lee, J.G. Hendricks, R.A. Rhodes and H.-H. Tai: *Plasma secretin concentrations in fasting and postprandial state in man. Am. J. Dig. Dis., 23:981-988, 1978.*

Chey, W.Y., M.S. Kim and K.Y. Lee: *Influences of the vagus nerve on release and action of secretin in dog. J. Physiol. (Lond.), 293:435-446, 1979.*

Chisholm, D.J., J.D. Young and L. Lazarus: *The gastrointestinal stimulus to insulin release I. secretin. J. Clin. Invest., 48:1453-1460, 1969.*

Fahrenkrug, J., O.B. Schaffalitzky de Muckadell and J.F. Rehfeld: *Production and evaluation of antibodies for radioimmunoassay of secretin. Scand. J. Clin. Lab. Invest., 36:281-287, 1976.*

Fahrenkrug, J. and O.B. Schaffalitzky de Muckadell: *Plasma secretin concentration in man: effect of intraduodenal glucose, fat, amino acids, ethanol, HCl, or ingestion of a meal. Europ. J. Clin. Invest., 7:201-203, 1977.*

Fahrenkrug, J., O.B. Schaffalitzky de Muckadell and J.J. Holst: *Plasma secretin concentration in anaesthetized pigs after intraduodenal glucose, fat, amino acids, or meals with various pH. Scand. J. Gastroenterol., 12:273-279, 1977.*

Gyr, K., C. Beglinger, M. Fried, U. Grötzinger, L. Kayasseh, G.A. Stalder and J. Girard: *Plasma secretin and pancreatic response to various stimulants including a meal. Am. J. Physiol., 246:G535-G542, 1984.*

Häcki, W.H. G.R. Greenberg and S.R. Bloom: *Role of sercetin in man I. In: Gut Hormones, edited by S.R. Bloom. Edinburgh: Churchill Livingstone, 1978, p.182-192.*

Hanssen, L.E. and P. Torjesen: *Radioimmunoassay of secretin in human plasma. Scand. J. Gastroenterol., 12:481-488, 1977.*

Hanssen, L.E., M. Osens, O. Flaten and J. Myren: *Immunoreactive secretin release and pure pancreatic juice after duodenal infusion of bile in man. In: Gastrointestinal Hormones and Pathology of the Digestive System, edited by M. Grossman, V. Speranza, N. Basso and E. Lozoche, New York: Plenum Press, 1978, p. 221-225.*

Jorpes, J.E. and V. Mutt: *On the biological activity and amino acid composition of secretin. Acta Chem. Scand., 15:1790-1791, 1961.*

Kim, M.S., K.Y. Lee and W.Y. Chey: *Plasma secretin concentrations in fasting and postprandial states in dog. Am. J. Physiol., 236: E539-E544, 1979.*

Kim, M.S., H.J. Park, Y.H. Jo, K.O. Kwon and Y.L. Lee: *Plasma gastrin concentration after a carbohydrate meal and a protein meal in normal human subjects. Kor. J. Physiol. (in Korean), 15:83-89, 1981.*

Kim, M.S., I.S. Chung, H.J. Park, C.C. Kim and H. Choi: *Plasma gastrin concentrations of peptic ulcer patients in fasting and postprandialstates. Kor. J. Gastroenterol. (in Korean), 14:79-89, 1982.*

Lauret, F. and J.L. Lassaigne: *Recherches Physiologiques et Chimiques pour Servir a l'Histoire de la Digestion. Paris: Madame Huzard, 1825. Cited from V. Mutt: Secretin: Isolation, structure, and functions. In: Gastrointestinal Hormones, edited by B. Jerzy Glass, New York: Raven Press, 1980, p.85-126.*

Lee, K.Y., H.-H. Tai and W.Y. Chey: *Plasma secretin and gastrin response to a meat meal and duodenal acidification in dogs. Am. J. Physiol., 230:784-789, 1976.*

Malagelada, J., G.F. Longstreth, T.B. Deering, W.H.J. Summerskill and V.L.W. Go: *Gastric secretion and emptying after ordinary meals in duodenal ulcer. Gastroenterology, 73:989-994, 1977.*

Mutt, V, S. Magnusson, J.E. Jorpes and E. Dahl: *Structure of porcine secretin. Biochemistry, 4: 2358-2362, 1965.*

Pelletier, M.J., J.A.P. Chayvialle and Y. Minaire: *Uneven and transient secretin release after a liquid test meal. Gastroenterology, 75:1124-1132, 1978.*

Rayford, P.L., P.J. Curtis, H.R. Render and J.C. Thompson: *Plasma levels of secretin in man and dogs: Validation of a secretin radioimmunoassay. Surgery, 79:658-665, 1976.*

Rhodes, R.A., H.-H. Tai and W.Y. Chey: *Observations on plasma secretin levels by radioimmunoassay in response to duodenal acidification and to a meat meal in humans. Am. J. Dig.*

*Dis., 21:873-879, 1976.*

Rominger, J.M., W.Y. Chey and T.-M. Chang: *Plasma secretin concentrations and gastric pH in healthy subjects and patients with digestive diseases. Dig. Dis. Sci., 26:591-597, 1981.*

Schaffalitzky de Muckadell, O.B. and J. Fahrenkrug: *Radioimmunoassay of secretin in man. Scand. J. Clin. Lab. Invest., 37:155-162, 1977.*

Schaffalitzky de Muckadell, O.B. and J. Fahrenkrug: *Secretion pattern of secretin in man: regulation by gastric acid. Gut, 19:812-818, 1978.*

Tai, H.-H. and W.Y. Chey: *Rapid extraction of secretin from plasma by XAD-2 resin and its application in the radioimmunoassay of secretin. Anal. Biochem., 87:376-385, 1978.*

Thompson, J.C., O.L. Llanos, A. Schafmayer, R. K. Teichman and P.L. Rayford: *Mechanisms of release and catabolism of secretin. In: Gut Hormones, edited by S.R. Bloom, Edinburgh: Churchill Livingstone, 1978, p.176-181.*

Young, J.D., L. Lazarus, D.J. Chisholm and F.F. V. Atkinson: *Radioimmunoassay of secretin in human serum. J. Nucl. Med., 9:641-642, 1968.*