Secretion pattern of secretin in man: regulation by gastric acid¹

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SUMMARY Median concentration of plasma secretin in the fasting state in 11 achlorhydria patients, 17 normal subjects, eight duodenal ulcer patients, and 11 Zollinger-Ellison patients was 0.3, 1.2, 2.5, and 5.9 pmol $\times$ 1⁻¹, respectively. Aspiration of gastric acid in normal subjects and duodenal ulcer patients was followed by a significant lowering of the plasma secretin concentration. In normal subjects insulin-induced hypoglycaemia resulted in increased secretin levels when gastric acid was allowed to enter the duodenum, whereas no changes were observed when gastric acid was aspirated. Simultaneous measurements of intraduodenal pH and plasma secretin concentration in the fasting state and in response to a meal showed that rapid falls in intraduodenal pH were followed by short-lived increments in plasma secretin concentration. These changes in pH and in secretin levels were diminished after cimetidine. It is concluded that gastric acid in man does trigger release of secretin and that secretin is secreted intermittently both in the fasting state and in response to a meal when boluses of acid enter the duodenum.

In recent years several radioimmunoassays for secretin have been presented. The assays all measure increased concentrations of secretin in human plasma after intraduodenal infusion of various, often unphysiologically large amounts of acid (Boden and Chey, 1973; Bloom, 1975; Byrnes and Marjason, 1976; O'Connor *et al.*, 1976; Rhodes *et al.*, 1976; Hanssen and Torjesen, 1977; Schaffalitzky de Muckadell and Fahrenkrug, 1977). Intraduodenal acidification is for the moment the only well-established stimulus to secretin release (Boden *et al.*, 1975; Fahrenkrug and Schaffalitzky de Muckadell, 1977; Fahrenkrug *et al.*, 1977). It has, however, been questioned whether the requirements for release of secretin are met in human physiology (Wormsley, 1973) and it has been difficult to demonstrate changes in secretin concentrations in plasma during physiological conditions in man (Rhodes *et al.*, 1976; Fahrenkrug and Schaffalitzky de Muckadell, 1977). If secretin has any significance in human physiology the

release of the hormone is expected to be dependent on acid in the duodenum.

The present study was therefore undertaken in order to examine the effect of endogenously released gastric acid on plasma secretin concentration in man.

Methods

Seventeen normal subjects, eight duodenal ulcer patients, 11 unoperated patients with the Zollinger-Ellison syndrome, and 11 patients with achlorhydria volunteered for the study after the purpose, nature, and possible risks had been carefully explained to them.

In normal subjects (median age 30 years, range 24-63 years, six females) none had any history of gastrointestinal or endocrine disease. In duodenal ulcer patients (age 33 years, range 24-66 years, three females) the diagnosis was based on the demonstration of ulcer by endoscopy and radiological examination. In Zollinger-Ellison patients (age 46 years, range 32-72 years, four females) the diagnosis was established on the basis of classical history, hypersecretion of acid, and hypergastrinaemia of extragastric origin. Achlorhydria was demonstrated by the augmented histamine test, the patients (age 57 years, 37-84 years, eight females) all had pernicious anaemia.

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INVESTIGATIVE PROCEDURES

I. EFFECT OF BASAL SECRETION OF ACID ON PLASMA SECRETIN CONCENTRATION

The effect of basal gastric acid secretion was studied in 10 of the normal subjects and all of the patients.

In normal subjects and duodenal ulcer patients a nasogastric tube was introduced after an overnight fast and the position of the tube checked by fluoroscopy. Blood samples were drawn from a peripheral vein 20, 17.5, 15, 12.5, 10, 7.5, 5, 2.5, and 0 minutes before and 10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5, and 30 minutes after the start of gastric acid aspiration. Intermittent machine suction (Egnell, Sweden) was used for the aspiration which lasted for 30 minutes. In six of the normal subjects additional samples were drawn 10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5, and 30 minutes after cessation of gastric aspiration.

In Zollinger-Ellison patients two samples and in achlorhydria patients four samples were drawn with 10 minute intervals after an overnight fast.

II. EFFECT OF STIMULATED GASTRIC ACID SECRETION ON CONCENTRATION OF SECRETIN IN PLASMA

Insulin test

In six normal, fasting subjects hypoglycaemia was induced by the intravenous injection of 0.2 IU insulin (Insulin Leo^(R) Neutral, Nordisk insulin laboratorium, Denmark) per kg body weight. Each subject was studied twice with seven days' interval. In one experiment gastric acid was allowed to enter the duodenum and in the other gastric acid was aspirated and secretion rate of acid measured. Blood samples for determination of plasma glucose and plasma secretin concentration were drawn from a peripheral vein 15 and 0 minutes before and 15, 30, 45, 60, 75, and 90 minutes after injection of insulin.

Meal test

Plasma secretin concentration and intraduodenal pH were measured simultaneously in seven normal subjects, before, during, and after ingestion of a meal consisting of 200 g beefsteak, sauce, vegetables, and a beer. A glass electrode (Radiometer glass electrode—calomel electrode system GK 282 C) (Rune, 1968) was positioned under fluoroscopic control in the upper second part of the duodenum, pH was read on a pH-meter 26 (Radiometer, Copenhagen, Denmark) and continuously recorded. The position of the glass electrode was checked by fluoroscopy before every blood sample was drawn or at least at five minute intervals. The

electrode system was calibrated at pH 2.00 and 7.41 and controlled at pH 4.00 before each experiment and the calibration was checked after the experiment. Blood samples were drawn from a peripheral vein at two to 10 minute intervals.

In four of the subjects an additional experiment was performed: 400 mg (two tablets) cimetidine (Smith, Kline, and French, UK) were given 30 minutes before introducing the glass electrode and another 200 mg were given together with the meal. Two hours after the meal 40 ml 0.1 mol $\times$ 1⁻¹ hydrochloric acid was infused into the duodenum over three minutes through a thin polyethylene tube attached to the pH electrode. Blood samples were drawn 10, five, and 0 minutes before and five and 10 minutes after the start of the infusion.

Gel-permeation-chromatography studies

The antiserum used contains an antibody population reacting with different sequences at the secretin molecule (Fahrenkrug *et al.*, 1976). To ensure that the detected values are due to measurements of intact secretin molecules, gel-permeation-chromatography of plasma samples was performed. Samples of 90 ml human plasma obtained in the fasting state and during intraduodenal acidification and one sample of 50 ml porcine plasma were treated as follows: plasma was extracted with ethanol as previously described (Schaffalitzky de Muckadell and Fahrenkrug, 1977) and dried under vacuum. The dried product was dissolved in 10 ml of 0.5 mol $\times$ 1⁻¹ acetic acid and lyophilised. The sample was then dissolved in 2 ml of elution buffer, mixed with trace amounts of ¹²⁵I-labelled albumin and ²²Na. One millilitre was applied to Sephadex G-50 superfine columns (11 $\times$ 1000 mm) calibrated with pure natural porcine secretin (GIH, Stockholm). The columns were eluted with NH₄HCO₃ solution (0.25 mol $\times$ 1⁻¹) containing 72.5 μ mol of human albumin and 200 000 kIU of aprotinin (Trasylol^(R)) per litre at 4°C. Ten minutes' fraction was collected and assayed.

Laboratory analyses

Plasma secretin was measured after extraction with ethanol as previously described (Schaffalitzky de Muckadell and Fahrenkrug, 1977) except that sample volume was increased to 800 μ l in order to increase the sensitivity of the assay. Antiserum 5595-3 (Fahrenkrug *et al.*, 1976) was used and hormone free plasma (plasma blanks) was prepared from every subject (in the meal test both fasting and postprandial plasma were used). All samples were assayed in triplicate. The detection limit of the assays used, calculated from the slope of standard curves and precision in the determination of response

metameter at zero hormone concentration (Ekins and Newman, 1970), was 0.5 pmol per litre sample. With correction for extraction recovery, the lowest plasma secretin concentration to be distinguished from zero with 95% confidence by means of a triple determination was 0.6 pmol per litre plasma. The theoretically attainable sensitivity of the assay (Ekins and Newman, 1970) is 0.1 pmol per litre plasma. The extraction recovery of plasma samples obtained from different individuals did not differ significantly. Analytical precision of the assays used was checked as follows: 30 of the blood samples were divided into two and the secretin concentration determined in each portion.

Between assay variation (15 assays) calculated from 15 duplicate samples was $0.6 \text{ pmol} \times 1^{-1}$ (SD) at a level of $4.8 \text{ pmol} \times 1^{-1}$ and within assay variation (15 duplicates) was $0.3 \text{ pmol} \times 1^{-1}$ (SD) at a level of $1.8 \text{ pmol} \times 1^{-1}$.

Plasma glucose was measured by a glucose-oxidase method (Trinder, 1969). Aspirated gastric acid was titrated with $0.1 \text{ mol} \times 1^{-1}$ NaOH (pH meter PHM 26, Titrator 11, Autoburette ABU 12, Radiometer, Copenhagen, Denmark) to pH 3.5.

Calculations and statistical analyses

With an operational detection limit (defined as above) of $0.6 \text{ pmol} \times 1^{-1}$ the assay does not allow a differentiation of values less than $0.6 \text{ pmol} \times 1^{-1}$ apart. However, the slope of standard curves and the precision in determination of response metameter is constant within the range of concentrations from zero to $5 \text{ pmol} \times 1^{-1}$, and for that reason a figure between $0.1\text{--}0.6 \text{ pmol} \times 1^{-1}$ is estimated with the same confidence as a figure above $0.6 \text{ pmol} \times 1^{-1}$ (but below $5 \text{ pmol} \times 1^{-1}$). The secretin concentrations read from the standard curve were therefore used for calculations whether the figure was below operational detection limit or not, as the read value is a better estimate of the true concentration than statements like 'zero, equal to detection limit, or unmeasurable.'

Measured values—that is, the difference between sample and blank—were positive in all normal subjects, duodenal ulcer patients, and Zollinger-Ellison patients. In achlorhydria patients occasional negative values (within the range expected from estimate of detection limit) were found. Negative values were included in the calculations of individual medians.

In the study of fasting levels in achlorhydria patients, normal subjects, duodenal ulcer patients, and Zollinger-Ellison patients individual median secretin concentration was calculated on the basis of four, nine, nine, and two triplicate determinations respectively. Individual median secretin concentra-

tions following gastric aspiration in normal subjects and duodenal ulcer patients were also based on nine triplicate determinations.

Statistical analyses were performed by the Mann Whitney U-test, Wilcoxon matched-pairs signed-ranks test, and the Friedman two-way analysis of variance (Siegel, 1956). Results are given, if nothing else is indicated, as medians followed by total ranges in parentheses. The level of statistical significance was 0.05.

Results

FASTING LEVELS OF SECRETIN

In the 10 normal subjects, and eight duodenal ulcer patients, aspiration of gastric acid was followed by a significant lowering of the concentration of secretin in plasma (Fig. 1).

Fig. 1 Median concentration and total ranges (hatched area) of secretin in plasma of 10 normal subjects (upper panel) and eight duodenal ulcer patients (lower panel) before and during aspiration of gastric acid (horizontal bar).

In the group of normal subjects individual median secretin concentration before aspiration ranged from 0.9 to $2.8 \text{ pmol} \times 1^{-1}$ with a median of $1.3 \text{ pmol} \times 1^{-1}$; median intra-individual range was $1.6 (1.1\text{--}3.2) \text{ pmol} \times 1^{-1}$. Compared to normal subjects duodenal ulcer patients had significantly

higher fasting levels of secretin; individual median secretin concentration ranged from 2.0 to 4.8 pmol $\times$ 1⁻¹ with a median of 2.5 pmol $\times$ 1⁻¹; median intra-individual range was 3.8 (1.8-6.0) pmol $\times$ 1⁻¹.

Median post-aspiration levels in the group of normal subjects and duodenal ulcer patients did not differ significantly, the values being 0.5 and 0.8 pmol $\times$ 1⁻¹, respectively. After gastric aspiration individual median values ranged from 0.2 to 1.4 pmol $\times$ 1⁻¹ both in normal subjects and in duodenal ulcer patients. In this period median intra-individual ranges were 1.4 (0.9-2.9) and 1.6 (0.8-2.2) pmol $\times$ 1⁻¹, respectively.

In the group of six subjects in whom additional blood samples were drawn after cessation of gastric aspiration median concentration of secretin decreased significantly from 1.2 (0.9-2.0) pmol $\times$ 1⁻¹ to 0.5 (0.2-1.4) pmol $\times$ 1⁻¹ after aspiration and increased significantly to 1.9 (1.0-2.8) pmol $\times$ 1⁻¹ after aspiration ended.

In achlorhydria patients median concentration of secretin was 0.3 (0.0-1.6) pmol $\times$ 1⁻¹; this value does not differ significantly from zero or post-aspiration secretin levels in normal subjects and duodenal ulcer patients, but is significantly lower than median secretin level found in normal fasting subjects before aspiration of gastric acid. In Zollinger-Ellison patients median concentration of secretin in plasma was 5.9 (2.7-10.4) pmol $\times$ 1⁻¹, which is significantly higher than the median level found in duodenal ulcer patients.

Basal secretion rate of gastric acid in normal subjects, duodenal ulcer patients, and Zollinger-Ellison patients was 3.0 (0.6-11.4), 5.5 (1.6-14.2), and 32.4 (20.2-120.0) mmol $\times$ h⁻¹ respectively. In this respect the difference between normal subjects and duodenal ulcer patients was not statistically significant, whereas basal acid output was significantly higher in Zollinger-Ellison patients than in duodenal ulcer patients.

INSULIN-INDUCED HYPOGLYCAEMIA (Fig. 2)

Basal glucose concentration was 4.6 (4.4-5.4) mmol $\times$ 1⁻¹, and nadir concentration, 1.5 (1.2-1.7) mmol $\times$ 1⁻¹, was reached 30 minutes after insulin injection. The concentration of glucose in plasma was not affected by gastric aspiration.

Secretion rate of gastric acid was 3.4 (2.0-11.8) mmol $\times$ h⁻¹ in the basal state and reached a maximum of 32.2 (25.6-39.2) mmol $\times$ h⁻¹ after 60-75 min.

The concentration of secretin in plasma increased significantly when acid was allowed to enter the duodenum. The fasting levels ranged from 0.2 to 2.2 pmol $\times$ 1⁻¹ with a median of 1.4 pmol $\times$ 1⁻¹. A maximum of 8.6 pmol $\times$ 1⁻¹ (6.6-15.8) was

Fig. 2 Median concentration of glucose and secretin in plasma and median gastric acid output of six normal subjects before and during hypoglycaemia induced by intravenous injection of 0.2 IU insulin per kg body weight. With (○—○) and without (●—●) aspiration of gastric acid. Abscissa: minutes after injection of insulin. Hatched area: total ranges.

reached 75 minutes after injection of insulin.

No significant changes in secretin levels occurred after stimulation with insulin when gastric acid was aspirated.

MEAL TEST (Figs. 3 and 4)

In every subject rapid falls in duodenal pH were followed by increments in plasma secretin concentration both in the fasting state and after the meal. Peak concentrations of secretin were observed two to five minutes after the fall in pH. Thus secretin was released intermittently at various intervals. In the fasting state individual median concentration of secretin ranged from 0.5 to 2.0 pmol $\times$ 1⁻¹ with a median of 1.2 pmol $\times$ 1⁻¹ and the pH spikes occurred with 15 (three to 45) minute intervals. Postprandially the pH-spikes occurred with eight (two to 25) minute intervals and secretin concentrations increased significantly; individual median concentration of secretin in plasma ranged from 0.8 to 3.0 pmol $\times$ 1⁻¹ with a median of 1.8 pmol $\times$ 1⁻¹. Postprandial individual maximum values ranged from 2.6 to 6.8 pmol $\times$ 1⁻¹ with a median of 3.6 pmol $\times$ 1⁻¹. Cimetidine suppressed the changes in intraduodenal pH as well as in plasma secretin levels (Fig. 4).

Fig. 3 Individual concentration of secretin in plasma and intraduodenal pH of three normal subjects before, during, and after a meal (horizontal bar).

Integrated secretin concentration—that is, the area below the curve of secretin concentration—divided by the observation time was 0.9 (0.7–2.0) pmol × l⁻¹ in the fasting state and increased significantly to 1.7 (0.9–3.1) pmol × l⁻¹ after the meal. Cimetidine significantly reduced the integrated concentration of secretin both in the fasting state and postprandially to 0.7 (0.5–1.0) pmol × l⁻¹ and 0.7 (0.6–1.5) pmol × l⁻¹, respectively. The secretin response to duodenal acidification by exogenous acid after cimetidine, however, was similar to earlier results without cimetidine, the concentration of secretin being 18.3 (11.8–20.2) pmol × l⁻¹ five minutes after infusion of acid (normal range 4.5–23.6 pmol/l (Schaffalitzky de Muckadell and Fahrenkrug, 1978)). The pH electrode was stable throughout the experiment, the difference between first and last pH control measurement being 0.0 (–0.1 to 0.1).

Fig. 4 Individual concentration of secretin in plasma and intraduodenal pH of four normal subjects before, during, and after a meal (left panel). An additional experiment (right panel) was performed in the same subjects after cimetidine.

GEL-PERMEATION-CHROMATOGRAPHY STUDIES

These studies (Fig. 5) demonstrated that the used antiserum only detects one component of secretin in human and porcine plasma and that the elution position is identical with that of pure natural porcine secretin (used as standards in the secretin assay).

Discussion

The present study has shown that the plasma secretin concentration in man is regulated by endogenous acid and that secretin under physiological conditions is secreted in minute short-lived

Fig. 5 Elution diagram of secretin immunoreactivity from plasma samples. Results from gel-permeation-chromatography on a 11×1000 mm Sephadex G-50 superfine column calibrated with ^{125}I -labelled albumin, pure natural porcine secretin, and ^{22}Na . Upper panel: human fasting plasma (●) and human plasma obtained during intraduodenal acidification (○). The concentration of secretin in the plasma samples were 3.1 and 11.1 $\text{pmol} \times \text{l}^{-1}$ respectively. Lower panel: porcine plasma (secretin concentration 20.7 $\text{pmol} \times \text{l}^{-1}$) obtained during intraduodenal acidification. V_0 , Void volume; $V_{e(s)}$, elution position of pure natural porcine secretin; V_t , total mobile phase.

spikes. The influence of gastric acid on the fasting secretin concentration is clearly demonstrated by comparing the secretin levels in the four groups of acid secretors. The influence of gastric juice is further substantiated by the decline in secretin levels after gastric aspiration in normal subjects and duodenal ulcer patients. In these studies the concentration of secretin in plasma did not reach zero, probably because of incomplete aspiration of acid, since the median secretin concentration in pernicious anaemia patients was indistinguishable from zero.

In previous reports (Bloom and Ward, 1975; Isenberg *et al.*, 1977; Schaffalitzky de Muckadell and Fahrenkrug, 1977) secretin levels in duodenal ulcer patients were lower than or equal to the values found in normal controls, whereas higher levels were found in the present study. This discrepancy is most probably explained by the more precise estimate of basal levels in the present study, as the values are based on nine samples drawn with short intervals from each person and the present assay is perhaps more sensitive than other secretin assays.

The maximum median concentration of secretin found after insulin-induced hypoglycaemia was

8.6 $\text{pmol} \times \text{l}^{-1}$. This figure is in fair accord with those observed in normal human subjects after intraduodenal instillation of $23\text{--}45$ mmol hydrochloric acid per hour (Isenberg *et al.*, 1977). In our study the median peak secretion of acid was 32.2 $\text{mmol} \times \text{h}^{-1}$ when gastric acid was aspirated, this rate of secretion, however, is probably higher than the secretion during the experiments in which gastric content was allowed to enter the duodenum, as the presence of acid in the duodenum is known to inhibit gastric secretion (Johnston and Duthie, 1964). Thus the determination of acid load is inaccurate and the results not directly comparable.

The unchanged secretin levels found after insulin-induced hypoglycaemia with aspiration of acid suggest that vagal activity by itself has no effect on the release of secretin.

The results of the meal test demonstrate that secretin both in the fasting state and during the meal is released intermittently when boluses of acid enter the duodenum. The timing of secretin release varies from individual to individual and for that reason intra-individual changes instead of median or mean changes were studied. The intermittent release of secretin explains the considerable fluctuations that were observed in the study of fasting levels (Fig. 1) in which intra-individual variation in secretin concentration during the 20 minute basal period were several times greater than analytical variation. The changing fasting levels and different timing in secretin release might in part explain the previous difficulties in demonstrating an effect of a meal on *mean* plasma secretin concentration at fixed time points in *groups* of normal subjects (Rhodes *et al.*, 1976; Fahrenkrug and Schaffalitzky de Muckadell, 1977).

Another explanation is that the sensitivity of most assays has been inadequate to detect the small rises in secretin concentration.

The short-lived changes in intraduodenal pH merely indicate the time at which acid enters into that part of the duodenum and is not necessarily correlated to acid load and the length of intestine acidified—that is, the intensity of stimulation. Furthermore, it is unlikely that the pH value measured is identical with the pH at the surface of the stimulated secretin cells. Thus it is not possible in these studies to determine the pH-threshold for release of secretin.

It was not possible to maintain the position of glass-electrode in the first part of the duodenum during a meal and an acidification of the most proximal part of the duodenum without a concomitant detectable fall in pH in the second part can therefore not be excluded. However, the results (Figs. 3 and 4) indicate that, if only the duodenal

bulb had been exposed to acid, secretin response would be minute if detectable, as all rises were preceded by a fall in intraduodenal pH.

The increments in secretin concentration during the spikes were small and consequently the question arises whether these changes are sufficient to stimulate pancreatic secretion of bicarbonate. A constant infusion of natural porcine (GIH) secretin in a dose which increases the plasma secretin level from 0.6 to $2.3 \text{ pmol} \times \text{l}^{-1}$ has been shown to increase bicarbonate secretion into the duodenum from 0.41 to $3.68 \text{ mmol} \times \text{h}^{-1}$ (Schaffalitzky de Muckadell *et al.*, 1978). Still, the effectiveness of intermittently released secretin on pancreatic bicarbonate secretion mimicked by intravenous injections of small boluses of exogenous secretin needs to be investigated.

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