

Human Secretin

Biologic Effects and Plasma Kinetics in Humans

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The action of synthetic human secretin, which differs in two amino acid residues from porcine secretin, was compared with synthetic porcine secretin in 6 healthy volunteers. Pancreatic secretion was assessed by a marker perfusion technique and plasma secretin concentrations were assessed by a specific radioimmunoassay. Increasing doses of either human or porcine secretin produced increasing bicarbonate output ($p < 0.01$), whereas trypsin and lipase were not stimulated over basal. The highest doses of secretin induced a significant increase in pancreatic amylase secretion. The two secretin preparations were found to be equipotent with respect to pancreatic secretion and plasma kinetics. Significant increases of plasma secretin were observed after a steak meal in 15 volunteers ($p < 0.001$). When human secretin was infused at postprandial concentrations, significant increases in pancreatic bicarbonate output were observed ($p < 0.05$). We conclude (a) that the substitution of two amino acids in human secretin does not affect biologic activity and plasma metabolism of the compound; (b) secretin does not stimulate pancreatic enzyme secretion at physiologic concentrations; and (c) the stimulatory effects of secretin on pancreatic amylase remain to be elucidated. The study suggests that human secretin is a true hormone.

The amino acid sequence of porcine secretin was first described by Mutt and Jorpes in 1966 (1). Subsequent synthesis was achieved in the same year by Bodanszky and coworkers (2). Some 15 yr later secretin was isolated from two other species, cow and chicken (3,4). Only recently, however, has the group of Viktor Mutt succeeded in isolating and sequencing the primary structure of human secretin (4). Human secretin is composed of 27 amino acid residues, but differs from porcine secretin at posi-

tions 15 and 16. These positions are Asp and Ser in pig, and Glu and Gly in human secretin.

The biologic effects of human secretin have not been characterized so far. Therefore, the present study was designed to compare the biologic activity and the plasma kinetics of synthetic human secretin with that of synthetic porcine secretin in healthy volunteers. Furthermore, an attempt is made to reevaluate the effect of secretin on pancreatic enzyme secretion, an issue that has remained controversial (5).

Materials and Methods

Peptides

Synthetic human secretin was purchased from Peninsula Laboratories (Belmont, Calif.). The peptide was dissolved in 0.9% saline containing 0.5% human serum albumin and prepared under aseptic conditions by the University of Basel Hospital Pharmacy. Vials were stored in ampoules of 40 μ g secretin at -20°C . Synthetic porcine secretin (Sekretolin) was purchased from Hoechst Pharma (Zurich, Switzerland).

Subjects

Six healthy male volunteers (mean age 24 ± 1 yr, mean weight 68 ± 2 kg) with no history of gastrointestinal disorders and no medication were studied. All subjects gave written informed consent to the studies undertaken. The studies were approved by the local Ethical Human Research Committee.

Experimental Procedure

Each subject was studied on different days and in random order.

Rapid intravenous infusion. After an overnight fast, 6 subjects received 125- μ g/kg doses of either synthetic human or synthetic porcine secretin, each peptide deliv-

ered over a 15-s period. Blood samples were taken at intervals of 1-15 min for plasma secretin radioimmunoassay.

Exocrine pancreatic secretion studies. After an overnight fast, gastric and duodenal secretions were collected as described previously (6). In brief, a multilumen tube was positioned under fluoroscopic guidance with the tip lying at the ligament of Treitz. Gastric and duodenal secretions were collected separately and continuously in 15-min aliquots. Polyethylene glycol (PEG) 4000 was perfused into the duodenum (2 g/L at a rate of 2 ml/min) as a nonabsorbable marker to correct for intestinal volume losses. After basal secretions had been collected for 45 min, exocrine pancreatic secretion was stimulated in each volunteer with graded consecutive doses of human or porcine secretin, respectively (15.5, 62.5, 250 ng/kg · h equaling 5, 20, 82 pmol/kg · h). Each dose was given for 45 min and only one peptide was studied on an experimental day. The peptides were dissolved in 0.9% NaCl containing 0.1% human serum albumin (wt/vol). The doses were chosen to produce threshold to near maximal pancreatic secretion (6).

Two blood samples were obtained at baseline and two further samples during the last 15 min of each dose of secretin administration for plasma secretin measurements. Blood was collected in ice-chilled ethylenediaminetetraacetic acid tubes containing 5000 KIU aprotinin per 5 ml blood. Samples were immediately centrifuged at 4°C and the plasma was stored at -20°C until assayed.

Secretory volumes were measured to the nearest milliliter; bicarbonate concentration of the duodenal juice was measured by the backtitration method (7), trypsin by the method of Wiggins (8), amylase according to Rick and Stegbauer (9), lipase by the method of Grenner et al. (10) using a commercial kit (a gift of Behring Werke, Zurich, Switzerland), and PEG turbidimetrically (11). Isoamylase activities were also measured in the duodenal aspirates using an inhibitor assay (Boehringer, Mannheim, F.R.G.), which uses a wheat protein to distinguish pancreatic from salivary isoamylase (12). Polyethylene glycol served as a volume marker to calculate the duodenal fluid secretion for a given period by the following equation:

$$V = [F \times (PEG)_{\text{perf}}] / [PEG]_{\text{meas}}$$

where V is the calculated duodenal volume (ml/15 min), F is the flow of the PEG solution perfused (30 ml/15 min), $(PEG)_{\text{perf}}$ is the concentration of the perfusate (2 g/L), and $(PEG)_{\text{meas}}$ corresponds to the concentration of PEG in the duodenal juice collected during 15 min. The recovery of the duodenal marker averaged $77\% \pm 3\%$ (mean \pm SEM), whereas the average percentage of PEG recovered from the stomach was $<1\%$. The last two 15-min periods during basal secretion and during each dose of secretin were used to calculate outputs per minute in each volunteer.

Secretin Radioimmunoassay

Secretin was measured as previously described (6) and all measurements were done with antibody 20. This antibody has been characterized previously (13) and was shown in the present study to react equally with human

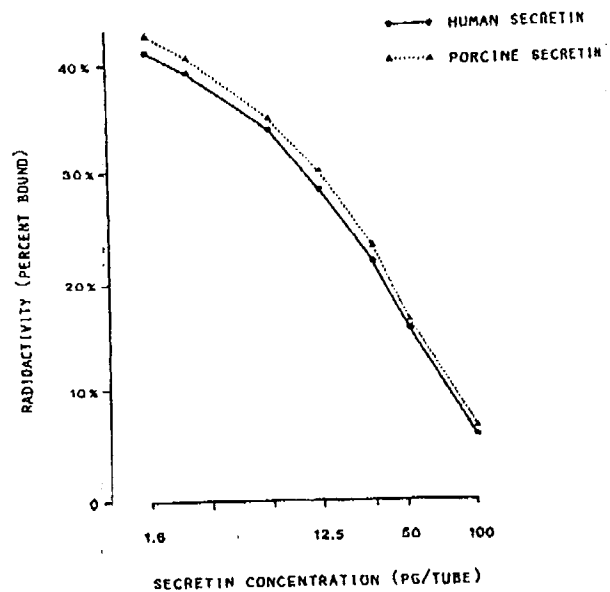


Figure 1. Inhibition curves of synthetic porcine and synthetic human secretin against binding of ^{125}I -labeled synthetic porcine secretin by antibody 20. Ratio of bound to total label is plotted against peptide concentration (pM in assay tube).

and porcine secretin (Figure 1). The sensitivity of the assay was 0.8 ± 0.02 pmol/L plasma. The intraassay variation was $<10\%$ at 3.3 pmol/L. All samples were assayed in the same run.

Calculations and Statistics

Values from the three 15-min periods during basal collection and during each dose of stimulant were averaged and used to calculate output per 15 min for bicarbonate (millimoles), trypsin (KIU), amylase (KIU), isoamylase (KIU), or lipase (milligrams). Basal secretory values were subtracted to give incremental values. Dose-response curves of the two peptides were compared by calculating the slopes obtained by regression analysis in each subject. Differences between slopes were tested by Student's t -test for paired data according to Elashoff (14). The relative molar potencies of both secretin preparations were compared by computing the ratio of the doses required to produce the same response. A 3-by-3 parallel line assay was used (15) and statistical differences between potencies were tested by one-way analysis of variance.

Results

Pancreatic Secretory Responses

Pancreatic secretory fluid and bicarbonate output in response to graded doses of either synthetic human or synthetic porcine secretin infusions are depicted in Figure 2. Both peptides produced a

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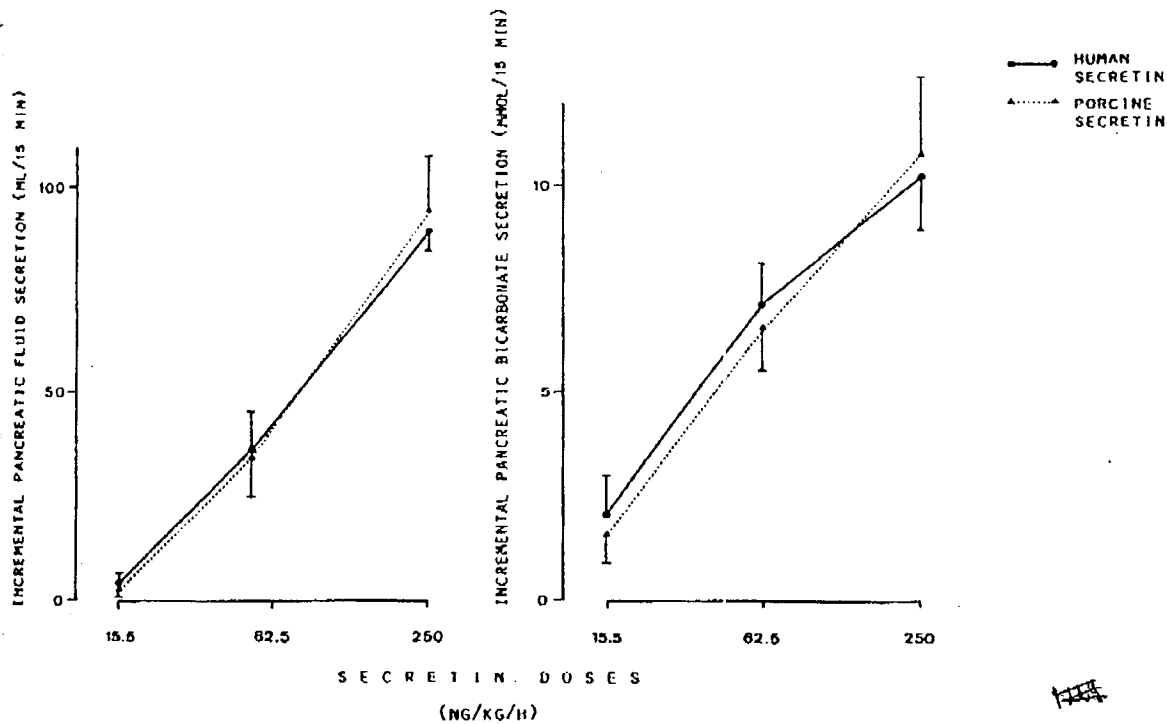


Figure 2. Pancreatic fluid (left) and bicarbonate (right) secretion in response to intravenous infusions of graded doses of either synthetic porcine or synthetic human secretin in 6 healthy volunteers. Fluid secretion is depicted as milliliters per 15 min and bicarbonate output as millimoles per 15 min. Data are shown as mean \pm SEM.

dose-dependent increase in fluid and bicarbonate secretion. The dose-response curves of the two secretin preparations were parallel and, in addition, nearly identical (Figure 2). A significant increase in fluid and bicarbonate output above fasting secretory rates was already elicited with the lowest doses used (15.5 ng/kg · h) ($p < 0.05$). The concentration of bicarbonate increased threefold with increasing doses of both secretin preparations, which paralleled increases in fluid output. The highest doses of secretin did not produce maximal pancreatic bicarbonate secretion for either peptide.

The molar potencies of the two peptides with respect to pancreatic fluid and bicarbonate secretion were then compared. The potency of synthetic porcine secretin was taken as the reference and assigned a potency of 1.0; the relative molar potencies of synthetic human secretin with respect to pancreatic fluid and bicarbonate secretion were 1.04 (0.82–1.49) and 1.02 (0.84–1.40), respectively (95% confidence intervals are given in parentheses). The data demonstrate that equimolar amounts of synthetic porcine and synthetic human secretin produced responses that were almost identical. The characteristics of the

responses to human secretin were in all respects (volume, bicarbonate, amylase, trypsin, and lipase outputs) similar to those with porcine secretin and no statistically significant differences were found.

Figure 3 shows the pancreatic enzyme responses to graded doses of either porcine or human secretin. Infusion of step doses of either secretin produced small increases in trypsin and lipase outputs that were not statistically different from basal values. However, both secretin preparations induced a significant increase of amylase secretion, but this increase was only observed at the highest doses of secretin (250 ng/kg · h). The mean maximal amylase secretion observed during the highest dose of secretin was 35% for human secretin and 38% for porcine secretin, compared with a maximal dose of caerulein (25 ng/kg · h) combined with secretin (50 ng/kg · h). Salivary isoamylase was detected only in one test: in two 15-min aliquots we measured isoamylase concentrations that amounted to 15% and 29%, respectively, of total amylase output in these periods. The contribution of salivary isoamylase to total amylase output was therefore insignificant, suggesting that the increase in amylase output in response to the

highest dose of secretin was due to increased pancreatic amylase secretion.

Plasma Secretin Concentrations

Fasting plasma secretin concentrations were comparable in the different experiments (2.4 ± 0.8 pmol/L for human secretin and 2.7 ± 0.6 pmol/L for porcine secretin). Exogenous porcine or human secretin produced dose-dependent increases in plasma secretin concentrations (Table 1). The correlations between secretin doses infused and plasma secretin levels were as follows: $y = 1.71x - 54.5$ ($r = 0.89$, $p < 0.001$) for human secretin and $y = 1.26x - 36.7$ ($r = 0.91$, $p < 0.001$) for porcine secretin. No statistical difference could be detected between

Table 1. Incremental Plasma Secretin Concentrations in Response to Graded Doses of Either Synthetic Porcine or Synthetic Human Secretin in 6 Healthy Volunteers

Dose of secretin (ng/kg · h)	Increment in plasma secretin levels	
	Human secretin (pM)	Porcine secretin (pM)
15.5	1.7 ± 0.4	2.0 ± 0.9
62.5	15.1 ± 0.8	17.7 ± 2.7
250	330.2 ± 41.0	284.0 ± 14.1

For each volunteer, the incremental value (the increase over basal) represents the average of two plasma samples taken at the end of each dose.

the two curves. The data were used to calculate metabolic clearance rates of both secretin preparations. The metabolic clearance rates were determined as follows:

$$\text{MCR} = \frac{\text{Secretin infused per minute}}{\text{Mean plasma secretin}}$$

where MCR is the metabolic clearance rate and mean plasma secretin is the mean value of the two samples taken at 120 and 135 min. The mean (\pm SEM) metabolic clearance rate of human secretin amounted to 13.5 ± 1.7 ml/min · kg and the mean metabolic clearance rate of porcine secretin was 14.8 ± 0.7 ml/min · kg, giving almost identical responses.

Plasma secretin concentrations measured after rapid intravenous injections of human and porcine secretin are shown in Figure 4. One- and two-compartment models were fit to decay curves, but somewhat better results were obtained with the one-compartment model, as attempts to fit a two-compartment model failed in 2 of 6 subjects because of lack of the iteration procedure. When weights inversely proportional to the secretin levels were used, the one-compartment model gave half-lives of 2.83 ± 0.52 min for human secretin and 2.84 ± 0.62 min for porcine secretin (mean \pm SEM).

For comparison, plasma secretin concentrations were measured after a steak meal in 15 volunteers. The meal consisted of 200 g of steak, 150 g of potatoes, a salad, and 3 dl of apple juice. No sustained increase of plasma secretin was observed, but secretin release occurred in spikes confirming our previous observations with a liquid meal (6). The maximal postprandial secretin release over basal levels amounted to 2.6 ± 0.5 pmol/L ($p < 0.001$, mean \pm SEM).

Discussion

The results of the present study indicate that the biologic activity of synthetic human secretin is

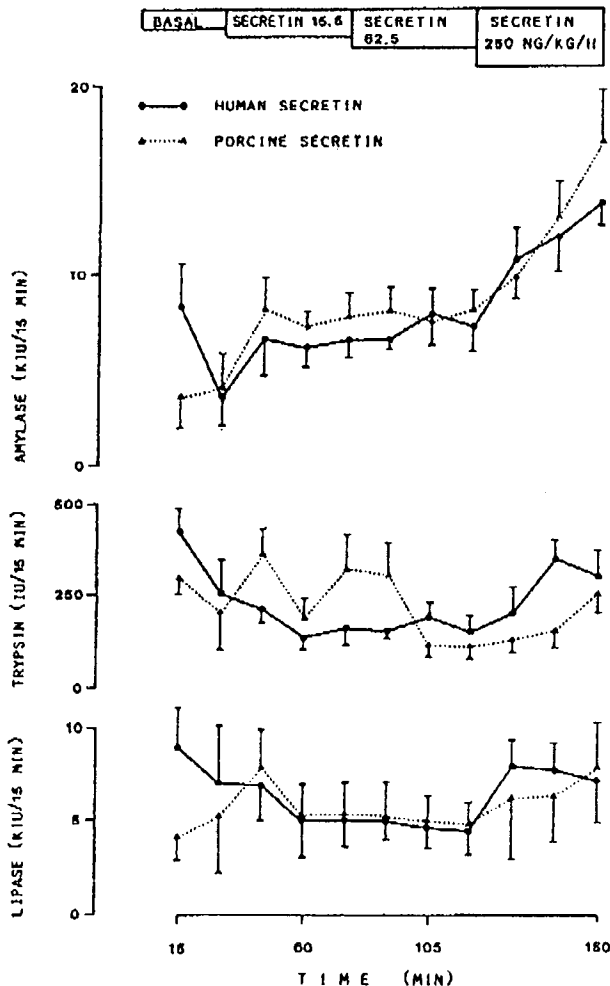


Figure 3. Pancreatic enzyme responses during infusion of graded doses of porcine and human secretin. $n = 6$. Data are shown as mean \pm SEM.

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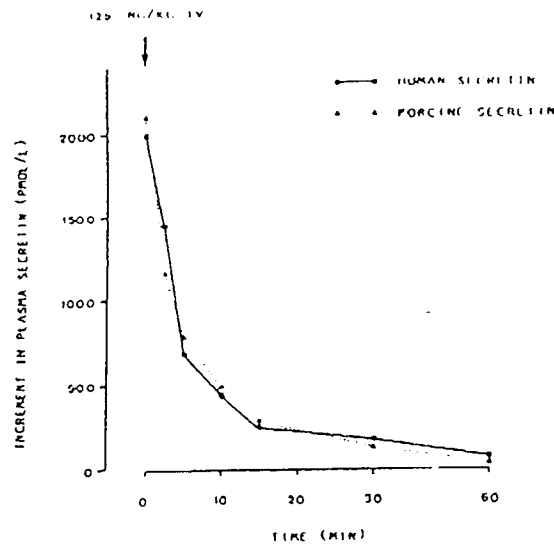


Figure 4. Disappearance of immunoreactive secretin after rapid intravenous bolus infusions of 125 µg/kg of synthetic porcine or synthetic human secretin in 6 healthy subjects. Points are mean values obtained after subtraction of basal values.

equivalent to that of synthetic porcine secretin on an equimolar basis. This equivalence applies to pancreatic secretory responses as well as to immunoreactive plasma secretin concentrations, which showed a nearly identical time-course of plasma levels. Furthermore, the calculated plasma half-lives agreed extremely well with each other and with values for synthetic porcine secretin obtained previously (16) in a different group of volunteers in our laboratory. The results are in the same order of magnitude (2-4 min) reported by other groups for porcine secretin preparations in humans (17,18).

The present study shows that low doses of exogenous secretin can stimulate pancreatic bicarbonate secretion with concomitant increases in plasma secretin concentrations. A similar increase in plasma secretin concentration was detected postprandially, but no data can be given on pancreatic fluid and bicarbonate secretion because of the technical problems involved in measuring pancreatic bicarbonate output in response to food in humans. These results demonstrate that human secretin is a true hormone, and we consider it reasonable to assume that the postprandial bicarbonate response involves secretin.

Both peptides produced similar effects on pancreatic enzyme secretion. The data of the present study demonstrate that the effect of secretin on pancreatic

enzyme secretion is rather complex. The results confirm our previous observations that secretin does not stimulate trypsin outputs over basal levels in humans. Furthermore, we did not see an increase in lipase secretion, which is in agreement with Niederau et al. (19), but at variance with the results of Gullo et al. (20), who in their study on pure pancreatic juice observed a significant increase in lipase secretion in response to higher doses of secretin. On the other hand, we observed a significant increase in amylase secretion for both human and porcine secretin, but only at the highest dose administered (250 ng/kg · h). Amylase, however, was not measured by Gullo et al. It could be argued that a contamination of duodenal aspirates with salivary isoamylase caused the increase in amylase output, as salivary amylase was found in duodenal juices after hormonal or meal stimulation in a variety of studies (21-23). This possibility would be supported by the fact that high doses of intravenous secretin inhibit gastric acid secretion (24), which could prevent inactivation of the salivary amylase (21). In the above study, salivary amylase was present only in trace amounts, making it unlikely that the significant increase in duodenal amylase output was due to contamination.

Thus, the question whether secretin does stimulate pancreatic secretion remains controversial and represents an issue beyond the scope of this paper. If ever secretin induces enzyme release, it is certainly a pharmacologic event (6,19,25-27).

In conclusion, the present study has shown that synthetic human and synthetic porcine secretin are equipotent on a molar basis and display identical plasma kinetic characteristics. These findings establish that human secretin is a true hormone. Furthermore, both secretin preparations induced a significant increase in amylase secretion at the highest doses of secretin, but no changes in trypsin or lipase outputs, respectively. The significance of these observations remains to be defined.

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