

## Vagal, Cholinergic Regulation of Pancreatic Polypeptide Secretion

T. W. Schwartz, ... , O. B. Schaffalitzky de Muckadell, F. Stadil

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### Research Article

The effect of efferent, parasympathetic stimulation upon pancreatic polypeptide (PP) secretion was studied in three ways: (a) Plasma PP concentrations increased in response to insulin-induced hypoglycemia in both normal subjects, from 11 pM (9.5-12.5) to 136 pM (118-147),  $n = 8$  (median and interquartile range) and in duodenal ulcer patients, from 33 pM (21-52) to 213 pM (157-233),  $n = 7$ . The PP response to hypoglycemia was diminished by atropine in normal subjects ( $P < 0.005$ ) and completely abolished by vagotomy in the duodenal ulcer patients. (b) Electrical stimulation, 8 Hz, of the vagal nerves in anesthetized pigs induced an increase in portal PP concentrations within 30 s from 32 pM (28-39) to 285 pM (248-294),  $n = 12$ . Minimal stimulatory frequency was 0.5 Hz and maximal stimulatory frequency 8-12 Hz. Atropine inhibited the PP response to electrical stimulation. Median inhibition with 0.5 mg of atropine/kg body wt was 74%, range 31-90%,  $n = 6$ . The response was eliminated by hexamethonium. Adrenergic alpha and beta blockade did not influence the release of PP in response to vagal stimulation. (c) Acetylcholine stimulated, in a dose-dependent manner, the secretion of PP from the isolated perfused porcine pancreas, half-maximal effective dose being 0.19  $\mu$ M; maximal PP output in response to 5 min stimulation was 228 pmol, range 140-342 pmol,  $n = 5$ . Atropine [...]

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# Vagal, Cholinergic Regulation of Pancreatic Polypeptide Secretion

T. W. SCHWARTZ, J. J. HOLST, J. FAHRENKRUG, S. LINDKÆR JENSEN, O. V. NIELSEN, J. F. REHFELD, O. B. SCHAFFALITZKY DE MUCKADELL, and F. STADIL, *Institute of Medical Biochemistry, University of Aarhus, Århus; Department of Clinical Chemistry, Bispebjerg Hospital, Copenhagen; Department of Surgical Gastroenterology C, Rigshospitalet, Copenhagen, Denmark*

**ABSTRACT** The effect of efferent, parasympathetic stimulation upon pancreatic polypeptide (PP) secretion was studied in three ways: (a) Plasma PP concentrations increased in response to insulin-induced hypoglycemia in both normal subjects, from 11 pM (9.5–12.5) to 136 pM (118–147),  $n = 8$  (median and interquartile range) and in duodenal ulcer patients, from 33 pM (21–52) to 213 pM (157–233),  $n = 7$ . The PP response to hypoglycemia was diminished by atropine in normal subjects ( $P < 0.005$ ) and completely abolished by vagotomy in the duodenal ulcer patients. (b) Electrical stimulation, 8 Hz, of the vagal nerves in anesthetized pigs induced an increase in portal PP concentrations within 30 s from 32 pM (28–39) to 285 pM (248–294),  $n = 12$ . Minimal stimulatory frequency was 0.5 Hz and maximal stimulatory frequency 8–12 Hz. Atropine inhibited the PP response to electrical stimulation. Median inhibition with 0.5 mg of atropine/kg body wt was 74%, range 31–90%,  $n = 6$ . The response was eliminated by hexamethonium. Adrenergic alpha and beta blockade did not influence the release of PP in response to vagal stimulation. (c) Acetylcholine stimulated, in a dose-dependent manner, the secretion of PP from the isolated perfused porcine pancreas, half-maximal effective dose being 0.19  $\mu$ M; maximal PP output in response to 5 min stimulation was 228 pmol, range 140–342 pmol,  $n = 5$ . Atropine completely abolished this response.

The results of the present study together with the previously demonstrated poor PP response to food in vagotomized patients, indicate that vagal, cholinergic stimulation is a major regulator of PP secretion.

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## INTRODUCTION

Human pancreatic polypeptide was isolated by Chance et al. as a side fraction during insulin purification (1). It is a 36 amino acid straight chain polypeptide with no similarity in sequence to any other known hormone. One or two aminoacids distinguish the known mammalian pancreatic polypeptides (PP)<sup>1</sup> (1), whereas they have only  $\approx 16$  amino acids in common with the avian counterpart (2).

PP has been localized to secretory granules in a specific endocrine cell type in the pancreas distinct from the A, B, D, and D<sub>1</sub> cells (3, 4). PP cells are found both in the islets and in the acinar tissue and duct epithelium. In some species PP cells are numerous in the extra-insular tissue and concentrated towards the duodenal part of the pancreas, whereas e.g., in the islets of the sheep the PP cells nearly outnumber the insulin cells (4).

The action of PP has not yet been established although PP has been shown to affect many gastrointestinal functions (5). At present it seems likely that PP acts on the exocrine pancreatic tissue as a local regulator of secretion, in a way that may be conceived as an anticholecystokinin and secretin-enhancing action (6, 7). It has recently been suggested that PP may also affect satiety (8).

During a meal PP concentrations in plasma increase rapidly (9–13). The food-mediated PP response consists of a rapid primary phase (5–30 min) which is eliminated by truncal vagotomy (10) and a prolonged secondary phase (0.5–6 h) which in man is reduced but not abolished by vagotomy (10). The present study was undertaken to investigate the effect of efferent parasympathetic stimulation on PP secretion.

<sup>1</sup> Abbreviations used in this paper: DU, duodenal ulcer; PP, pancreatic polypeptides; VIP, vasoactive intestinal polypeptide.

## METHODS

### Radioimmunoassay for pancreatic polypeptide

**Antiserum.** Anti-PP serum (146-5), a generous gift from Drs. R. E. Chance and N. E. Moon (Eli Lilly & Co., Indianapolis, Ind.), was raised in a rabbit against subcutaneously injected highly purified bovine PP (BPP) coupled to albumin and mixed with complete Freund's adjuvant. The antiserum was used at a final dilution of  $1/7.5 \times 10^6$ .

**Tracer.** Highly purified bovine PP was iodinated by a chloramine T method: 1 mCi  $^{125}\text{I}$  (Behring-Werke AG, Marburg/Lahn, West Germany), 0.24 nmol (1  $\mu\text{g}$ ) highly purified BPP (a gift from Dr. Chance, Eli Lilly & Co.), and 18 nmol (5  $\mu\text{g}$ ) chloramine T was mixed in a total volume of 0.023 ml/0.05 M sodium phosphate buffer, pH 7.4. The reaction was terminated after 20 s by the addition of 26 nmol (5  $\mu\text{g}$ ) sodium metabisulfite in 0.010 ml sodium phosphate buffer. Then 1.2  $\mu\text{mol}$  of potassium iodide in 0.1 ml was added followed by 0.2 ml of equine serum. The tracer was purified on a  $10 \times 1,000$  mm Sephadex G-50 superfine column eluted with ammonium bicarbonate, 0.25 M, pH 8.2, at  $4^\circ\text{C}$ . The fractions from the ascending part of the  $^{125}\text{I}$ -PP peak gave the lowest unspecific binding without antibody (1–3%) and the highest binding in antibody surplus (90–95%). The specific radioactivity of the tracer was calculated to 200–250  $\mu\text{Ci}/\mu\text{g}$  ( $0.8\text{--}1.05 \times 10^6$  Ci/mol).

**Standard.** Highly purified human PP (a gift from Dr. Chance, Eli Lilly & Co.) was used as standard.

**Assay conditions.** Incubation was carried out in 0.02 sodium barbitone buffer, pH 8.4, with 0.2% bovine serum albumin (Ortho Pharmaceutical Corp., Raritan, N. J.) and 0.6 mM thiomersal. The incubation volume was 1.5 ml. In assays of plasma the sample volume comprised 1/20 of the incubation volume and in other assays at most 1/10 of the incubation volume. To obtain comparable incubation conditions "hormone-free" plasma, twice treated with charcoal, was added to the standard and the same volume of buffer was added to the plasma samples. Incubation for 3 days was used in all assays except in assays on column eluates, where preincubation for 2 days was followed by 1 day of incubation with tracer.

**Separation.** Separation was performed with plasma-coated charcoal (Merck article 2186, Merck AG, Darmstadt, West Germany). To each tube 0.5 ml of a preincubated charcoal slurry, 0.25 ml charcoal 40 g/liter and 0.25 ml outdated plasma, was added. Centrifugation was performed 20 min after addition of charcoal. Control samples were included in each centrifugation.

**Specificity.** No displacement of the tracer from antiserum could be demonstrated with up to 10 nM of: monocomponent human insulin (Novo Research Institute, Bagsvaerd, Denmark); monocomponent porcine pancreatic glucagon (Novo Research Institute); synthetic ovine somatostatin (Beckman Instruments, Geneva, Switzerland); synthetic human gastrin-17 (ICI Pharmaceutical Co., Alderly Park, Cheshire, England); highly purified porcine cholecystokinin (CCK 33); highly purified porcine secretin; and highly purified porcine vasoactive intestinal polypeptide (VIP) all generous gifts from Dr. V. Mutt, Karolinska Institute, Stockholm, Sweden, and highly purified porcine gastric inhibitory polypeptide, a generous gift from Dr. J. Brown, University of British Columbia, Vancouver, Canada.

**Sensitivity and precision.** The experimental detection limit during equilibrium incubation was 2.8 pM and in preincubation assays 1.0 pM. The within-assay coefficient of variation was 6.8, 7.1, 8.0, and 6.8% in buffer standards of 12, 25, 60, and 120 pM. The coefficient of variation was 9.7 and 12.7% in plasma samples of 15.7 and 49.7 pM. The be-

tween-assay coefficient of variation tested in 10 consecutive assays at concentrations of 30 and 75 pM was 12.8 and 12.1%.

**Accuracy.** Measurements of PP in plasma with added PP, and in dilutions of perfusate from the isolated pancreas (see below), porcine plasma, and human plasma yielded results which deviated  $<20\%$  from the expected values. The following specimens were exposed to gel permeation chromatography: (a) perfusate from the isolated porcine pancreas during stimulation with acetylcholine; (b) porcine plasma obtained from the portal vein during electrical stimulation of the vagus nerve; and (c) human plasma obtained during insulin hypoglycemia. The gel permeation chromatography was performed at  $4^\circ\text{C}$  on  $10 \times 1,000$  mm Sephadex G-50 superfine columns eluted with 1.0 M acetic acid with 2% equine serum and 0.2% bovine serum albumin. The fractions were lyophilized and reconstituted in barbitone buffer 0.02 M, pH 8.4. In every case only one major immunoreactive peak with elution position corresponding to purified PP was found, Fig. 1. When plasma

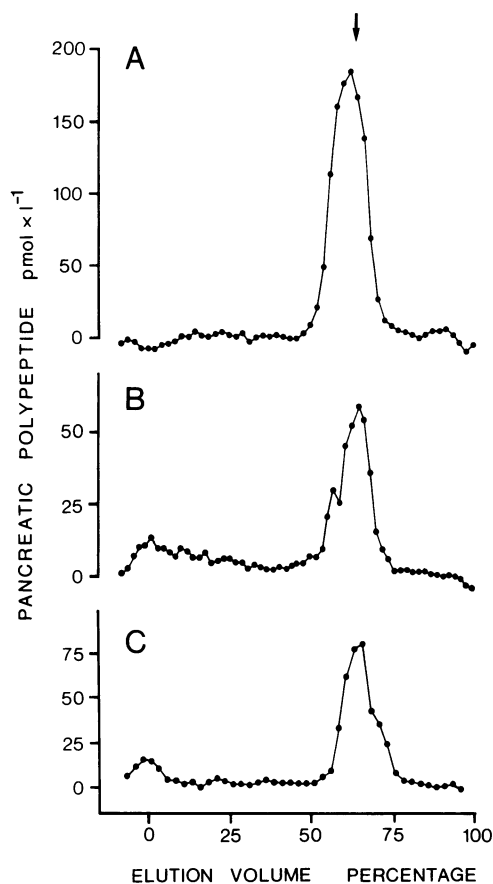


FIGURE 1 Elution diagrams of PP immunoreactivity in: A perfusate from the isolated perfused porcine pancreas, B human plasma obtained during insulin hypoglycemia, C porcine plasma obtained during electrical stimulation of the vagus. The samples were applied to Sephadex G-50 superfine columns ( $10 \times 1,000$  mm) eluted at  $4^\circ\text{C}$  with 1.0 M  $\text{CH}_3\text{COOH}$  containing 2% equine serum and 0.2% bovine serum albumin. The columns were all calibrated with  $^{22}\text{NaCl}$  and column A also with  $^{125}\text{I}$ -albumin. Abscissa: percent of volume between the elution volume of  $^{125}\text{I}$ -albumin and  $^{22}\text{NaCl}$ . The arrow on top indicate the elution position of highly purified human PP.

was applied without internal void volume marker apparent PP immunoreactivity was determined in and closely after the void volume of the column (Fig. 1). A similar apparent immunoreactivity could be detected in the void volume when the hormone-free, charcoal-treated plasma that usually was added to the standards was applied. This apparent PP immunoreactivity could not be removed by specific PP immunoabsorption (antibody from the same rabbit was used for assay). We therefore conclude that the present assay measures only a peptide which elutes in the same position as the highly purified PP. Fasting levels of PP in plasma from young people (20–40 yr) were 13 pM (range 2–30),  $n = 50$ , in agreement with results obtained by Floyd et al. (13) with another PP antiserum, with iodinated highly purified human PP as tracer, and with another separation technique.

### *Insulin hypoglycemia*

**Patients.** Eight normal subjects, two females and six males, median age 22 yr, range 19–25, and seven duodenal ulcer (DU) patients, one female and six males, median age 52 yr, range 34–60 were studied after an overnight fast. Blood was taken from an antecubital vein, centrifuged at 4°C and serum was stored at –20°C before assayed. Gastric juice was aspirated continuously during the experiment. Data concerning this acid secretion and gastrin release have been published (14, 15).

**Stimulation.** Hypoglycemia was achieved by i.v. injection of 0.2 IU of insulin (Insulin LEO, Nordisk Insulin Laboratory, Gentofte, Denmark) per kilogram body weight. Blood glucose concentration, measured by the glucose oxidase method decreased in all subjects below 1.8 mM.

**Inhibition.** The normal subjects were studied on separate days with insulin alone or after i.m. injection of 30 µg atropine/kg body wt given 25 min before the insulin. The DU patients were studied before and 13 wk (8–26) after their operation, truncal vagotomy, and pyloroplasty.

### *Electrical stimulation of the vagal nerves*

**Preparation.** 28 female pigs, Danish Landrace, weight: 25 kg (20–32) were used. Food, but not water, was withdrawn 18 h before experiments. Azaperone (4 mg/kg i.m.) was used for premedication. Anesthesia was induced with Halothane/N<sub>2</sub>O per O<sub>2</sub> and maintained with chloralose (Merck AG, Darmstadt, West Germany) 70 mg/kg i.v. Polyethylene catheters were inserted into the portal vein and left femoral artery and vein. Intra-arterial blood pressure and rectal temperature were continuously monitored (EMT 34 transducer, mingograph, E. Schönander, Stockholm, Sweden, and temperature recorder, Z8, ellab, Copenhagen, Denmark). A laparotomy was performed, the pylorus was ligated, and gastric content was continuously drained by suction via an orogastric tube. The splanchnic nerves were cut bilaterally. Through a thoracotomy the vagal trunks were identified above the diaphragm and cut below the heart. The peripheral ends of the nerves were passed through a bipolar platinum electrode. Blood was collected from the portal vein and the femoral artery, in ice-chilled tubes, containing 500 kallikrein inactivator units aprotinin (Trasylol, The Bayer Co., New York) and 50 IU heparin/ml blood. Samples were centrifuged at 4°C and plasma stored at –20°C until assayed.

**Stimulation.** 5 min stimulations with constant-current (8 mA) square-wave impulses (frequency 0.25–20 Hz, impulse duration 5 ms) were carried out by means of an electronic square-wave stimulator (Palmer, England) Applied voltage

and current was monitored on a dual-beam oscilloscope (type 502A, tektronic, Guernsey Ltd., England).

**Inhibition.** Atropine, 0.005, 0.05, 0.5-mg/kg, was injected before repeated submaximal stimulations in one pig, atropine 0.5 mg/kg in six different pigs. Hexamethonium, 10 mg/kg was used in four different pigs. In four other pigs submaximal stimulations without inhibition were repeated. In five different pigs combined alpha and beta blockade was performed: propranolol (Inderal, ICI Pharmaceutical Co.) was injected i.v. in a dose of 1 mg/kg. Phenoxybenzamine chloride, (Alfred Benzon, Copenhagen, Denmark) in a dose of 1 mg/kg diluted in 100 ml isotonic saline was infused during 1 h, after which the vagal stimulation was repeated. In these experiments dextran, Macrodex 6%, Pharmacia, Uppsala, Sweden, was infused to carefully substitute blood and fluid losses.

### *Isolated, perfused, porcine pancreas; acetylcholine*

**Preparation.** The isolation and preparation procedure has been described in detail elsewhere (16). Five pigs, Danish Landrace, weight: 26–30 kg, median 28 kg, were used. The pancreas was isolated together with their entire vascular supply; the duodenum was not included in the preparation but the pancreaticoduodenal vessels remained intact. The pancreases were perfused at 38°C with Krebs-Ringer bicarbonate solution enriched with fumarate, glutamate, and pyruvate all 5 mM, Dextran T 70 (Pharmacia, Uppsala, Sweden) 4%, human serum albumin (Trocken Reinst, Behring Werke/Lahn, West Germany) 0.1%, and 5 mM glucose. The perfusate was oxygenated with 95% oxygen and 5% CO<sub>2</sub> and perfused through the pancreas at a flow rate of 30 ml/min, thus the oxygen consumption could be maintained at 0.006–0.008 ml O<sub>2</sub>/min per g tissue. Flow rate, pressure, temperature, PCO<sub>2</sub>, PO<sub>2</sub> and pH of the perfusate were measured at 20 min intervals. The effluent was collected every minute and stored at –20°C until assayed.

**Stimulation.** After a resting period of 30 min 5 min stimulation periods with acetylcholine 0.05, 0.1, 0.5, 2.5, and 100 µM (final concentration in perfusate) were carried out separately by 15 min resting periods.

**Inhibition.** Atropine, 0.5 µM (final concentration in perfusate), was infused 5 min before and during stimulation with acetylcholine, 0.5 µM.

### *Statistics*

The results are, if not otherwise indicated, presented as median concentration followed by the interquartile range in brackets. The Wilcoxon matched-pairs signed-ranks test or when appropriate the Mann-Whitney test or the Friedman two-way analysis of variance were used for statistical evaluation (17). Differences resulting in  $P$  values <0.05 were considered significant.

## **RESULTS**

### *Insulin hypoglycemia*

During insulin hypoglycemia the plasma concentrations of PP in the eight normal subjects increased from 11 pM (9.5–12.5) to a maximum of 136 pM (118–147) at 45 min after the injection of insulin; PP levels were still elevated 180 min after the injection, 46 pM (29–55), Fig. 2. Atropine did not significantly alter basal

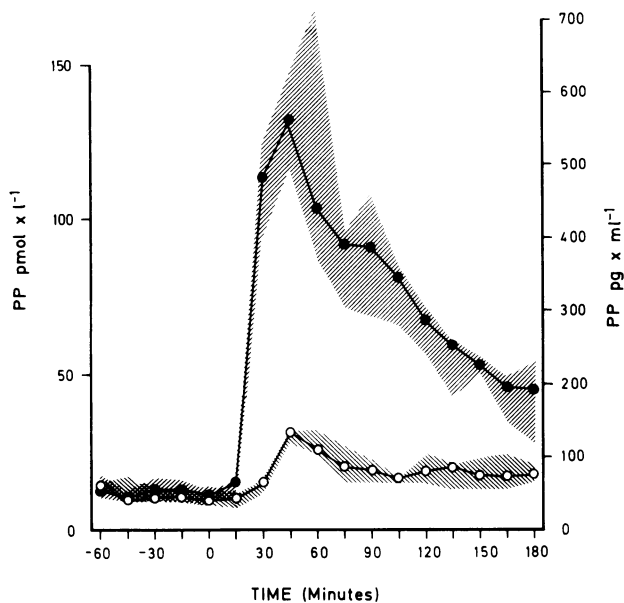


FIGURE 2 Plasma PP concentrations, median concentration and interquartile range (hatched area) in 8 normal subjects during insulin hypoglycemia ( $0.2 \text{ U} \times \text{kg}^{-1}$  body weight) without (●) or with (○) atropine ( $0.03 \text{ mg/kg}$ , i.m.) injected 25 min before insulin.

PP concentrations,  $10.1 \text{ pM}$  ( $9.3\text{--}11.5$ ) 5 min before and  $9.5 \text{ pM}$  ( $9.0\text{--}14.0$ ) 25 min after atropine. However, the response to insulin hypoglycemia, from  $9.5 \text{ pM}$  ( $9.0\text{--}14.0$ ) to a maximum of  $31.5 \text{ pM}$  ( $28.5\text{--}31.5$ ) at 45 min after insulin, was diminished by atropine ( $P < 0.005$ ) at all points from 30 to 120 min after insulin injection.

Basal PP concentrations in the DU patients before the operation were  $33 \text{ pM}$  ( $21\text{--}52$ ), significantly higher than those of the younger normal group. The PP response pattern to insulin hypoglycemia before the operation was similar to that of the normal group, Fig. 3. However, the maximal PP concentrations were higher in the DU patients,  $213 \text{ pM}$  ( $157\text{--}233$ ). After truncal vagotomy basal PP concentrations,  $17.5 \text{ pM}$  ( $9.5\text{--}27.0$ ), were lower than preoperative levels ( $P < 0.05$ ), and not different from the basal PP levels in the normal group. No increase in PP concentrations was observed during insulin hypoglycemia in the DU patients after truncal vagotomy, Fig. 3 ( $P > 0.10$ , Friedmann two-way analysis).

### Electrical stimulation

Frequency-response studies were carried out in three pigs, Figs. 4 and 5. Half-maximal stimulatory frequency was 2.5, 3.3, and  $5.6 \text{ Hz}$ , and maximal stimulation was achieved with frequencies of 8, 12, and  $12 \text{ Hz}$  respectively. Increase in portal plasma PP concentrations could be detected during stimulation with one

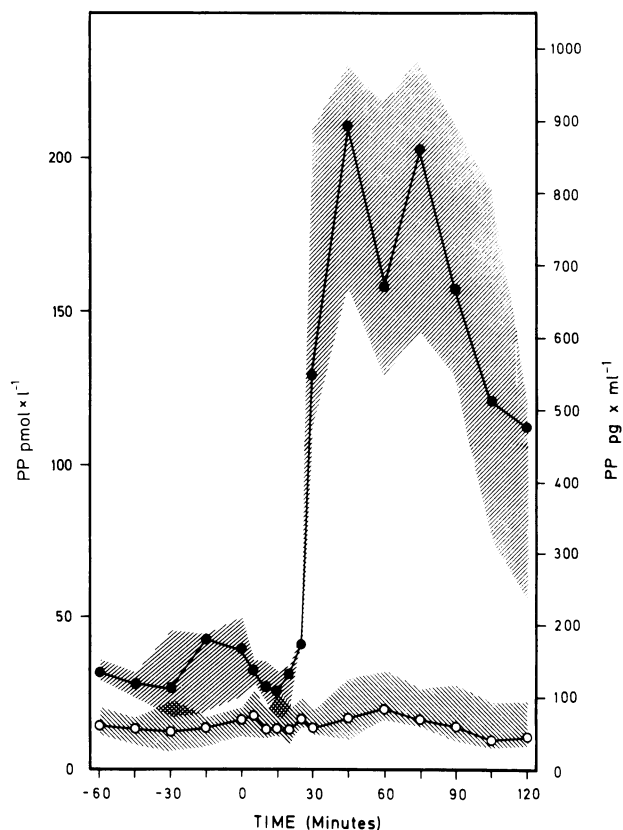


FIGURE 3 Plasma PP concentrations, median concentration and interquartile range (hatched area) in seven duodenal ulcer patients during insulin hypoglycemia ( $0.2 \text{ U/kg}$  body wt) before (●) and after (○) truncal vagotomy.

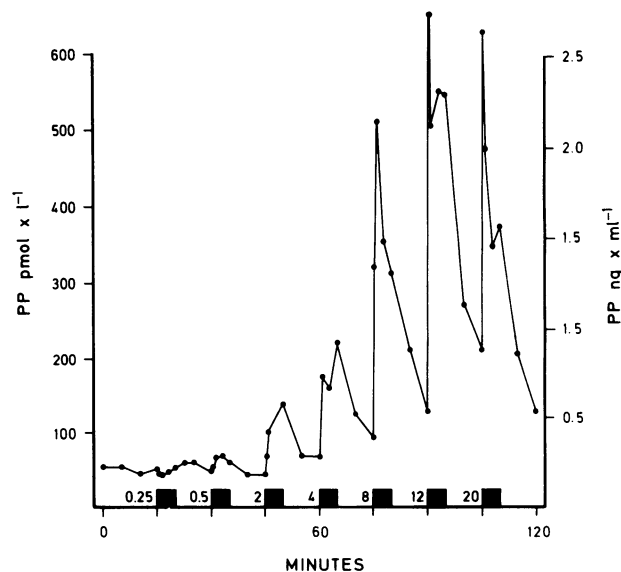


FIGURE 4 Portal plasma PP concentrations during graded electrical stimulation with frequencies from 0.25 to  $20 \text{ Hz}$ , indicated by bars, of the vagal nerves in a pig.

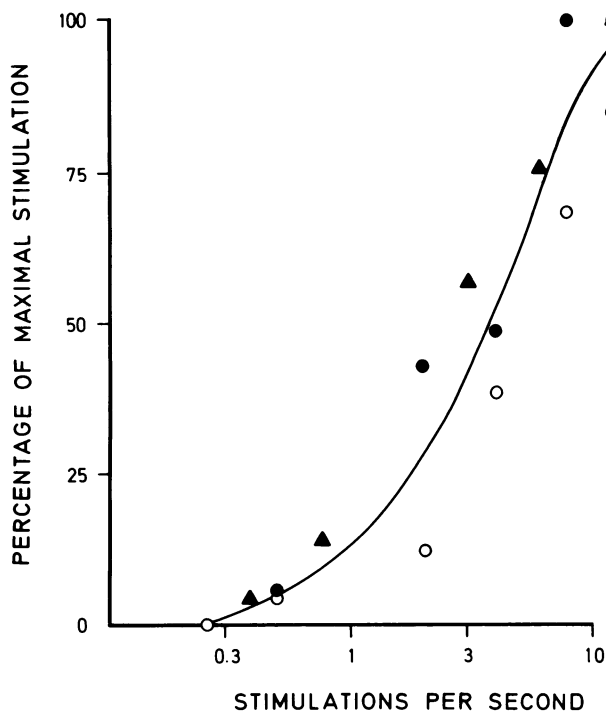


FIGURE 5 Integrated PP response in three individual pigs during graded stimulation of the vagal nerves. The experiment demonstrated in Fig. 4 is indicated by (○). The responses shown have been calculated by integration of PP concentration curves during stimulation periods minus prestimulatory levels, if basal PP levels was not reached before repeated stimulation a calculated basal level was subtracted.

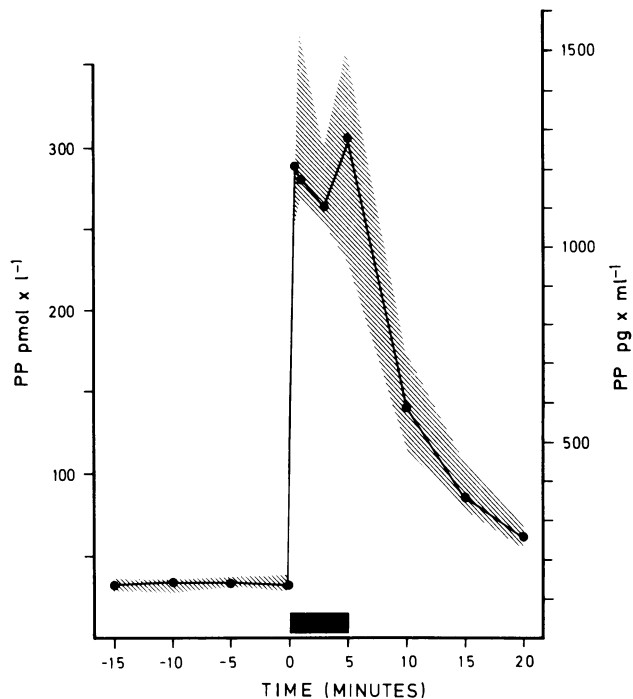


FIGURE 6 Portal plasma PP concentrations, median concentration and interquartile range (hatched area) in response to 8 Hz electrical stimulation, indicated by bar, of the vagal nerves in pigs,  $n = 12$ .

impulse every other second, but not with stimulations every 4th s.

During submaximal stimulation, 8 Hz, PP concentrations in portal plasma increased within 30 s from 32 pM (28–39) to 285 pM (248–294),  $n = 12$ , Fig. 6. PP concentrations in portal plasma, and in arterial plasma (Fig. 8), remained elevated throughout the 5 min stimulation period. The PP response could be reproduced in the same pig during repeated stimulation 3 h after the primary stimulation. The integrated PP response during restimulation averaged 91% of the primary response (range: 84–129%)  $n = 5$ , Fig. 7.

Atropine in a dose of 0.05 mg/kg was sufficient to inhibit the PP release in response to submaximal electrical stimulation of the vagal nerves, Fig. 8. However, even with 0.5 mg/kg of atropine the degree of inhibition varied; median inhibition: 74% (range: 31–90%)  $n = 6$ , Fig. 7. Injection of hexamethonium 10 mg/kg before vagus stimulation eliminated the PP response, Figs. 9 and 7. Combined alpha (phenoxybenzamine) and beta (propranolol) adrenergic blockade did not significantly influence the PP response to vagal stimulation,  $n = 5$ , Fig. 7.

Cessation of the electrical stimulation resulted in a

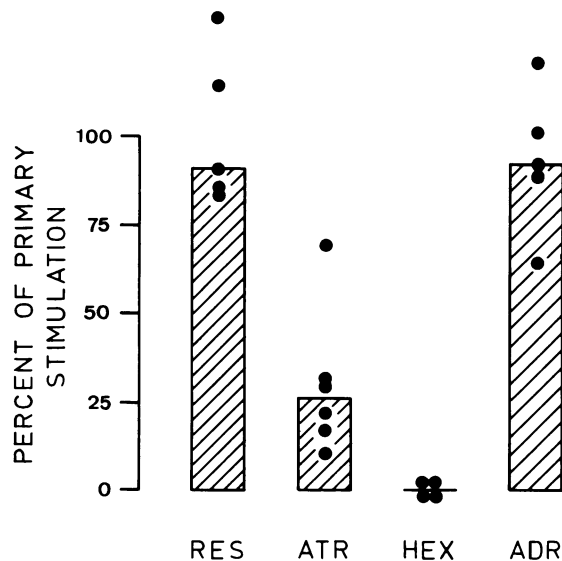


FIGURE 7 The effect of different blocking agent upon 8 Hz electrical, vagal stimulation of PP secretion. Ordinate indicates integrated PP response during restimulation as percent of integrated PP response during the primary stimulation. RES.-restimulation without any blocking agents. ATR. Atropine 0.5 mg/kg, HEX.-hexamethonium, 10 mg/kg, ADR-combined alpha, phenoxybenzamine 1 mg/kg, and beta, propranolol 1 mg/kg, adrenergic blockade.

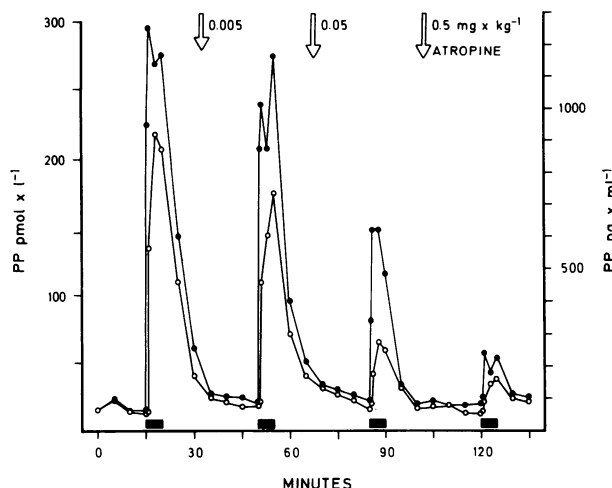


FIGURE 8 The effect of increasing doses of atropine on the plasma PP concentrations in the portal vein (●) and in the femoral artery (○) during repeated 8 Hz stimulations, indicated by bars, of the vagal nerves in a pig.

rapid decline in PP concentrations, Fig. 6 and 8. After a more rapid decrement during the first five min, the semilogarithmic disappearance curve for PP was linear from 5 to 15 min after cessation of the stimulation, suggesting a half time of disappearance for endogenously released PP of 5.7 (4.5–6.9) minutes;  $n = 12$ .

### *The isolated, perfused, porcine pancreas*

Basal output of PP from the isolated pancreas was 2.6 pmol/min (range: 1.4–3.6 pmol/min). Acetylcholine

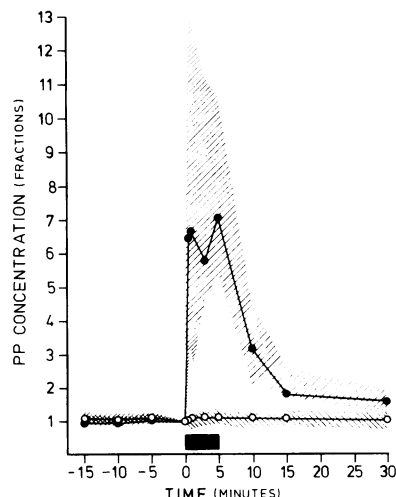


FIGURE 9 Portal plasma PP concentrations, median and total range (hatched area), during electrical stimulation, indicated by bar, of the vagal nerves without (●) or with (○) pretreatment with hexamethonium, 10 mg/kg body wt,  $n = 4$ . The ordinate indicates fractional PP concentration obtained by division of actual concentration with concentration at time zero.

increased the secretion of PP from the isolated pancreas in a dose-dependent manner, Fig. 10 and 11. The lowest dose tested, 0.05  $\mu\text{M}$ , stimulated the PP secretion in all experiments. Half-maximal effective dose of acetylcholine was 0.19  $\mu\text{M}$  (range: 0.07–0.80  $\mu\text{M}$ ). Maximal output of PP in response to 5 min stimulations was 228 pmol (range: 140–342 pmol). In all experiments the stimulation of PP secretion was abolished by atropine (Fig. 10).

## DISCUSSION

The present study shows that efferent, vagal stimulation releases PP, and that this is probably mainly mediated by acetylcholine. Together with the previously demonstrated poor PP response to food in vagotomized patients (10) these data indicate that vagal, cholinergic stimulation is of major importance for the secretion of PP.

**Insulin hypoglycemia.** The increase in concentration of PP in plasma during insulin hypoglycemia is similar to the increase observed during a meal (11, 13, 18). This response is closely correlated to the hypoglycemia (11, 13). The fact that atropine strongly inhibits the PP response (Fig. 2) indicates that vagal activity and not hypoglycemia per se stimulates the PP cell. Accordingly low glucose concentrations did not enhance the release of PP from the isolated pancreas (unpublished observations). On the other hand the study with atropine dose not prove that the PP response to hypoglycemia is mediated by a peripheral, cholinergic transmission, because atropine might also act on the central nervous system (19).

The total elimination of the PP response to hypo-

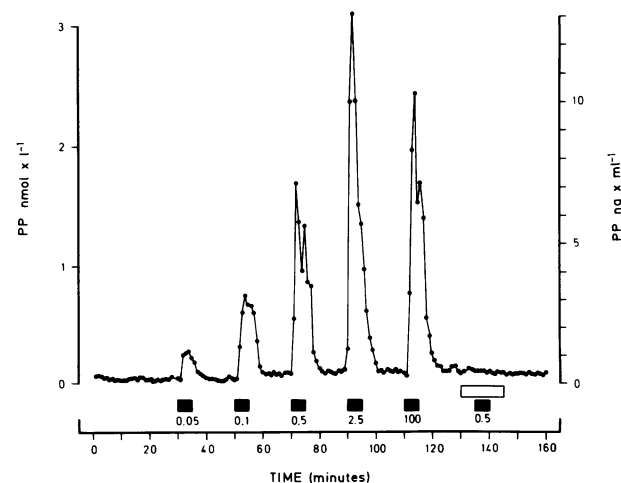


FIGURE 10 The effect of increasing concentrations of acetylcholine (0.05–100  $\mu\text{M}$ ), indicated by closed bars, upon PP concentrations in the perfusate from an isolated porcine pancreas. Addition of atropine, 0.5  $\mu\text{M}$ , is indicated by open bar.

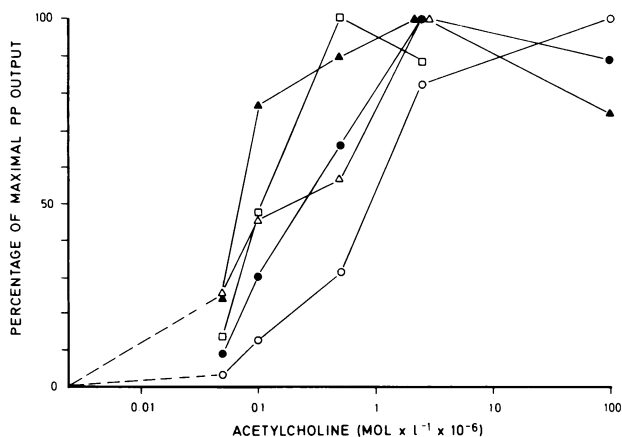


FIGURE 11 Integrated PP output from the isolated porcine pancreas in response to graded stimulation with acetylcholine in five individual pancreases. The output was calculated by integration of PP concentration curves from the infusion of acetylcholine started to PP levels returned to prestimulatory levels, minus this concentration, multiplied with the flow rate.

glycemia by truncal vagotomy (Fig. 3) indicates, in accordance with findings by Adrian et al. (18), that all of the increment in PP concentration during insulin hypoglycemia is caused by vagal activity. Other mechanisms activated during hypoglycemia, e.g. the adrenals and the sympathetic nervous system, which might possibly affect the secretion of PP, are entirely dependent on an intact vagal innervation.

**Electrical stimulation of the vagal nerves.** The large increase in PP concentrations in portal plasma during electric stimulation of the vagal nerves confirms that efferent vagal stimulation is a potent release mechanism for PP. The increase in plasma concentrations of PP during vagal stimulation does reflect increased PP secretion, since portal bloodflow was unchanged or increased up to 40% during these stimulations (20). Moreover, a similar increase could be detected in the systemic circulation, Fig. 8, which proves an actual increased splanchnic output.

That the threshold frequency for PP release is below one stimulation per second and that maximal secretion is achieved with 8–12 Hz stimulation is in agreement with the supposed physiological discharge rate of the peripheral autonomic nervous system. By recording from single or a few nerve fibres of both sympathetic and parasympathetic nerves a continuous discharge of impulses with frequencies at  $\approx 1$  Hz is found during basal conditions, and during intense reflex stimulation the discharge rate is in the order of 10 Hz (21). Furthermore, similar frequency-response curves were obtained in studies on electrical, vagal stimulation of acid secretion (22).

The elimination by hexamethonium of the PP re-

sponse during vagal stimulation (Fig. 9) indicates that a nicotinic receptor is intercalated in the peripheral vagal pathway distal to the diaphragm. This is in accord with the concept of a classic parasympathetic pathway with a peripheral, postganglionic neurone activated by nicotinic receptors.

The inhibitory effect of atropine (Fig. 8) indicates that the transmission from the postganglionic neurone to the PP cell is, at least in part, mediated by acetylcholine acting on a muscarinic receptor. In agreement with the effect of atropine upon postganglionic, vagal stimulation of other gastrointestinal functions (19), large amounts of atropine are necessary to inhibit electrical, vagal PP release. However, even with a large dose of atropine, 0.5 mg/kg, only 75% of the response could be abolished (Fig. 7); thus other transmitter substances apart from acetylcholine could be involved. It is not likely that the catecholamine containing vagal fibres (23) play any major role during electrical stimulation because combined alpha and beta adrenergic blockade does not influence the PP response, Fig. 7. Furthermore electrical stimulation of the splanchnic nerves does not enhance PP secretion (unpublished observations). Peptidergic transmission might be involved in the neural stimulation of PP because e.g. VIP (24) and gastrin (25) have been found in the vagus. Furthermore VIP containing cell bodies are in some species localized in pancreatic ganglia,<sup>2</sup> and VIP is released by an atropine-resistant mechanism during electrical, vagal stimulation (20). Finally, VIP and members of the gastrin family of gastrointestinal hormones can release PP (18, 26). However, the possible involvement of such substances in the electrical, vagal stimulation of PP secretion is presently difficult to delineate.

**The isolated porcine pancreas.** Low doses of acetylcholine stimulated the secretion of PP from the isolated pancreas. The half-maximal effective dose of acetylcholine (0.2  $\mu$ M) for release of PP from the isolated porcine pancreas is similar to the  $D_{50}$  of acetylcholine for stimulation of the motor activity in the gastrointestinal musculature (27) and for stimulation of insulin release from the isolated perfused rat pancreas (28). It is reasonable to assume that acetylcholine acting on muscarinic receptors also is involved in the neural stimulation of PP secretion, since atropine inhibited vagal stimulation, Figs. 7 and 8, and because the effect of acetylcholine on the isolated pancreas was completely abolished by an equimolar dose of atropine, Fig. 10. The amount of PP released during maximal stimulation with acetylcholine from the isolated porcine pancreas could very well account for the increase in plasma PP concentra-

<sup>2</sup> L.-I. Larsson, J. Fahrenkrug, J. J. Holst, and O. B. Schaffalitzky de Muckadell. Innervation of the pancreas by vasoactive intestinal polypeptide immunoreactive nerves. In preparation.



tions during maximal, vagal stimulation in the same species.

From these three studies taken together it can be argued: that efferent, vagal stimulation is a potent release mechanism for PP; that the vagal pathway includes a peripheral neurone activated by nicotinic receptors below the diaphragm; and that the final mediator probably mainly is acetylcholine acting on muscarinic receptors.

The importance of efferent, vagal stimulation of the PP secretion in human physiology is illustrated by studies on vagotomized patients. In a prospective study with each patient being his own control we found that truncal vagotomy eliminated the first 15–25 min of the otherwise rapid increase in plasma PP concentrations during a meal, and also significantly reduced the secondary prolonged response (10). These findings have partly been supported by the results of Adrian et al. (18) and have been confirmed by Taylor et al. (29). It has been suggested that the elimination by vagotomy of the primary PP response might be due to impaired gastric emptying after the operation (30). However, the afferent branch of the vagovagal reflex for PP release is activated by (pregastric) classic, cephalic stimulation (31), and by gastric food- and distention-receptors (26). We therefore conclude that vagal stimulation of the PP secretion is responsible for the rapid, primary increase in plasma PP concentrations during a meal and that it also contributes to the prolonged secondary PP response.

Vagal activity does not seem to influence the basal PP secretion in young normal subjects, because atropine does not suppress their basal PP concentrations (Fig. 2). However, the elevated PP levels found in some DU patients were normalized by vagotomy (10) (Fig. 3). The difference between the DU patients and the normal group may yet be due to difference in age because the patients studied were relatively old, and PP levels tend to increase with age (13). However, fluctuations in PP concentrations and in the basic acid output in DU patients are correlated, and moreover atropine normalizes the elevated PP (Schwartz, Olbe, and Stenquist, unpublished observations). Thus, possibly some DU patients have elevated vagal activity, reflected in increased basal PP concentrations.

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