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CYCLIC CHANGES OF PLASMA PANCREATIC POLYPEPTIDE AND PANCREATIC SECRETION IN FASTING DOGS

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SUMMARY

1. Fasting conscious dogs, each with a gastric fistula, Heidenhain pouch and Thomas duodenal fistula, were used.

2. Basal pancreatic secretion showed periodic increases in phase with the periodic contraction of the stomach and duodenum.

3. Periodic increases of plasma pancreatic polypeptide (PP), but not of gastrin, were observed in phase with the periodic contraction and secretion of the gut.

4. Ganglion blockade abolished the cyclical activity, both secretory and motor, of the gut and of plasma PP.

5. Intraduodenal infusion of lidocaine suppressed the spontaneous increase of pancreatic secretion and plasma PP.

6. It is concluded both that the cyclical release of PP and the increase in pancreatic secretion are under the control of the intrinsic nerves of the duodenum.

INTRODUCTION

In fasting dogs, basal pancreatic secretion changes periodically in phase with the motor activities of the upper gastrointestinal tract (Boldyreff, 1911). Recently, the plasma concentration of pancreatic polypeptide (PP), which is released from the endocrine pancreas, has been found to show a cyclical change concomitant with the spontaneous activities of the gut in fasting men (Schwartz, Stenquist, Olba & Stadil, 1979) and dogs (Keane, DiMagno, Dozois & Go, 1980). Food-stimulated release of PP has been studied extensively (Schwartz, Rehfeld, Stadil, Larsson, Chance & Moon, 1976; Adrian, Bloom, Besterman, Barnes, Cooke, Russell & Faber, 1977; Floyd, Fajans & Pek, 1977; Taylor, Feldman, Richardson & Walsh, 1978*a*; Taylor, Impicciatore, Carter & Walsh, 1978*b*) but little is known of its basal secretion. The purpose of this study is to elucidate the underlying mechanism that controls the cyclical release of PP in fasting dogs.

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METHODS

Six mongrel dogs (17–27 kg), each with a gastric fistula, Heidenhain pouch and Thomas duodenal fistula, were used once a week. They were 2–18 months after operation.

Animals were kept in Pavlov stands after an 18 hr fast. Secretions were collected every 10 min. Gastric secretion was collected by simple drainage, pouch secretion by the saline wash-out method (Magee & Nakajima, 1966) and pancreatic secretion by direct cannulation of the duct through the Thomas fistula. Motility of the stomach and the duodenum were recorded by small rubber balloons introduced through the gastric and the duodenal fistulae. Balloons filled with 1 ml. water were connected to a pressure transducer (Narco Bio System, Inc., Houston). Pressure changes were recorded on a polygraph (Narco Bio System, Inc., Houston). Blood was taken from a peripheral vein and plasma was kept at $-20°$ until assayed.

Control observations from 5 to 6 hr were made on each animal and an intravenous infusion of saline (150 mM-NaCl, 8 mM-KCl) at 15 ml./10 min was maintained to replace the fluid loss.

Two series of tests were done: (a) pentolinium tartrate, (Wyeth, Philadelphia, PA) in a dose of 20 mg, which is sufficient to paralyse the nictiating membrane for over an hour, was given subcutaneously 30 min after observing the pancreatic peak. (b) Two percent lidocaine HCl (Pfaltz & Bauer, Stanford, CT) adjusted to pH 7 was infused into the duodenum at 5 ml./10 min for 70 min. The infusion was started 50 min after the pancreatic peak.

Acid was titrated with 50 mM-NaOH (Radiometer). Peptic activities were estimated by the method of Anson (1938). Protein concentration was measured by spectrophotometry at 280 nm (Hitachi-Perkin Elmer). The number of contractions of the gut for each 10 min collection period was counted.

Chloramine T was used for the iodination (Kulneff-Herlin, Herlin, Chen, Gimmon, Murphy & Joffe, 1982) of gastrin 17 (synthetic: Research Plus, Bayonne, NJ) and pancreatic polypeptide (porcine; Novo Laboratories, Inc., Wilton, CT). Radiolabelled gastrin was purified by gel filtration followed by chromatography on DEAE Sephadex A25 (Kulneff-Herlin *et al.* 1982; Gimmon, Murphy, Chen, Nachbauer, Fischer & Joffe, 1982). Radiolabelled pancreatic polypeptide was purified on a column of SP Sephadex C25 eluted with a gradient of 0–0·5 M-NaCl in 0·05 M-NaH_2PO_4 (130 ml.) containing 0·2% human serum albumin (AB Kabi, Stockholm, Sweden). Peak chromatographic fractions containing the radiolabelled hormones were mixed with equal volumes of ethanol containing 1% (v/v) 10 N-HCl and stored at -60 °C.

Antibody raised against synthetic human gastrin 2–17I (Imperial Chemical Industries, Ltd., Macclesfield,) had comparable reactivities with gastrins 14, 17I, 17II, and 34* and less than 10% cross reactivity with cholecystokinin 8* at the highest concentration (0·5 p-mole peptide/ml.) used for standard curves. (I and II denote unsulphated and sulphated forms, respectively, and the arabic numerals* denote the number of amino acid residues in C-terminal sequences.) Antibody to bovine pancreatic polypeptide was a gift from R. E. Chance, Lilly Research Laboratories, Indianapolis, IN.

Radioimmunoassay procedures and constituents of assay mixtures were as described previously (Kulneff-Herlin *et al.* 1982) except that, in the case of pancreatic polypeptide, buffer A containing 0·2% human serum albumin was used for solutions of antiserum, standard quantities of hormone and radiolabelled hormone. The minimal detectable plasma concentration was 10 pg/ml. for both gastrin and PP. The intra- and interassay coefficients of variation were $< 10\%$ and $< 15\%$ respectively.

To analyse the periodical change, one sample which contained the peak pancreatic secretion was arbitrarily designated the zero period. Samples obtained before the peak were renumbered in reverse order from -1st to $-i$th and those after the peak from $+1$st to $+j$th (i and j correspond to the number of samples before and after the peak, respectively). Means and standard errors were calculated for each of the newly numbered samples and n is the number of dogs. Regression analysis was made with the method of least squares. A paired, t test was used for comparison and $P < 0·05$ was taken as the level of significance.

RESULTS

Basal secretion

Basal pancreatic secretion showed periodic changes with apogees and perigees (Fig. 1). The duration of one cycle was about 100 min. Basal gastric secretion also showed fluctuations but its periodicity was less obvious than that of pancreatic secretion. Plasma levels of PP followed a pattern similar to that of pancreatic secretion, whereas plasma gastrin did not.

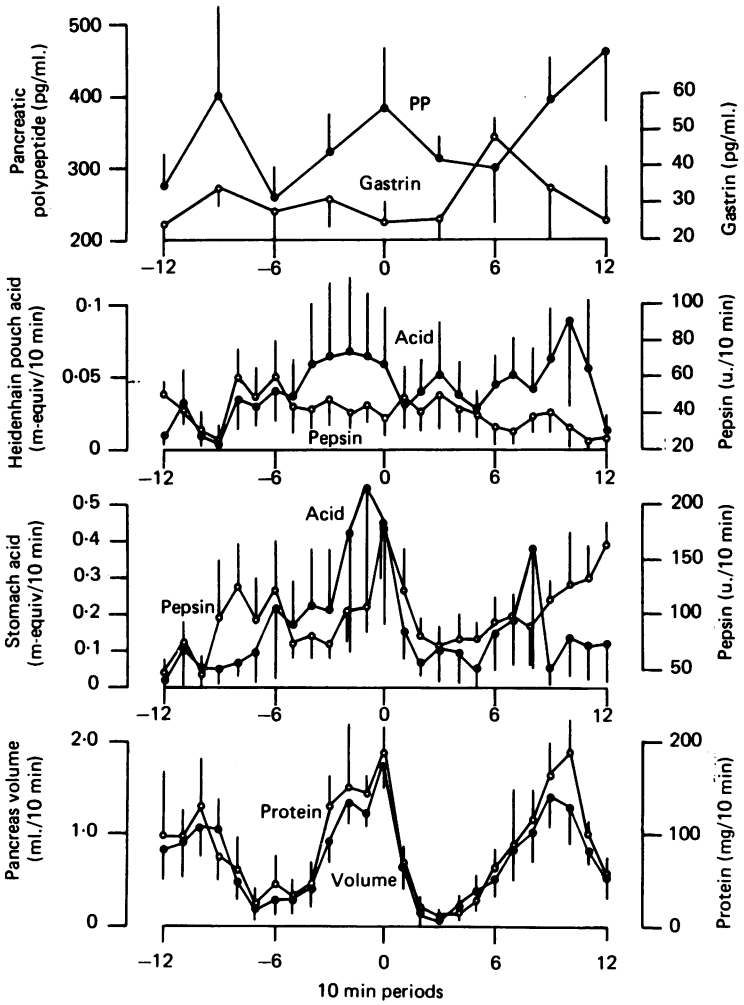

Fig. 1. Basal secretion. Mean values, S.E. of means ($n = 5$).

Pancreatic protein secretion was very high at its peak, though the volume of secretion was small (Fig. 2). Basal plasma levels of PP showed wide variability (59–760 pg/ml.). The apogee level (376 ± 23 (mean $\pm$ S.E. of mean, pg/ml.) was significantly ($P < 0{\cdot}01$) higher than the perigee (247 ± 17 pg/ml.). Plasma PP (Fig. 3) was significantly correlated with pancreatic secretion ($r = 0{\cdot}637$ and 0·540 for

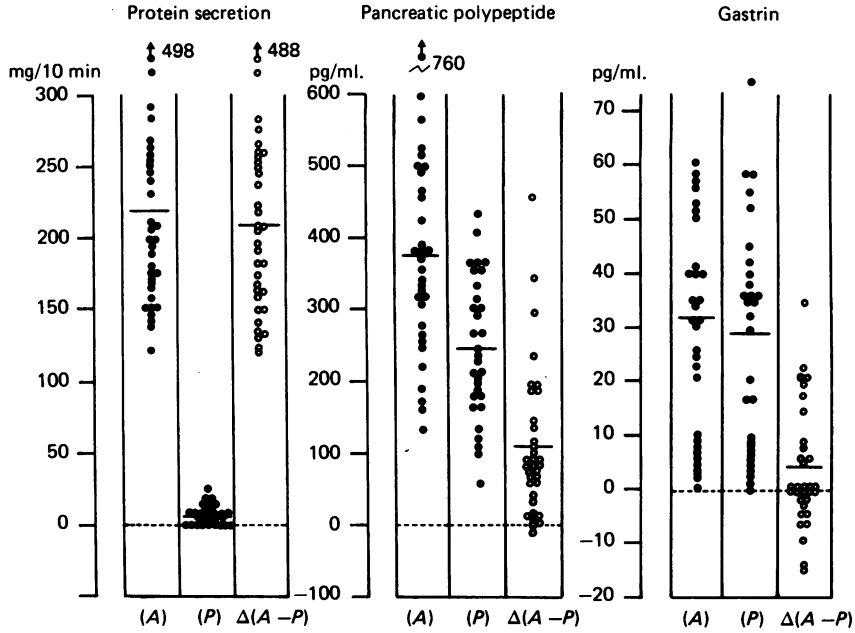

Fig. 2. Pancreatic protein secretion at apogee (*A*) and perigee (*P*) and corresponding plasma PP and gastrin levels in six dogs. $\Delta(A-P)$ indicate the difference between apogee and perigee levels in each of the cycles. Bars indicate the mean values.

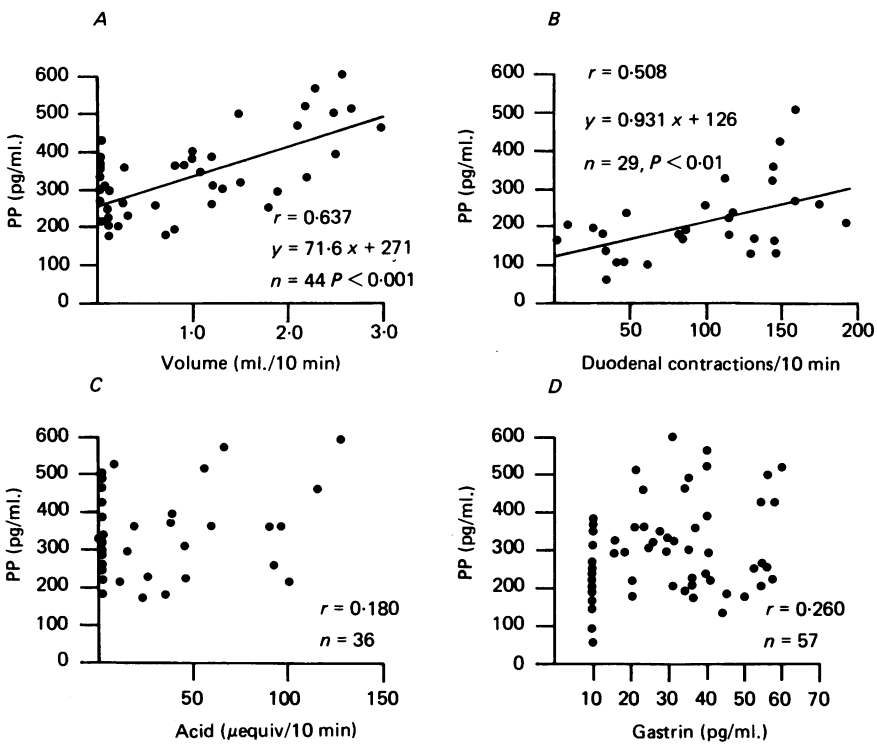

Fig. 3. Correlation of plasma PP with pancreatic secretion (*A*), duodenal motility (*B*), gastric acid secretion (*C*) and plasma gastrin (*D*).

volume and protein output respectively), as well as with duodenal motility ($r = 0{\cdot}508$). Plasma gastrin, on the other hand, showed no consistent change concomitant with secretory and motor apogees and perigees. Neither acid secretion nor plasma gastrin was significantly related to plasma levels of PP.

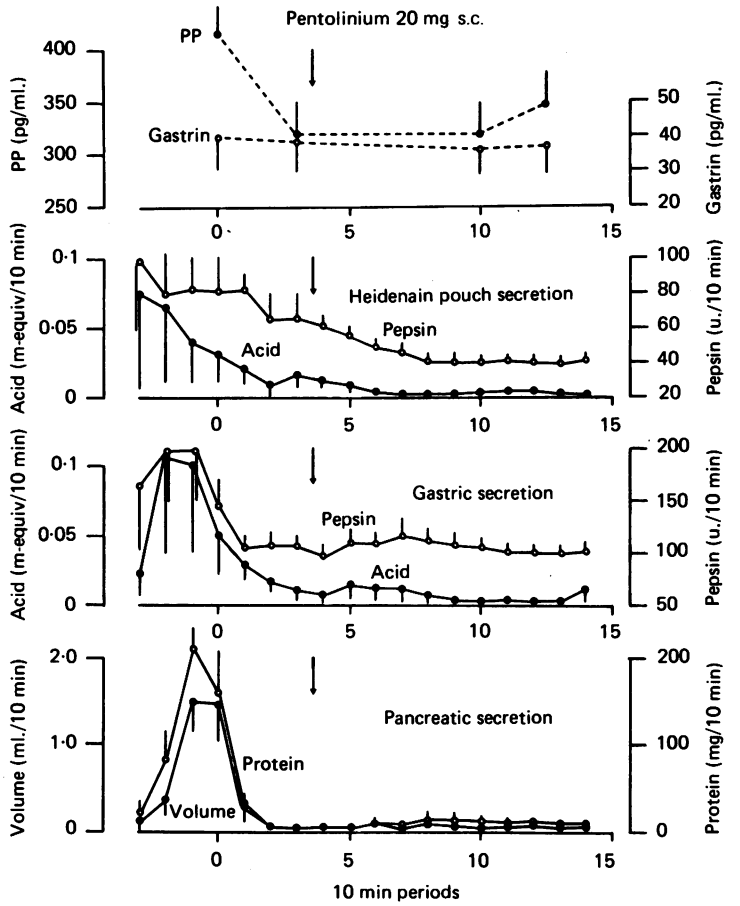

Fig. 4. Effect of pentolinium (20 mg s.c.) on basal secretion. Mean values ± S.E. are given ($n = 5$).

Effect of ganglionic blockade

Subcutaneous injection of pentolinium (20 mg/dog) abolished the periodic increase of pancreatic water and protein secretion and of plasma PP (Figs. 4 and 5). Basal acid and pepsin secretion were also suppressed.

Effect of duodenal anaesthetization

Basal pancreatic secretion, volume and protein, changed in phase with gastric and duodenal motility in control studies (Fig. 6). The peak secretion of the pancreas was just ahead of that of the duodenal motility.

Intraduodenal infusion of lidocaine suppressed the spontaneous increase in the duodenal motility. Activities of the stomach and the pancreas maintained their

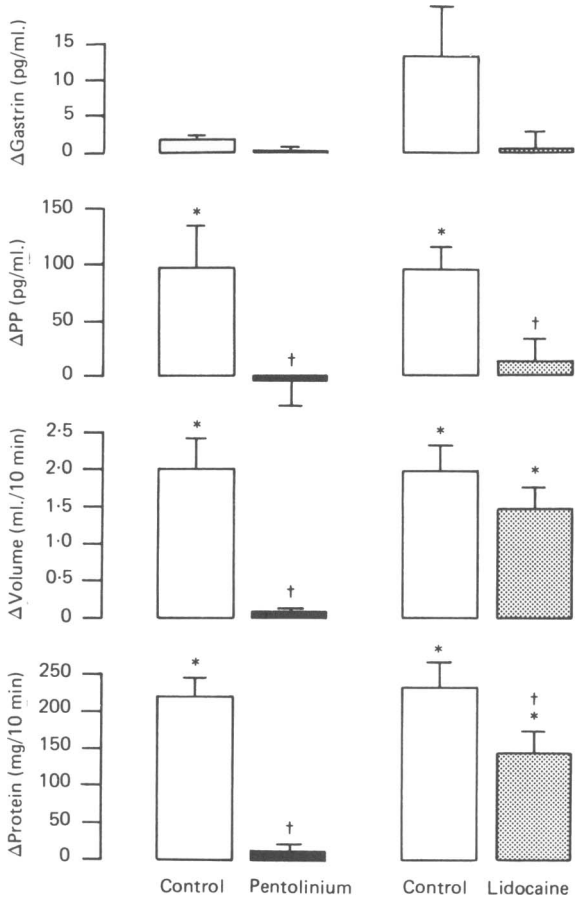

Fig. 5. Effect of pentolinium and duodenal infusion of lidocaine on the periodic increase of pancreatic secretion and plasma PP at the apogee. Controls are differences between apogee and perigee levels preceding the cycle of drug treatment. Since the apogee of secretion and motility of the gut were abolished by pentolinium, plasma levels obtained at the expected apogee time (i.e. 100 min after the last apogee) is used. Mean increase ± S.E. of mean over the perigee are given ($n = 5$). Asterisks indicate the significant increase from perigee. Daggers indicate significant decrease from control.

periodicity and reached their peaks at almost the same time as the controls. However, plasma PP failed to increase, the pancreatic secretion, volume and protein, were less than control (Fig. 5).

When the infusion of lidocaine was stopped, the duodenum recovered quickly from anaesthesia. Peaks of the gastric and duodenal motility, pancreatic secretion and the plasma PP were synchronized again but came earlier than those of the controls.

DISCUSSION

The present study has confirmed Boldyreff (1911) in showing that the fasting secretion of water and protein by the pancreas waxed and waned in phase with the

periodic motor activity of the empty upper gastrointestinal tract. Keane *et al.* (1980) have clearly shown the same in phase fluctuations in plasma PP in fasting dogs, i.e. lowest during the duodenal phase 1 (perigee) and highest during phase III (apogee). Fasting levels of PP range widely (Schwartz *et al.* 1979; Keane *et al.* 1980) and as a whole a significant overlapping between apogee and perigee levels was observed in

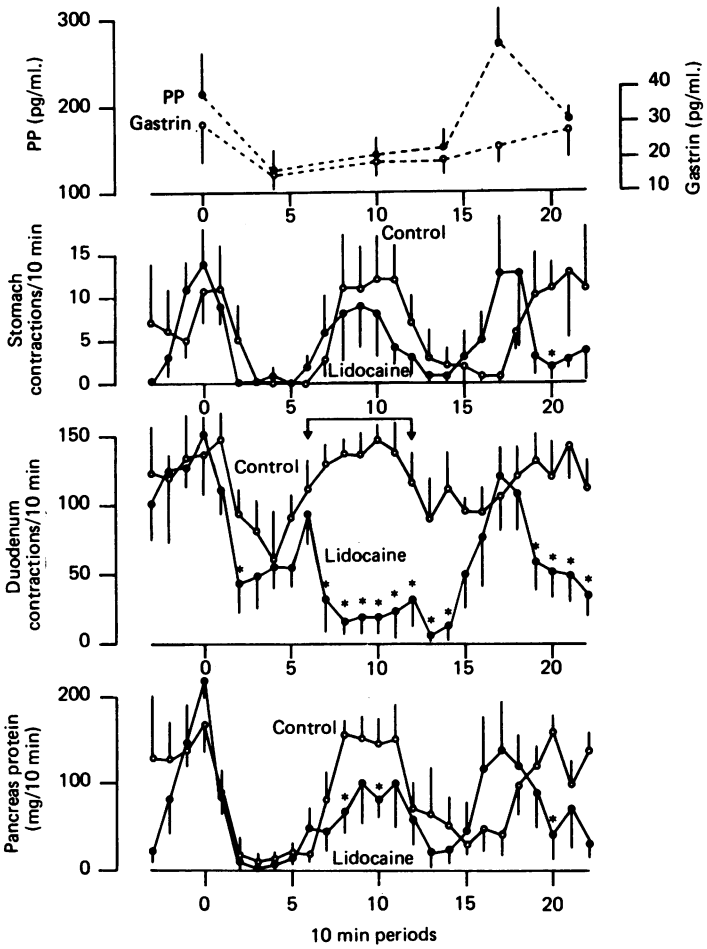

Fig. 6. Effect of duodenal infusion of 2 % lidocaine on periodic contraction and secretion. Open circles and filled circles indicate control and lidocaine, respectively. Mean values ± S.E. of means ($n = 5$) are given. Asterisks indicate significant difference from control.

this study. However the relationship between the consecutive apogee and perigee levels was consistent: the former was higher than the latter. For these reasons plasma levels of the hormones were analysed in relation to pancreatic apogees and perigees and the effect of treatment was tested on the difference from the preceding perigee level.

The entrance of gastric juice to the duodenum is unlikely to be the cause of the periodic secretion of the pancreas and PP. Gastric secretion was diverted to the

exterior in this study and the introduction of alkali into the duodenum did not abolish periodic secretion (Magee & Naruse, 1982). The high content of protein in pancreatic juice suggest that it is caused either by a nervous or cholecystokinin mechanism.

Involvement of a cholinergic mechanism seems to be certain, because both the periodic contractions of the gut (Ormsbee & Mir, 1978) and the spontaneous increase of plasma PP (Schwartz *et al.* 1979) are suppressed by atropine. In the present study, pentolinium also abolished the cyclical increase of plasma PP, pancreatic secretion and gut motor activity, suggesting that ganglionic mechanisms were also involved. Schwartz *et al.* (1979) believed that the spontaneous increase of plasma PP reflected the abdominal 'vagal tone', based on the assumption that spontaneous acid secretion is caused by the vagus in man. Gastrin levels, which are under the control of vagi, are not significantly correlated with the motor activities of the gut either in men (Peeters, Vantrappen & Janssens (1980) or dogs (Keane *et al.* 1980). Our observations on gastric secretion and on plasma gastrin were essentially the same as those by Boldyreff (1911), Keane *et al.* (1980) and Peeters *et al.* (1980). More direct evidence (i.e. vagotomy) will be necessary to clarify the role of vagi in this phenomenon.

Gastrointestinal mechanisms seem to play an important role in food-stimulated PP secretion, though conflicting results on vagal involvement have been obtained even by the same authors (Schwartz *et al.* 1976; Adrian *et al.* 1977; Floyd *et al.* 1977; Taylor *et al.* 1978*a*, *b*; Lamers, Diemel & Jansen, 1982). A great decrease in the spontaneous release of PP by duodenal anaesthesia is hardly a systemic effect of absorbed lidocaine, because periodic secretion reappeared within 30 min after lidocaine was stopped. Boyes, Adams & Duce (1970) have shown that plasma lidocaine levels are maintained for more than 2 hr after stopping its infusion. Since the vagus and the stomach were left intact during the study, this suggested that the basal cyclical release of PP is dependent on duodenal mechanisms. However, since local anaesthetics inhibit both neural and humoral mechanisms (Yamazaki, 1982), this does not exclude the possibility that duodenal hormones may also participate in this phenomenon. It may be concluded that both the cyclical release of PP and of pancreatic secretion are under the control of the intrinsic nervous system in the duodenum.

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