Secretion of Pancreatic Polypeptide in Man in Response to Beef Ingestion is Mediated in Part by an Extravagal Cholinergic Mechanism

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The effect of atropine, a muscarinic cholinergic blocking agent, on the response of plasma pancreatic polypeptide (hPP) to the ingestion of beef was investigated. Six healthy subjects ingested 250 g broiled ground beef on three occasions. After beef ingestion alone, the expected biphasic plasma hPP response was observed. On the two other occasions atropine (intravenous bolus followed by infusion) was begun either at 4 or 60 min after the beginning of beef ingestion so as to coincide with the early (first) and late (second) phases of hPP response to beef ingestion. On both occasions plasma hPP concentrations returned rapidly to baseline. Mean integrated incremental hPP responses in the absence of atropine were 9.1 ± 3.4 ng min ml^{-1} for the first phase (0–40 min) and 29.7 ± 5.7 ng min ml^{-1} for the second phase (60–180 min); with atropine at 4 min, respective responses were 0.8 ± 0.9 and -1.0 ± 1.3 ng min ml,$^{-1}$ and with atropine at 60 min they were 10.6 ± 5.0 and 1.3 ± 1.6 ng min ml.$^{-1}$ After atropine administration, the half-time of disappearance of hPP from the circulation was 4–6 min, suggesting the complete cessation of stimulated hPP secretion. We conclude that the mechanisms of both the early and late phases of beef meal-stimulated release of hPP involve muscarinic cholinergic-neural transmission. The portion of the second (late) phase response which has been shown to persist after truncal vagotomy must be mediated by a cholinergic mechanism which is extravagal in character.

THE INGESTION of a protein-rich meal causes biphasic release of human pancreatic polypeptide (hPP).[1,2,3] The initial phase of the response lasts 30–40 min, and has cephalic,[4,5] gastric[5] and small-intestinal[6] components; within the first 6 mo after vagotomy, this initial phase is abolished.[3,7] The second phase of hPP release occurs 30–40 min after meal ingestion, persists to some degree after vagotomy[3,4,5,7] and appears, therefore, to be vagally mediated only in part. Direct effects of absorbed nutrients upon the hPP cell to stimulate secretion of hPP are probably of minor consequence, since intravenously administered nutrients are at most only weak hPP secretagogues.[1,2,8,9,10] Gut hormones released into blood and/or extravagal neural mechanisms in addition to vagal neural transmission may be important in the second phase of PP responses.

Whereas others have investigated the effects of atropine when it was administered before food was ingested, we investigated its effect when administered after beef had been ingested and when early and late phase plasma hPP responses had already been initiated. When atropine was given after the initiation of either phase, the plasma hPP concentration quickly returned to baseline, suggesting cholinergic mediation of both phases of hPP secretion stimulated by the ingestion of protein-rich meals.

MATERIALS AND METHODS

All subjects gave written consent to the study which had been approved by the Human Use Committee of the University of Michigan Hospital, Ann Arbor.

Subjects

Six subjects (20–32 yr-old) were within 15% of ideal body weight[11] and had normal glucose tolerance.[12] None had a family history of diabetes or a history of cardiac disease. All had normal electrocardiograms.

Tests

Subjects fasted overnight and arrived at the Clinical Research Unit between 0730 and 0830 hr. All tests were done with the subjects seated in bed. A cannula was placed in an antecubital vein for blood sampling and kept patent with a dilute solution of heparin in saline (100 U/ml). A second cannula was placed in a vein in the opposite forearm for atropine infusion. Four basal samples were obtained at $-30, -15, -5$, and 0 min. Care was taken so that the subjects could neither see nor smell the food before the last basal sample was obtained. The subjects were then instructed to consume one 50 g (uncooked weight) patty of broiled beef and one 30 ml glass of water every 2 min for 10 minutes. Salt and pepper were allowed ad libitum. Venous blood samples were taken in heparinized tubes, 3, 4, 5, 7, 8, 10, 15, 20, 30, 40, 50, 55, 60, 65, 70, 80, 90, 100, 120, 135, 150, 165, 180 min after the beginning of beef ingestion.

Each subject ingested beef on three occasions in random order. On one occasion beef was ingested without atropine infusion. On another occasion, 15 μg/kg atropine was infused over 3 min, starting 4 min after beginning the meal and was followed by an infusion of 17 μg/kg/min which was maintained for 60 min. A third test was done using the same dose of atropine starting 60 min after beginning the meal. In some instances, when the atropine was given at 4 min, extra

water (30–60 ml) was needed to complete the meal. All subjects developed a dry mouth and sinus tachycardia during atropine infusion.

Laboratory Methods

Plasma was separated within 30 min by centrifugation and an aliquot taken for measurement of glucose. The remainder was stored at −20°C for hormone assay. For each hormone measured, all samples from an individual were assayed in a single assay.

Pancreatic Polypeptide Assay

The plasma hPP concentrations were measured by a radioimmunoassay (RIA);[13] rabbit anti-hPP serum (Lot 615-1054B-248-18*), hPP (Lot 615-1054B-200*) as reference standard, and ^{125}I labeled bovine PP (bPP) (Lot 615-D63-188-9*) tracer was used. The mean least-detection limit of the assays was 49 pg/ml plasma. The intraassay coefficient of variation was 12%.

Insulin Assay

Immunoreactive insulin (IRI) was measured by a double antibody RIA.[14] The least detection limit was 1.5 μU/ml plasma. The intraassay variation was 5.0%.

Glucagon Assay

Plasma levels of "pancreatic" glucagon (IRG) were measured using a double antibody RIA (15). The antiserum, G9-1 (Ann Arbor, MI) used in the assay detects the C-terminal region of the glucagon molecule and has negligible (2%–5%) cross reactivity with intestinal glucagon-like material (GLI). It does, however, detect glucagon of varying molecular weight. The least detection limit was 34 pg/ml plasma, the intraassay variation was 4.1%.

Plasma glucose was measured by the procedure of Worthington Biochemical Company based on the coupled-enzyme method of Slein,[16] using hexokinase and glucose-6-phosphate dehydrogenase, with modifications by Bondar and Mead.[17]

Statistical Methods

The results are presented as means ± SE and are given both as hPP concentrations (pg/ml) at time points indicated and as incremental response areas above basal (ng min ml^{-1}) for each of the two phases. Phase I (early phase of beef meal-induced hPP release) was defined as the period from 0 to 40 min. Phase II (late phase) was defined as 60–180 min; this definition made it possible to compare control and atropine test results for only that portion of phase II that followed the time (60 min) at which atropine began to be administered. For the six control tests the nadir between phases I and II occurred at 32 ± 4 min (mean ± SE). Significant differences in plasma hPP incremental area responses of the pairings of the three experimental conditions were determined using the paired t test. A p value of <0.05 was considered significant. The half-time (t ½) of disappearance of hPP from plasma was calculated by linear regression of log hPP concentration vs. time, where t ½ = 0.693/Ke. Ke is the slope of disappearance of hPP from the circulation. Time points 7, 8, 10, 15, and 60, 65, 70 min respectively were used for the calculation of the half-time of disappearance when atropine had been administered at 4 and 60 min.

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*Kindly donated by R. Chance, Eli Lilly and Company, Indianapolis, Indiana.

RESULTS

Ingestion of Beef; Control Test

The biphasic plasma hPP response to beef ingestion is shown in Fig. 1A. The mean ± SE of the basal plasma hPP concentrations was 98 ± 8 pg/ml. The mean ± SE of maximal hPP concentrations was 499 ± 172 pg/ml (not shown in the figure) for phase I and

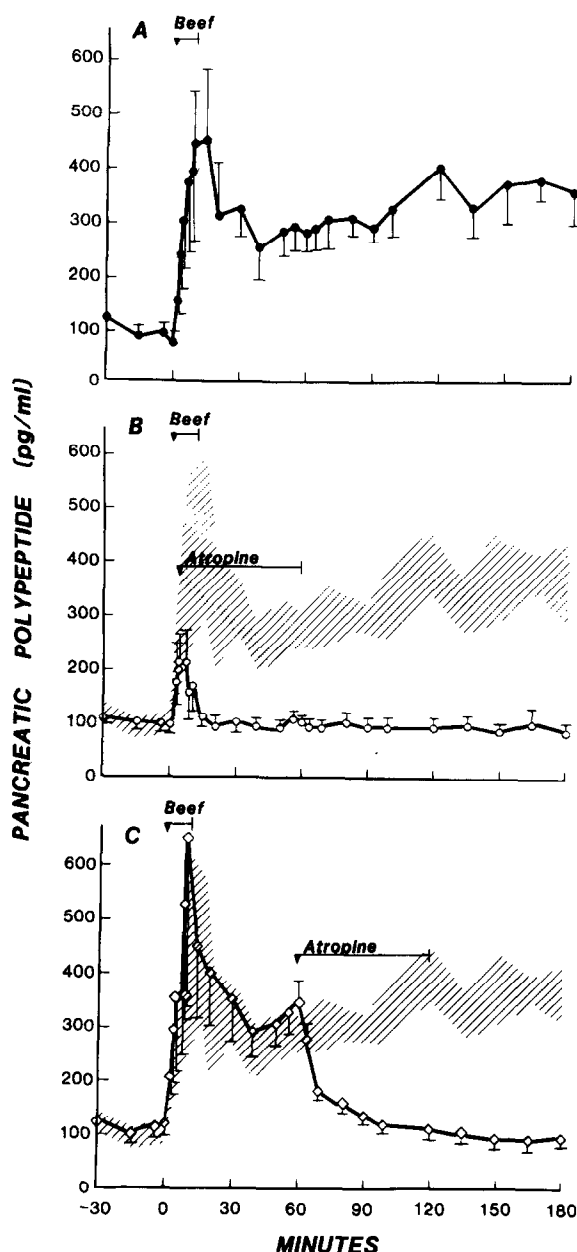Fig. 1. Plasma hPP response of 6 healthy subjects to ingestion of 250 g beef plus 150 ml water over 10 min: (a) without atropine, (b) with infusion of atropine begun at 4 min and (c) atropine begun at 60 min. See Materials and Methods for details of atropine administration. The shaded area in panels B and C encompasses the mean ± SE of the plasma concentration during the control test (panel A).

Table 1. Incremental Plasma hPP Response Areas to Beef Ingestion

	Phase I (0 to 40 min.)	Phase II (60 to 180 min.)
	(ng.min.ml^{-1} ± SE)	
Control	9.1 ± 3.4	29.7 ± 5.7
Atropine at 4 min	0.8 ± 0.9*	−1.1 ± 1.3*
Atropine at 60 min	10.6 ± 5.0	1.3 ± 1.6*

*$p < 0.05$ Compared to Control.

occurred 16.7 ± 2.8 min after beginning the meal ingestion. Plasma hPP levels fell to a mean nadir at 30 min, after which a second rise began and reached a mean maximal concentration of 465 ± 57 pg/ml (not shown in figure), at 144 ± 17 min. The incremental area (mean ± SE) of plasma hPP response for the first phase (0–40 min) was 9.1 ± 3.4 ng min ml^{-1} and for the second phase (60–180 min) was 29.7 ± 5.7 ng min ml^{-1} (Table 1). Incremental area responses of glucose, insulin (IRI), and glucagon (IRG) are shown in Table 2. IRI rose significantly above basal during phase I (289 ± 112 μU min ml^{-1}) and phase II (1718 ± 407) of the test (Table 2).

Ingestion of Beef; Atropine Administered at 4 Min

During this test (Fig. 1B) mean hPP concentration rose rapidly from a basal of 105 ± 13 pg/ml to 198 ± 54 pg/ml at 4 min when atropine was begun. Mean plasma hPP concentration continued to rise for another 1 min after which it fell to reach baseline at 15 min where it remained for the duration of the test. The mean maximal hPP concentration during phase I was 237 ± 58 pg/ml reached at 5.7 min. The incremental response areas for phase I, 0.8 ± 0.9 ng min ml,$^{-1}$ and for phase II, −1.1 ± 1.3 ng min ml,$^{-1}$ were significantly less ($p < 0.05$) than the control responses (Table 1). The insulin response was abolished in both phase I and phase II (Table 2). In both phases glucagon and glucose increased less than during the control test.

Ingestion of Beef; Atropine Administered at 60 Min

Phase I plasma hPP concentrations (Fig. 1C) and plasma hPP, IRI, IRG and glucose response areas

(Tables 1 and 2) were similar to those during the control test. Following the phase I peak, hPP concentrations decreased to a mean nadir of 281 ± 45 pg/ml at 41 ± 4 min which was similar to the control response (Fig. 1C). The mean plasma hPP then rose to 345 ± 35 pg/ml at 60 min at which time atropine administration was begun. Within 5 min mean plasma hPP concentration had begun to fall and had returned to the basal level by 90 min. Thus, for phase II the incremental integrated hPP response area, 1.3 ± 1.6 ng min ml,$^{-1}$ was significantly less than the response during the control test (Table 1). Phase II IRI, IRG, and glucose responses were also less than those during the control test.

In all subjects, the administration of atropine resulted in a rapid fall of hPP concentrations to basal levels. The mean half-time of disappearance of hPP from plasma was 4.17 min when atropine was given at 4 min, and 5.88 min when it was given at 60 min.

DISCUSSION

These studies demonstrate that both the early (phase I) and late (phase II) phases of the plasma hPP response to protein ingestion are rapidly terminated by atropine infusion and, therefore, are cholinergically mediated. Taylor et al. had shown in the dog that administration of cholinergic blocking agents prior to meal ingestion prevents development of both phases.[7] In the present study we administered atropine in separate experiments soon after the early and soon after the late phases had been established. In this way the observed effects of cholinergic blockade could be related more particularly to mechanisms which contribute to these two response phases.

The early phase of hPP release by food ingestion appears to be mediated by the vagus nerve as it is eliminated by truncal vagotomy.[3,5,7,9] It was, therefore, not unexpected that an early phase response already initiated would be abolished by atropine administration.

The mechanisms mediating the second phase of the plasma hPP response which becomes discernable at 30–40 min after protein ingestion appear to be more

Table 2. Incremental Plasma Insulin, Glucagon and Glucose Response Areas to Beef Ingestion

	Phase I (0–40 min.)			Phase II (60–180 min.)		
	Insulin μU.min.ml^{-1}	Glucagon pg.min.ml^{-1}	Glucose mg.min.dl^{-1}	Insulin μU.min.ml^{-1}	Glucagon pg.min.ml^{-1}	Glucose mg.min.dl^{-1}
Control	†289 ± 112	514 ± 336	56 ± 25	†1,718 ± 407	3,891 ± 1,746	385 ± 326
Atropine at 4 min	23 ± 69*	−22 ± 87	−100 ± 90	53 ± 237*	−1,026 ± 704*	−136 ± 295*
Atropine at 60 min	†304 ± 145	391 ± 64	−17 ± 54	†546 ± 216*	1,041 ± 395	−234 ± 257*

*Significant decrease from control area $p < 0.05$.

†Significant response above basal area $p < 0.05$.

complex than those which mediate phase I response. The stimulus for phase II release probably includes stretching of the stomach[18] and duodenum[6] and the effects of particular nutrients in the stomach and intestine.[1,10,19,20] The degree to which the signals for phase II meal-induced release of hPP originate in the stomach as compared to the intestine is unknown, but the persistence of the plasma hPP response for up to 6 hr after the ingestion of protein[1] suggests a major role for the intestine. Circulating absorbed products of digestion evidently contribute little or none[1,2,9,10,21] to the phase II response. It can be presumed that the intestinal component of the phase II response was present at the time of late (60 min) atropine administration since a significant degree of gastric emptying would have occurred by that time. Some phase II response is demonstrable consistently early (<6 mo) and late after truncal vagotomy and pyloroplasty in man[3,5,9] and dog;[7] vagal denervation was confirmed by the Hollander test[9] and pentagastrin stimulation.[3] The persistence of a phase II response in the absence of vagal inervation indicates the participation in the response of an extravagal mechanism. The abrupt complete termination of the response by atropine as shown in this study indicates that the phase II extravagal mechanism is cholinergic in nature.

This extravagal mechanism may be humoral, neural or both.[5] One or more GI hormones released into blood in response to a meal may contribute as a humoral extravagal mechanism(s) for hPP release. Secretin[22,23] CCK-8[22] and bombesin,[24] each stimulates the release of hPP, but this has not been demonstrated at physiologic doses. As is the case of phase II beef response, the stimulating effect of secretin is demonstrable after truncal vagotomy but is entirely blocked by atropine.[23] Neural extravagal signal transmission requires postulation of nerves directly connecting the gastrointestinal tract and the pancreatic endocrine cells, as well as a means by which particular nutrients in the gastrointestinal tract activate this transmission system.

The increases in IRI and IRG plasma levels were relatively small and response phases not as distinct as with hPP. The effects of atropine upon the IRI and IRG response areas during phases I and II, however, were qualitatively similar to the effects upon the hPP response areas and, therefore, may have been mediated through a similar mechanism(s). Knowledge of the character and route of signals transmitted from the GI tract to the hPP cell may have a more general applicability, i.e., to the regulation of secretion of other pancreatic endocrine cells.

REFERENCES

1. Floyd JC Jr, Fajans SS, Pek S, et al: A newly recognized pancreatic polypeptide: Plasma levels in health and disease. Recent Prog Horm Res 33:519–570, 1977
2. Adrian TE, Bloom SR, Besterman HS, et al: PP-physiology and pathophysiology, in Bloom SR (ed): Gut Hormones, Edinburgh, Churchill Livingstone, 1978, pp 254–260
3. Schwartz TW, Rehfeld JF, Stadil F, et al: Pancreatic polypeptide response to food in duodenal-ulcer patients before and after vagotomy. Lancet 1:1102–1105, 1976
4. Schwartz TW, Stenquist B, Olbe L: Cephalic phase of pancreatic-polypeptide secretion studied by sham feeding in man. Scand J Gastroenterol 14:313–320, 1979
5. Taylor IL, Feldman M, Richardson CT, et al: Gastric and cephalic stimulation of human pancreatic polypeptide release. Gastroenterology 75:432–437, 1978
6. Fink AS, Floyd JC Jr, Fiddian-Green RG: Release of human pancreatic polypeptide and gastrin in response to intraduodenal stimuli: A case report. Metabolism 28:339–342, 1979
7. Taylor IL, Impicciatore M, Carter DC, et al: Effect of atropine and vagotomy on pancreatic polypeptide response to a meal in dogs. Am J Physiol 235:E443–E447, 1978
8. Marco J, Hedo J, Villaneuva M: Control of pancreatic polypeptide secretion by glucose in man. J Clin Endocrinol Metab 46:140–145, 1978
9. Adrian TE, Bloom SR, Besterman HS, et al: Mechanism of pancreatic polypeptide release in man. Lancet 1:161–163, 1977
10. Taylor IL, Byrne W, Walsh JH, et al: Pancreatic polypeptide (PP) release by amino acids. Regulatory Peptides (Supplement 1). Abstracts of the Third International Symposium on Gut Hormones, Cambridge, U.K., September 14–18, 1980

11. Metropolitan Life Insurance Company Statistical Bulletin 40:Nov–Dec, 1959
12. National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 28:1039–1057, 1979
13. Berger D, Crowther RC, Floyd JC Jr, et al: Effect of age on fasting plasma levels of pancreatic hormones in man. J Clin Endocrinol Metab 47:1183–1189, 1978
14. Hayashi M, Floyd JC Jr, Pek S, et al: Insulin, proinsulin, glucagon and gastrin in pancreatic tumors and in plasma of patients with organic hyperinsulinism. J Clin Endocrinol Metab 44:681–694, 1977
15. Pek S: Glucagon and insulin, in Rothfeld B (ed): Nuclear Medicine: Endocrinology, Philadelphia, JB Lippincott, 1978, pp 122–140
16. Slein MW: D-glucose determination with hexokinase and glucose-6-phosphate dehydrogenase, in Bergmeyer HU (ed): Methods of Enzymatic Analyses, New York, Academic Press, 1963, pp 117–123
17. Bondar RSL, Mead D: Evaluation of glucose-6-phosphate dehydrogenase from leuconostoc mesenteroids in the hexokinase method for determining glucose in serum. Clin Chem 20:586–590, 1974
18. Schwartz TW, Grötzinger U, Schöön I-M, et al: Vago-vagal stimulation of pancreatic-polypeptide secretion by graded distention of the gastric fundus and antrum in man. Digestion 19:307–314, 1979
19. Taylor IL, Kauffman GL, Walsh JH, et al: Release of pancreatic polypeptide (PP) by intestinally perfused liver extract. Gastroenterology 76:1259A, 1979

20. Wilson RM, Boden G, Owen, OE: Pancreatic polypeptide responses to a meal and to intraduodenal amino acids and sodium oleate. Endocrinology 102:859–863, 1978

21. Hedo JA, Villanueva ML, Marco J: Influence of plasma free fatty acids on pancreatic polypeptide secretion in man. J Clin Endocrinol Metab 49:73–77, 1979

22. Scarpello JH, Vinik AI, Owyang C: Relative potencies and additive effects of secretin, pentagastrin and CCK-8 as secretogogues of human pancreatic polypeptide. Clin Res 28:284A, 1980

23. Glaser B, Vinik AI, Sive AA, et al: Plasma human pancreatic polypeptide responses to administered secretin: Effects of surgical vagotomy, cholinergic blockade and chronic pancreatitis. J Clin Endocrinol Metab 50:1094–1099, 1980

24. de Magistris L, Delle Fave G, Kohn A, et al: Stimulation of pancreatic-polypeptide and gastrin secretion by bombesin in man. Life Sci 28:2617–2621, 1981