# Synthetic and Secretory Effects of Cholecystokinin–Pancreozymin on the Pigeon Pancreas[1,2,3] (34870)

M. M. SAHBA, J. A. MORISSET, AND P. D. WEBSTER

*Section of Gastroenterology, Veterans Administration Hospital, and Medical College of Georgia, Augusta, Georgia 30904*

There is little information concerning mechanisms involved with control and integration of pancreatic zymogen secretion and synthesis. The "secretory cycle" as outlined by Heidenhain (1875) postulated that exocrine cells secrete and then initiate processes whereby product was synthesized. In some manner secretion initiated synthesis (1). Hokin and co-workers suggested that secretion and synthesis were independently controlled processes and that secretion might proceed without synthesis (2). The latter point of view would receive support if it were demonstrated *in vivo* that enhancement of secretion could occur without perceptible changes in rates of synthesis.

This work results from efforts to extend previous reports concerning mechanism of action of cholecystokinin–pancreozymin on the pigeon exocrine pancreas (3).

These studies establish three points: (1) Cholecystokinin-pancreozymin (CCK-PZ) administered intravenously to pigeons in doses ranging from 10–100 Ivy units/kg was followed promptly by amylase secretion into the duodenum. Increased secretion was apparent within 5 min, approached maximal rates within 20–30 min, and returned toward basal rates within 60–80 min. (2) Amylase content of pancreatic tissue from cholecystokinin-pancreozymin (40 Ivy units/kg)-treated birds was less than amylase content of control birds. (3) Cholecystokinin-pancreozymin administration (40 Ivy units/kg) was not associated with a demonstrable increase in incorporation of L-phenylalanine-$^{14}$C into pancreatic proteins when compared with control studies.

The data demonstrate, at least for the time periods under consideration, that *in vivo* secretion can be augmented without apparent changes in synthesis. The data suggest that, perhaps, within physiologic range, cholecystokinin–pancreozymin acts to alter rates of release of pancreatic zymogens (secretion) but has little influence over rates of zymogen synthesis.

*Materials and Methods.* Studies were performed using white Carneau pigeons (450-500 g weight, 6–8 weeks of age) either fasted 3 days or fed *ad libitum* (3). All birds had free access to water. Variability between groups of birds in amino acid-$^{14}$C incorporation and amylase content in pancreatic tissue has been commented on previously (4).

Cholecystokinin–pancreozymin was obtained from Gastrointestinal Hormone Research Laboratories under the direction of Doctors Jorpes and Mutt, Chemistry Department, Karolinska Institute, Stockholm, Sweden. Urethane was purchsed from Fisher Scientific Company, Atlanta, Georgia. Sources of other materials have been described (3, 5).

*Studies of pancreatic amylase secretion.* Fed pigeons were anesthesized with urethane (1.0 mg/kg) given intramuscularly in 1 ml of saline. Methods employed for intubation of the duodenum and collection of pancreatic secretions have been described (5). Amylase activity (units) represents amounts of

[1] Supported by Research Grant AM 13131-01 from the National Institute of Arthritis and Metabolic Diseases and a Medical Investigator Award from the Veterans Administration.

[2] Presented in part at the Gastroenterology Research Forum held in Washington, D. C. May 1969.

[3] Doctor Morisset is a Postdoctoral Research Fellow, National Research Council of Canada and Medical Research Council of Canada.

amylase secreted per 10-min period of collection.

At the beginning of each experiment, the duodenum was irrigated with 150 ml saline. The first 10 ml of this washed solution was collected and identified as the zero time sample. After irrigation of the duodenum, four 10-min (10 ml each) fractions were collected (Fractions 1–4); cholecystokinin–pancreozymin was given through a wing vein in 10, 20, 40, and 100 Ivy units/kg doses in 1-ml volume of saline; fractions 5–15 collected; a second injection of cholecystokinin–pancreozymin given; and fractions 16–20 collected.

L-*Phenylalanine-$^{14}C$ incorporation studies.* Three pigeons were given intravenously either physiologic saline solution or indicated doses of cholecystokinin–pancreozymin dissolved in 0.5 ml saline. The pigeons were killed, pancreatic tissue slices prepared, and incubated in tissue culture media with 1 $\mu$Ci of L-phenylalanine-$^{14}C$ as described (4, 5).

*Assay of protein.* Protein content was measured using methods described by Gornall *et al.* (6).

*Assay of amylase activity.* Amylase activity was assayed using methods described by Bernfeld (7). A unit represents that amount of amylase which catalyzes the formation of 1 mg of maltose in 3 min at 30°.

*Assay of radioactivity.* This has been described previously (4, 8).

*Results.* The secretory response of the pigeon pancreas to intravenous pancreozymin (5, 10, 20, and 50 Ivy units/500 g body weight) is demonstrated in Fig. 1. Amylase activity appearing in the duodenum is graphed on the ordinate, time in minutes along the abscissa. Within 5–10 min after administration of cholecystokinin-pancreozymin, there was an increase in amounts of amylase secreted into the duodenum. The peak secretory response was observed within 20–40 min and within 60–80 min, the rate of amylase secretion had returned to basal levels. A second injection of cholecystokinin–pancreozymin was given at 160 min and again a prompt increase in amylase secretion appeared. The response appeared similar to that observed after the initial dose.

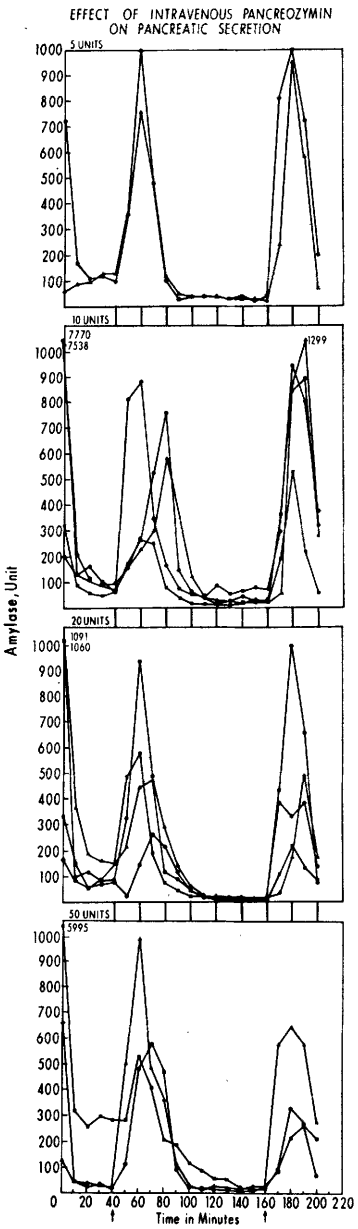


FIG. 1. Rate of amylase secretion into pigeon duodenum after 5, 10, 20, and 50 Ivy units/500 g body weight of intravenous CCK–PZ. Ordinate represents time in minutes. ↑ indicates time of CCK–PZ injection.

There was considerable variability from bird to bird in amounts of amylase secreted after a given dose of cholecystokinin–pancreozymin. However, there was fairly uni-

TABLE I. Effect of *in Vivo* Cholecystokinin–Pancreozymin on L-Phenylalanine-$^{14}$C Incorporation by Pigeon Pancreas Slices.

| Fed or fasted[a] | *In vivo* incubation (min)[b] | Dose of CCK-PZ[c] (units) | No. of exp.[d] | L-Phenylalanine-$^{14}$C[e] mg protein | | % Diff.[f] | *p* value[g] |
|---|---|---|---|---|---|---|---|
| | | | | Saline | CCK-PZ | | |
| Fed | 30 | 40 | 5 | 1038 ± 171 | 1068 ± 274 | + 3.0% | >0.1 |
| Fed | 60 | 40 | 8 | 1484 ± 403 | 1534 ± 311 | + 3.3% | >0.1 |
| Fed | 90 | 40 | 9 | 1604 ± 579 | 1852 ± 452 | +15.4% | >0.1 |
| Fed | 120 | 40 | 11 | 1136 ± 499 | 970 ± 437 | −14.6% | >0.1 |
| Fasted | 30 | 40 | 3 | 491 ± 227 | 521 ± 86 | + 6.1% | >0.1 |
| Fasted | 60 | 40 | 3 | 593 ± 263 | 548 ± 173 | + 7.5% | >0.1 |
| Fasted | 90 | 40 | 3 | 612 ± 55 | 629 ± 121 | + 2.7% | >0.1 |
| Fasted | 120 | 40 | 3 | 593 ± 183 | 645 ± 84 | + 8.9% | >0.1 |
| Fasted | 180 | 40 | 3 | 518 ± 128 | 454 ± 217 | −13.0% | >0.1 |

[a] Fasted or fed pigeons were given either saline or cholecystokinin–pancreozymin intravenously.

[b] *In vivo* incubation period represents time from injection of saline or CCK–PZ until sacrifice.

[c] Dose of CCK–PZ in Ivy units/kg body weight.

[d] Number of experiments.

[e] Pancreatic slices were incubated in tissue-culture media and incorporation of L-phenylalanine-$^{14}$C into whole tissue protein determined. Mean value ± SD.

[f] Percent difference between saline and CCK–PZ groups.

[g] Statistical difference between groups of paired animals.

form response as to onset and duration of secretion.

Table I shows the effects of 40 Ivy units/kg of cholecystokinin-pancreozymin given intravenously on L-phenylalanine-$^{14}$C incorporation by pancreatic tissue slices prepared from fasted or fed pigeons. Fed pigeons given cholecystokinin–pancreozymin (40 Ivy units/kg) and killed after 30, 60, 90, or 120 min showed a +3%, +3.3%, +15.4%, and −14.6% difference in L-phenylalanine-$^{14}$C incorporation into pancreatic tissue protein when compared with control pigeons. Fasted pigeons given cholecystokinin-pancreozymin and killed after 30, 60, 90, 120, and 180 min showed a +6%, −7.5%, +2.7%, +8.9%, and −13% difference in L-phenylalanine-$^{14}$C incorporation into pancreatic tissue protein when compared with control pigeons. There was no statistical difference between any group for any time period studied. As previously reported, fed pigeons incorporated greater amounts of L-phenylalanine-$^{14}$C into tissue proteins than fasted pigeons (3). These data demonstrate that paired groups of pigeons show considerable variability; for example, +15% to −14.6% difference. However, there was no pattern of differences, *i.e.*, experimental always greater than control, despite the fact that up to 11 experiments were performed for one time period. Despite the rather large distribution of values around zero, it was concluded that there was no real difference in amounts of L-phenylalanine-$^{14}$C incorporation between fed or fasted pigeons given 40 Ivy units/kg of cholecystokinin–pancreozymin or saline.

Three experiments were performed to examine effects of intravenous cholecystokinin–pancreozymin, 100 Ivy units/kg, on L-phenylalanine-$^{14}$C incorporation using pancreatic tissue slices prepared from fed pigeons. There was no difference between control and cholecystokinin–pancreozymin-treated groups when the animals were killed 90 min after injection (control, 1320 cpm/mg protein; cholecystokinin–pancreozymin, 1220 cpm/mg proteins).

Three experiments were performed to determine whether the present batch of

cholecystokinin–pancreozymin used in these experiments would elicit an enhancement of pancreatic protein synthesis as previously reported (3). A 177% increase in rates of L-phenylalanine-$^{14}$C incorporation into whole tissue proteins was obtained when 3-day fasted pigeons were given 100 Ivy units/kg of cholecystokinin–pancreozymin and killed 90 min later (control, 450 cpm/mg protein; cholecystokinin–pancreozymin, 1250 cpm/mg protein). These studies confirm our previous report (3).

Table II shows amylase content of pancreatic tissue obtained from fed pigeons given saline or cholecystokinin–pancreozymin. For all times groups (30, 60, 90, and 120 min) amounts of amylase per milligram tissue wet weight were less in birds pretreated with cholecystokinin-pancreozymin when compared with those given saline. Thus, not only was secretion obtained, but secretion was of sufficient magnitude to result in decreased amylase content of the tissue.

*Discussion.* The secretory studies were performed to answer three questions: (1) Does porcine cholecystokinin-pancreoyzmin have a secretory effect in pigeons? (2) When is secretion initiated after intravenous cholecystokinin-pancreozymin? (3) What is the duration of this response? These findings document the temporal secretory response of the pigeon pancreas to 10, 20, 30, 40, and 100 Ivy units/kg of porcine cholecystokinin–pancreozymin administered intravenously. Not only was secretion initiated after 20 Ivy units of cholecystokinin–pancreozymin, but sufficient amylase was transported from the pancreas to effect a decrease in total amylase content.

TABLE II. Amylase Content of Pancreatic Tissue from Pigeons Given Cholecystokinin–Pancreozymin or Saline.[a]

| *In vivo* incubation[b] (min) | No. exp.[c] | Amylase units[d] mg tissue wet weight Saline | CCK–PZ | % Diff.[e] |
|---|---|---|---|---|
| 30 | 3 | 14.0 ± 2.0 | 6.6 ± 1.7 | —53% |
| 60 | 2 | 10.8 ± 0.4 | 7.4 ± 0.3 | —31% |
| 90 | 4 | 12.0 ± 1.2 | 6.0 ± 0.9 | —49% |
| 120 | 5 | 12.4 ± 2.0 | 7.8 ± 1.0 | —36% |

[a] Fed pigeons given saline or CCK–PZ, 4 units/kg iv.

[b] *In vivo* incubation period represents time from injection of saline or CCK–PZ until sacrifice.

[c] Number of experiments.

[d] Amylase activity of pancreatic tissue. Mean value ± SD.

[e] Percent difference between saline or CCK–PZ treated groups.

Forty Ivy units/kg doses of cholecystokinin–pancreozymin when given to fasted or fed pigeons were not associated with measurable increases in L-phenylalanine-$^{14}$C incorporation into pancreatic tissue proteins. In addition, 100 Ivy units/kg doses of cholecystokinin–pancreozymin to fed pigeons were not associated with detectable increases in amounts of L-phenylalanine-$^{14}$C incorporation.

We were able to elicit increased L-phenylalanine-$^{14}$C incorporation only when 100 Ivy units/kg doses of cholecystokinin–pancreozymin were given to previously fasted pigeons. These experiments agree with previous observations showing 100 Ivy units/kg doses of cholecystokinin–pancreozymin administered to fasted pigeons effected an increase in L-phenylalanine-$^{14}$C incorporation of 126% after 60 min, 200% after 90 min, 60% after 120 min, and 30% after 180 min of *in vivo* incubation (3).

One problem basic to exocrine cell function concerns the manner in which processes of secretion and synthesis are integrated. Heidenhain suggested that after stimulation exocrine cells secrete and then synthesize new product. His morphologic observations have been interpreted by some to suggest a feedback mechanism whereby synthetic processes were activated after decreases of stored product. On the other hand, Hokin and coworkers suggested on the basis of observations derived from *in vitro* systems that secretion of zymogens could occur without measurable increases in rates of zymogen synthesis.

It is far easier to describe rather than explain these observations. However, it seems plausible to suggest that the gastrointestinal hormone, cholecystokinin–pancreozymin, acts primarily as a secretogogue or releasing

factor and that the hormone has little influence on rates of protein synthesis.

*Conclusion.* Studies of effects of cholecystokinin-pancreozymin on pancreatic secretion and synthesis have revealed the following: 40 Ivy units/kg iv to fed or fasted pigeons, enhances secretion without an effect on synthesis; 100 Ivy units/kg to fed pigeons, enhances secretion without change in synthesis; 100 Ivy units/kg doses to 3-day fasted pigeons, enhances secretion as well as synthesis. In order to elicit a synthetic response in the pigeon with intravenous cholecystokinin–pancreozymin, large doses as well as previously fasted animals were required.

These studies indicate that secretory and synthetic responses can be disassociated. The results support the thesis that while synthesis and secretion frequently change in a parallel fashion they can be disassociated and most likely, therefore, have independent, though integrated, control systems.

1. Heidenhain, R., Pflügers Arch. **10**, 557 (1875).

2. Hokin, L. E., and Hokin, M. R., *in* "The Exocrine Pancreas" (A. V. S. de Reuck and M. P. Cameron, eds.), p. 186. Little, Brown, Boston, Massachusetts (1961).

3. Webster, P. D., and Tyor, M. P., Amer. J. Physiol. **211**, 157 (1966).

4. Webster, P. D., Gastroenterology **55**, 375 (1968).

5. Webster, P. D., Proc. Soc. Exp. Biol. Med. **132**, 1072 (1969).

6. Gornall, A. G., Bardawell, C. J., and David, M. M., J. Biol. Chem. **177**, 751 (1949).

7. Bernfeld, P., *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), p. 149. Academic Press, New York (1955).

8. Patterson, M. S., and Greene, R. C., Anal. Chem. **37**, 854 (1965).

Received Mar. 13, 1970. P.S.E.B.M., 1970, Vol. 134.