A New Collection Method for Pancreatic Juice and its Secretory Response to Wing Vein Injection of Cholecystokinin, Glucose and Lysine in Chicks

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Abstract A new collection method for pancreatic juice in chickens was developed. Duodenum of the chick anesthetized with diethyl ether was cannulated with Tygon® tube, through which physiological saline was continuously infused with the aid of a peristaltic pump in order to prevent a plug problem. Pancreatic secretions were successfully collected during an experimental period of 60 min as the mixture of the saline with intestinal juice. By this method, neither the blockage problem nor the irregular secretion of the pancreatic juice was observed. A steady, relatively small basal output of enzymes, amylase, trypsinogen and chymotrypsinogen, occurred when physiological saline or the end products of digestion (glucose, methionine and lysine) were administered by the wing vein injection. Cholecystokinin had an immediate effect on pancreatic enzyme secretion and this response was in a dose dependent fashion. The injection of cholecystokinin seemed to have selective stimulation favoring the secretion of chymotrypsinogen followed by amylase and trypsinogen. The injection of cholecystokinin plus lysine increased the secretion of all enzymes more than that observed with cholecystokinin alone, whereas the injection of cholecystokinin plus glucose stimulated only an amylase secretion. The regulatory mechanism of pancreatic digestive enzyme secretion is discussed.


Key words: pancreatic juice collection, amylase, trypsinogen, chymotrypsinogen, cholecystokinin, chicken

To study the mechanism of pancreatic digestive enzyme secretion in response to nutrients, it is necessary to collect pancreatic juice. In chickens, however, the presence of three or four pancreatic ducts almost precludes the total quantitative collection. In addition, there are difficulties in cannulation even for the major duct, so that complete data on the volume of pancreatic juice secreted are not available. Heatley et al.1) and Dal Borgo et al.2,3) succeeded the cannulation of the main pancreatic duct and reported a flow rate of 9.7 ml/kg body weight per day, but there was no information on the total volume of juice produced by the pancreas and the flow rate was too irregular to accept as quantitative measurements.

The exocrine pancreas synthesizes and secretes a number of enzymes that are
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involved in the digestion of macromolecular food products in the diet. The pancreas is capable of changing the proportion of enzymes in response to changes in the composition of the diet.4,5) Such changes, which occur over a period of time from hours to days, are modulated at the level of biosynthesis of exocrine proteins 6) and may result in an enrichment of enzymes required for hydrolyzing principal substrates present in the diet. Short-term changes in pancreatic secretion following the hormonal stimulation have been observed in human pancreas.7,8) Within 15 min after cholecystokinin (CCK) stimulation, the secretion of lipase and chymotrypsinogen was more stimulated than that of amylase. In rat pancreas, DAGORN et al.9) also observed greater stimulation of the secretion of chymotrypsinogen than that of amylase in response to oleic acid ingestion. ROTHMAN,10) and ROTHMAN and WILKING11) have observed preferential discharge of trypsinogen relative to chymotrypsinogen after CCK stimulation in rabbit pancreas. STEER and MANABE,12) in contrast, found no change in the ratio of the two enzymes. Recently, GRENDELL et al.13) showed that glucose selectively evoked the release of amylase from the pancreas, whereas lysine promoted the selective release of trypsinogen. ROTHMAN14,15) showed that a variation in the proportion of different enzymes could occur in response to the presence of end products of digestive processes in the intestine and blood.

The present study was undertaken to develop a new collection method for pancreatic juice and to investigate the effect on pancreatic secretion of intravenous injection of CCK and/or end products of digestion in chickens.

Materials and Methods

Day-old male white Leghorn chicks, purchased from a local supplier (Hattori Hatchery Co. Ltd. Nagoya) were housed in electrically-heated battery brooders, and given a commercial chick diet ad libitum until two to three weeks of age, weighing between 130 and 160g. Before the operation the chicks were fasted overnight with free access to water. The chick anesthetized with diethyl ether was placed on an operating table with its right side up. All feathers around the operation site were removed. An incision, approximately 2 cm long perpendicular to the last rib, was made through the abdominal skin and muscle layers with a scalpel. The cystic and hepatic ducts were tied up at the end close to the intestine with ligature. The duodenum was brought into position for cannulation by picking it up, and incised 9 mm upper and 9 mm lower parts from the entrance of pancreatic duct. The isolated piece of duodenum was cannulated with Tygon® tube (ID. 1.59, OD. 3.18 mm) to collect the secreted juice from pancreatic duct and the isolated duodenum. The same length of the other part of the duodenum was also cannulated as a blank control to collect the secretion from the intestine itself (Fig. 1). The uncannulated parts of the duodenum were returned to the abdominal cavity. The isolated duodenum was washed twice with physiological saline solution (0.85% NaCl) to clean up the contents. Physiological saline solution was infused from the upper cannula with a peristaltic mini-pump at a rate of 0.07 ml/min. The incision with the protruding duodenum was then covered with gauze.
Fig. 1. A simplified scheme of the collection method of pancreatic juice in chicks. Physiological saline was perfused with a mini-pump at a rate of 0.07 ml/min. Arrows (†) indicate the site at which ligature was made.

moistened with warm saline. The mixtures of saline, intestinal and pancreatic juice were collected for every 10 min from the lower cannula in a tube stood in ice in a thermos bottle. Secretion for the first 10 min following the cannulation was discarded. At 10 min. after the commencement of the collection, each chick received a 0.25 ml injection into the wing vein over 0.5 to 1.0 min. In the 0.25 ml injection solution included were the followings:

1) 0.85% NaCl,
2) 15 mM or 30 mM glucose in 0.85% NaCl,
3) 0.5 mM L-lysine or L-methionine in 0.85% NaCl,
4) 0.06, 0.15, or 0.30 IU CCK in 0.85% NaCl,
5) 0.06 IU CCK plus 0.5 mM L-lysine in 0.85% NaCl, or
6) 0.06 IU CCK plus 15 mM glucose in 0.85% NaCl.

Secretions collected for six 10-min intervals were placed in a screw cap vial, immediately frozen and stored at −20°C until the assays for amylase, trypsinogen and chymotrypsinogen contents. The contents of pancreatic digestive enzymes were corrected by taking account of the contamination of intestinal juice.

The amylase activity was estimated by the method of CARAWAY using the Wako Amylase test (Wako pure Chemical Industries Ltd.) and expressed as Caraway units. Trypsinogen was converted to trypsin by incubating with purified enteropeptidase (Miles Laboratories Ltd. South Africa) for 30 min at 37°C. Based on the method of HUMMEL, the rate of hydrolysis of p-toluenesulfonyl-L-arginine methyl ester (Sigma Chemical, St. Louis, USA) was measured by the increase in absorbance at 247 nm.
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Trypsin activity was expressed in terms of micrograms, using crystalline trypsin (Sigma Chemical, St. Louis, USA). Chymotrypsinogen was converted to chymotrypsin as described in the procedure for the activation of trypsinogen. The procedure used to determine chymotrypsin activity was based on the rate of hydrolysis of N-benzoyl-L-tyrosine ethyl ester (Sigma Chemical, St. Louis, USA) by determining the change in absorbance at 256 nm according to the method of HUMMEL.\(^{17}\) Chymotrypsin activity was expressed in terms of micrograms, using crystalline chymotrypsin (Sigma Chemical, St. Louis, USA).

Analysis of variance was used to analyze the data statistically and for comparison of the individual treatment differences, the Duncan’s multiple range test (DUNCAN)\(^{18}\) was applied.

![Graphs showing enzyme secretion](image)

Fig. 2. Effect of wing vein injection of physiological saline (●—●), 15 mM glucose (○—○) or 30 mM glucose (◇—◇) on the pancreatic secretion of amylase, trypsinogen and chymotrypsinogen. For each experiment enzyme secretion was expressed as percentage of the preinjection level (0 min) to compensate variance between chicks in basal (preinjection) enzyme secretion. Vertical bars represent pooled SEM of 5 observations.
Results

Figure 2 shows the effect of the wing vein injection of 15 mM or 30 mM glucose on the pancreatic secretion of amylase, trypsinogen and chymotrypsinogen. For each experiment enzyme output was expressed as percentage of preinjection level (0 min) to compensate the variance between chicks in basal (preinjection) enzyme output. Enzyme secretions in general decreased gradually over the time with the injections of saline, 15 mM glucose or 30 mM glucose and there was no significant difference in the pattern of each enzyme secretion between the treatments.

Figure 3 shows the effect of wing vein injection of 0.5 mM lysine or methionine on the pancreatic secretion of amylase, trypsinogen and chymotrypsinogen. With the

![Graphs showing enzyme secretion over time](image)

Fig. 3. Effect of wing vein injection of 0.5 mM lysine (○—○) or 0.5 mM methionine (●—●) on the pancreatic secretion of amylase, trypsinogen and chymotrypsinogen. For each experiment enzyme secretion was expressed as percentage of the preinjection level (0 min) to compensate variance between chicks in basal (preinjection) enzyme secretion. Vertical bars represent pooled SEM of 4 and 5 observations for lysine and methionine treatments, respectively.
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Lysine injection the enzyme secretions slightly increased for the first 10 min and thereafter trypsinogen maintained its secretion rate for 50 min but amylase and chymotrypsinogen secretions decreased and then increased. With the methionine injection the secretions of all enzymes analyzed decreased gradually.

Figure 4 shows the effect of wing vein injections of 0.06, 0.15 or 0.30 IU CCK on the pancreatic secretion of amylase, trypsinogen and chymotrypsinogen, expressed in terms of percentage of the preinjection level. Pancreatic enzyme secretions increased with increase in CCK in a dose-dependent fashion and the peak secretion were rapidly reached at 10 to 20 min. The pattern of enzyme output was generally in parallel, but 0.30 IU CCK showed a tendency of a higher secretion of chymotrypsinogen than amylase and trypsinogen, being lowest in the latter.

Figure 5 shows the effect of 0.06 IU CCK alone or in combination with 15 mM glucose or 0.5 mM lysine on the pancreatic secretion of amylase, trypsinogen and chymotrypsinogen. By the addition of 15 mM glucose to 0.06 IU CCK, amylase secretion tended to increase whereas trypsinogen and chymotrypsinogen secretions remained to be comparable to the reaction to CCK alone. Simultaneous injection of 0.5 mM lysine with CCK significantly increased all enzyme secretions relative to those observed with CCK alone.

Table 1 shows the effect of varying levels of CCK from 0.06 to 0.30 IU and

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**Fig. 4.** Effect of wing vein injection of 0.06 (●), 0.15 (○) or 0.30 (△) IU cholecystokinin (CCK) on the pancreatic secretion of amylase, trypsinogen and chymotrypsinogen in chicks. For each experiment enzyme secretion was expressed as percentage of the preinjection period (0 min) to compensate variance between chicks in basal (preinjection) enzyme secretion. Vertical bars represent pooled SEM of 5 observations (except for amylase in 0.15 and 0.30 IU CCK, and trypsinogen in 0.15 IU CCK injection, being 4 observations).
Fig. 5. Effect of wing vein injection of 0.06 IU CCK (○—○) alone or in combination with 15 mM glucose (●—●) or 0.5 mM lysine (◆—◆) on the pancreatic secretion of amylase, trypsinogen and chymotrypsinogen in chicks. For each experiment enzyme secretion was expressed as percentage of the preinjection level (0 min) to compensate variance between chicks in basal (preinjection) enzyme secretion. Vertical bars represent pooled SEM of 5 observations (except for amylase in CCK plus lysine injection, being 4 observations).

Table 1. Effect of the administration of cholecystokinin (CCK) alone or in combination with glucose or lysine on pancreatic amylase, trypsinogen and chymotrypsinogen secretions for 10 and 30 minutes after the wing vein injection

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Amylase (10 min)</th>
<th>Trypsinogen (10 min)</th>
<th>Chymotrypsinogen (10 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Caraway unit)</td>
<td>(µg)</td>
<td>(µg)</td>
</tr>
<tr>
<td>0.06 IU CCK</td>
<td>82 ± 6.9</td>
<td>6.6 ± 1.6</td>
<td>19.1 ± 4.8</td>
</tr>
<tr>
<td></td>
<td>270 ± 58.5</td>
<td>23.1 ± 5.1</td>
<td>101.1 ± 22.8</td>
</tr>
<tr>
<td>0.15 IU CCK</td>
<td>166* ± 11.9</td>
<td>12.9* ± 2.0</td>
<td>50.6 ± 22.5</td>
</tr>
<tr>
<td></td>
<td>409 ± 70.5</td>
<td>35.8 ± 2.4</td>
<td>135.0 ± 22.5</td>
</tr>
<tr>
<td>0.30 IU CCK</td>
<td>353* ± 25.8</td>
<td>21.6* ± 3.0</td>
<td>138.3* ± 19.5</td>
</tr>
<tr>
<td></td>
<td>500* ± 11.0</td>
<td>43.4 ± 7.0</td>
<td>261.7* ± 34.2</td>
</tr>
<tr>
<td>0.06 IU CCK + Glucose</td>
<td>± 23.4 ± 9.6</td>
<td>9.6 ± 2.6</td>
<td>± 23.6 ± 8.3</td>
</tr>
<tr>
<td></td>
<td>70.2 ± 4.6</td>
<td>28.1 ± 4.6</td>
<td>96.3 ± 34.5</td>
</tr>
<tr>
<td>0.06 IU CCK + Lysine</td>
<td>117 ± 19.2</td>
<td>9.6 ± 1.9</td>
<td>± 43.0 ± 16.0</td>
</tr>
<tr>
<td></td>
<td>374 ± 12.8</td>
<td>44.8 ± 4.3</td>
<td>149.5 ± 16.0</td>
</tr>
</tbody>
</table>

Values are means±SEM. n=5 except for n=4 for amylase in the injection of 0.15, 0.30 IU CCK and 0.06 IU CCK + lysine, and trypsinogen in 0.15 IU CCK injection. *Significantly different (P<0.01) from 0.06 IU CCK.
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Simultaneous addition of glucose or lysine to CCK at the lowest dose on the secretions of each enzyme at 10 and 30 min. Pancreatic enzyme secretion increased with the increase in CCK administration. Secretions of amylase and trypsinogen tended to be increased by CCK plus glucose administration compared with those by CCK alone. Injection of 0.06 IU CCK plus lysine significantly increased amylase and trypsinogen outputs by two-fold at 10 min compared with those observed with 0.06 IU CCK alone, amounting to a similar magnitude by 0.15 IU CCK alone. Chymotrypsinogen output by the administration of CCK plus lysine was elevated 1.5 to 2 times in comparison with that by 0.06 IU CCK alone but no significant difference was detected at either 10 min or 30 min.

Discussion

In the present paper, the authors developed a simplified new collection method for pancreatic juice through the intestine with the aid of a peristaltic mini-pump. The method worked reasonably well. Neither the blockage problem nor the irregular secretion of the pancreatic juice was observed. This procedure, however, required several incision to be made. In addition, a pure flow rate of the pancreatic juice could not be measured since the collected juice was diluted about five- to ten-fold by infusates. The pancreatic juice was collected as the mixture with saline and intestinal juice, but the correction made by the measurement of intestinal juice per se made no difference in the conclusions reached, because the juice had only negligible enzyme activities.

In the present experiment, there were three distinct responses of pancreatic enzyme secretion in chicks. First, in the absence of CCK a steady and relatively small basal output of enzymes occurred when physiological saline or end products of digestion was administered by the wing vein injection. Secondly, CCK, the intestinal hormone, had an immediate effect on pancreatic enzyme secretion and the response was in a dose dependent fashion. Thirdly, CCK with lysine or glucose showed a synergistic action on stimulative secretion of some pancreatic digestive enzymes.

A nonparallel stimulatory effect on digestive enzyme secretion in response to CCK in rats was reported by Dagorn7), who showed that chymotrypsinogen secretion was largely enhanced. Tseng et al.19) also reported a nonparallel stimulation by CCK which caused a greater secretion of amylase than trypsinogen in rats. The present experiment showed that pancreatic digestive enzymes in chicks responded to wing vein injection of CCK in a dose dependent fashion, which seemed to be parallel at doses of 0.06 and 0.15 IU, while 0.30 IU CCK seemed to have a selective stimulation favoring a greatest increase in chymotrypsinogen followed by amylase and trypsinogen.

Grossman20) reported that CCK actions were on the stimulation of pancreatic enzyme secretion and of contraction of the gallbladder. Dagorn21) postulated the existence of two types of acinar cells, one continuously secreting digestive enzyme and unresponsive to stimuli, such as CCK, and the other being induced only by CCK. These two groups of cells could contain different proportions of enzymes, and hence secretion
would be nonparallel as a result of the hormone addition. However, Dagorn\textsuperscript{21} did not provide evidence for his hypothesis. The selective secretion of digestive enzymes has recently been examined at a cellular level. Glucose evoked the release of amylase from zymogen granules in a concentration-dependent fashion without affecting trypsinogen release, whereas lysine was found to have the opposite effect (Grendell and Rothman).\textsuperscript{22} Grendell \textit{et al.}\textsuperscript{13} reported that the selective enzyme release was produced by glucose and lysine and suggested that such end products of digestion could regulate the digestive process by modifying the secretory response of pancreas to CCK. In the present experiment with chicks, wing vein injection of CCK alone or in combination with end products of digestion (glucose or lysine) also showed the specific induction of a particular enzyme secretion (Fig. 5).

Although the mechanism of the pancreatic digestive enzyme secretion is speculated by various hypotheses\textsuperscript{21-24}, the present results would support the idea that end products of digestion were effective on the pancreatic digestive enzyme secretion in cooperation with CCK, presumably due to a change in permeability of zymogen granule cell membrane as postulated by Grendell \textit{et al.}\textsuperscript{13} Further studies have to be done for the understanding of the detailed nature of this regulatory mechanism.

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\textbf{References}

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鶏ヒナにおける腸液の新しい採取方法とコレシストキニン、
グルコースおよびリジンの翼下静脈注射に対する
腸液消化酵素分泌反応

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鶏ヒナの腸液を採取する新しい方法を開発した。すな
わち，麻酔した鶏ヒナの十二指腸を腸管開口部の前後で
切断して Tygon チューブを設置し，低速ポンプを用い
て生理食塩水を連続的に流入洗浄することにより，分泌
された腸液は生理食塩水と腸液との混合物として採取さ
れる。この方法では，腸液消化酵素の分泌障害や不規則
的な分泌は見られなかった。アミラーゼ，トリプシン
ガーゲンおよびキモトリプシンガーゲンの分泌は生理食塩水ま
たはグルコース，メチオニンおよびリジンなどの消化最
終産物を翼下静脈に注射した後に相対的に低い安定した
反応として観察された。コレシストキニン投与により腸
液酵素分泌はすばやく反応し，この反応は投与した量に
比例した。またコレシストキニンは，アミラーゼおよび
トリプシンガーゲン分泌よりキモトリプシンガーゲン分泌を
選択的に促進するようであった。コレシストキニンをリ
ジンとともに投与するとコレシストキニン単独投与に比
して，アミラーゼ，トリプシンガーゲンおよびキモトリプ
シンガーゲン分泌が増加した。これに反し，コレシストキ
ニンをグルコースとともに投与するとコレシストキニン
単独に比べて，アミラーゼ分泌が増加した。

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