THE ENDOGENOUS SECRETIN IN CHICKEN:
MINOR PHYSIOLOGICAL ROLE IN EXOCRINE Pancreatic secretion

Ei-ichi KOKUE and Toyoaki HAYAMA

Department of Veterinary Science, Faculty of Agriculture, Tokyo University
of Agriculture and Technology, Fuchu, Tokyo 183, Japan

Abstract The action of secretin (SN) on the exocrine pancreas in the chicken was studied in comparison with the rat and dog. Hydrochloric acid (0.1 N), pepton (5%) and olive oil were introduced into the small intestine of conscious chickens with chronic pancreatic fistula and caused the volume and protein output to increase slightly in about two-thirds of the chickens tested. The percent increase of the volume output in anesthetized chickens by HCl instillation was only one-tenth of that in the rat. Acid extracts of the intestinal mucosa (80 mg) from chickens when given intravenously to rats caused the volume of secretion from the rat pancreas to increase about twofold, whereas the extracts from the dogs' and rats' intestine increased the volume output 5.9 and 5.5 times respectively. The increase of pancreatic secretion in chickens by intravenous injection of commercial SN was about one-third of that in the rat. The present studies indicate that SN or a SN-like hormone may be present in the chicken, but it plays only a minor physiological role.

Hormonal control of the exocrine pancreatic secretion is well established in mammals (HARPER, 1967). In birds, however, there are few papers on the subject (IVANOV and GOTEV, 1962; HEATLEY et al., 1965; DAL BORGO et al., 1968). We have reported previously that the chicken pancreas secretes juice continuously even after long starvation and that food intake causes only a slight increase in output (KOKUE and HAYAMA, 1972). It was the object of this investigation to determine if intestinal hormones, especially secretin (SN), had a physiological role in regulating chicken pancreatic secretion.

MATERIALS AND METHODS

Experiments in conscious chickens. Young male chickens (White Leghorn),
weighing 0.5–0.8 kg, were used. They were fed a commercial mash. At least five days before the experiment a chronic pancreatic fistula was installed using the method described in the previous report (Kokue and Hayama, 1972). To measure the pancreatic secretion the chicken was confined in a cage (Kokue and Hayama, 1969). Each observation was carried out in the conscious bird after starvation for 24 hr. Observations on each chicken were made no more than twice a week. The following solutions (endogenous SN releasers) were instilled through a cannula inserted into the duodenum: 0.1 n HCl, 5% pepton, 1% casein, 1% trypsin inhibitor (Sigma Co., Ltd), 0.85% NaCl solution and olive oil. A perfusion pump (syringe type) was used for the instillation. In conscious birds a pump rate of 0.14 ml/min was used. The secretion was collected every 15 min.

Experiments in anesthetized chickens and rats. Chickens and male rats (Wistar), weighing 0.4–0.6 kg and 0.3 kg respectively, were anesthetized with intraperitoneal urethan (1.3 g/kg) after 24 hr starvation. Pancreatic fistula in the chickens were constructed by the same method as used in the chronic experiments. The solutions (SN releaser) were instilled through a cannula inserted through duodenostomy into the duodenum of the chickens at a rate of 0.25 ml/min. Pancreatic fistula in rats were constructed by the method described by Lin and Alphin (1959). The duodenum and common bile duct in the rat were gently exposed after making a mid-line incision in the abdomen. The upper end of the common bile duct was doubly ligated below its bifurcation and cut between the ligatures. The lower end of the duct just before its entrance into the duodenum, was cannulated with a polyethylene tube of the proper size. The free end of the tube was led to the exterior through a needle puncture on the right side of the body wall. The rate of pancreatic secretion was determined by measuring the distance travelled by the juice in a vinyl tube (0.5 mm in inside diameter) which was connected to the polyethylene tube. Several solutions were instilled into the duodenum through a cannula inserted through the pylorus.

The pancreatic secretory responses to varying intravenous doses of commercial SN (Vitrum) were also observed in chickens and rats.

Contents of intestinal SN-like substance in chicken, rat, and dog. The SN-like substances of three species were extracted from the intestinal mucosa according to the method of Bayliss and Starling (1902). Chickens and rats, weighing 0.7 kg and 0.3 kg respectively, were used. They were killed by decapitation. Mongrel dogs, weighing approximately 10 kg, were anesthetized with intravenous sodium pentobarbital (30 mg/kg) and bled out. Immediately after death, the entire small intestine was removed. The mucosa was stripped off the intestine by blunt dissection.

The SN activity of the extract was measured biologically by Love’s method (1957) using 11 rats. The neutralized acid extracts of the intestinal mucosa, equivalent to 20 and 80 mg of wet weight, were given intravenously, usually every 30 min, to the rats. The estimations of the activity were expressed as Rat Unit
SECRETIN IN CHICKEN PANCREATIC SECRETION

(RU), which were the ratios of the secretions during 30 min after injection to those before injection.

The volume output was determined by measuring the distance travelled by the juice in a vinyl tube (0.5 mm inside diameter: 10 mm = 2.0 μl). The juice sampled during a given period was diluted 200 times with 0.01 N HCl. Protein concentration was determined by comparing the absorption of the diluted juice at 280 μm in a spectrophotometer (Beckman type, Shimazu QV-50) with a standard solution of bovine serum albumine. The secretion was collected every 15 min. Results are expressed as means of the last two control periods and the two collection periods immediately after the treatments. Significance of differences between these was determined by Student’s t above the 95% probability level. No response is claimed in the text unless this criterion is met. The t test was used also to compare the magnitude of responses of the rat pancreas with the mucosa of chicken, rat, and dog.

RESULTS

Duodenal instillations in conscious chickens

Various solutions were instilled into the duodenum. Little change of flow rate and protein concentration was observed during the instillation of 0.85% NaCl. Instillation of 0.1 N HCl caused the flow to increase slightly in 10 out of 16 chickens (Table 1). The percent increase in juice volume over basal was less than 30%. No increase was observed in four chickens and in two the secretion was suppressed by the acid. Pepton solution elicited a small increase in volume compared with the other solutions. However, two out of eight chickens did not respond to pepton. Olive oil was given to the 16 chickens. Eight of them showed an obvious increase in juice volume. But in four chickens the secretion was

<table>
<thead>
<tr>
<th>Substances</th>
<th>No. of animals</th>
<th>Volume (%)</th>
<th>Protein content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.85% NaCl</td>
<td>8</td>
<td>94 ± 19</td>
<td>93 ± 24</td>
</tr>
<tr>
<td>HCl (0.1 N)</td>
<td>16</td>
<td>127* ± 29</td>
<td>114 ± 21</td>
</tr>
<tr>
<td>Pepton solution (5%)</td>
<td>8</td>
<td>129* ± 14</td>
<td>148* ± 20</td>
</tr>
<tr>
<td>Olive oil</td>
<td>12</td>
<td>148* ± 43</td>
<td>153* ± 44</td>
</tr>
<tr>
<td>Trypsin inhibitor solution (1%)</td>
<td>4</td>
<td>115 ± 24</td>
<td>119 ± 22</td>
</tr>
<tr>
<td>Casein solution (1%)</td>
<td>4</td>
<td>99 ± 18</td>
<td>105 ± 13</td>
</tr>
</tbody>
</table>

Values are means ± SD. The secretion was collected every 15 min. The responses were expressed as percent of the control value (two samples before the treatment). The volumes and protein contents were increased slightly by the instillation of 0.1 N HCl, 5% pepton solution and olive oil.

*P < 0.05
unaltered or suppressed. Four birds suffered from diarrhea after the instillation of olive oil and the results for these were discarded. The instillation of casein solution or trypsin inhibitor solutions failed to increase the juice volume (Table 1).

There was no significant change in the protein concentration of the juice after intraduodenal instillation of any of the solutions except pepton and olive oil.

Intraduodenal hydrochloric acid (0.1 N) in anesthetized chickens and rats

Figure 1 shows a comparison of the effects of the intraduodenal 0.1 N HCl on the juice volume of anesthetized chickens and rats. Instillation of acid in eight rats caused the flow to increase within 15 min and continue for 30 min.

![Graph showing effects of 0.1 N HCl instillation into the upper small intestine on the pancreatic secretion of anesthetized chicken and rat. Nine chickens and eight rats were used under anesthesia after 24 hr starvation. Vertical lines indicate the standard deviation. The solution was infused at 0.25 ml/min for 60 min in chicken and for 15 min in rat. Intestinal hydrochloric acid was less effective in chicken compared with the rat.](image)

Fig. 1. Effects of 0.1 N HCl instillation into the upper small intestine on the pancreatic secretion of anesthetized chicken and rat. Nine chickens and eight rats were used under anesthesia after 24 hr starvation. Vertical lines indicate the standard deviation. The solution was infused at 0.25 ml/min for 60 min in chicken and for 15 min in rat. Intestinal hydrochloric acid was less effective in chicken compared with the rat.

On the other hand, intraduodenal hydrochloric acid in three out of nine chickens failed to increase the juice volume. In the remaining six the flow increased within 15 min but the increase was slight compared with the rat. The responses varied greatly from chicken to chicken.

Table 2 shows the average increases of pancreatic secretions in chickens, rats and dogs, when the HCl solutions were administered into the duodenum. Some values listed are taken from the literature. Calculation of the percent increase
Table 2. Changes of pancreatic volume output in chicken, rat, and dog following instillation of 0.1 N HCl into the upper small intestine.

<table>
<thead>
<tr>
<th>Animals</th>
<th>Experiments</th>
<th>Volume change</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken</td>
<td>Conscious</td>
<td>127%</td>
<td>Kokue and Hayama</td>
</tr>
<tr>
<td></td>
<td>Anesthetized</td>
<td>130%</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>Anesthetized</td>
<td>345%</td>
<td>RAMIREZ et al. (1966)</td>
</tr>
<tr>
<td></td>
<td>Anesthetized</td>
<td>421%</td>
<td>Kokue and Hayama</td>
</tr>
<tr>
<td>Dog</td>
<td>Conscious</td>
<td>259%</td>
<td>WANG and GROSSMAN (1951)</td>
</tr>
<tr>
<td></td>
<td>Conscious</td>
<td>620%</td>
<td>FARELL and IVY (1926)</td>
</tr>
</tbody>
</table>

The changes of the volume output were expressed as a percent of the control value (volume secretion during HCl solution instillation / volume secretion during the same period before the instillation x 100). The values in chickens were much lower than in rats or dogs.

for individual experiments discloses that the average increase was 27–30% in chickens. However, in rats or in dogs the percent increases of the volume of pancreatic juice were much higher than in chickens.

Sensitivity of chickens and rats to exogenous SN

The sensitivity of the pancreas to the intravenous injection of SN was determined in nine anesthetized chickens and six rats with acutely cannulated fistulas. Three out of nine chickens did not respond to the exogenous SN. Figure 2 shows that the magnitude of the responses to the intravenous injection of SN are proportional to the doses (in Boots unit/kg, i. v.), in six of the chickens and in all of the rats. The sensitivity of chicken pancreas to SN is clearly lower than that of the rat. For example, the increase in juice volume following one unit/kg of SN in the chicken was about one-third of that in the rat.

Contents of SN-like substance in chickens, rats, and dogs

Two doses of the neutralized crude extracts of the intestinal mucosas, equivalent to 20 mg/kg and 80 mg/kg wet weight, were given intravenously to the rat for bioassay of the SN activity. The chicken mucosal extract doubled the secretion from the rat pancreas whereas similar extracts of dog and rat mucosas increased the secretion 5.9 and 5.5 times respectively (Table 3).

DISCUSSION

We have previously examined the exocrine pancreatic secretion in chicken using permanent fistula (KOKUE and HAYAMA, 1972). It could be in this study the higher resting secretion per body weight in chicken and rat compared with dog. We also knew that the chicken pancreas will continuously secrete a considerable amount of juice regardless of the starvation time (24–72 hr), the presence of ingesta the digestive canal and the existence of the vagus nerve. Moreover, in the present study we found that the SN-like activity in the intestinal mucosa of chickens was
Table 3. Contents of SN-like substances of the intestinal mucosa of chicken, dog and rat.

<table>
<thead>
<tr>
<th>Intestinal mucosa (wet weight)</th>
<th>Animal number</th>
<th>Chicken SN-like activity (RU)</th>
<th>Dog SN-like activity (RU)</th>
<th>Rat SN-like activity (RU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 mg</td>
<td>1</td>
<td>2.1</td>
<td>4.7</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.8</td>
<td>2.6</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.9</td>
<td>3.7</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.6</td>
<td></td>
<td>2.7</td>
</tr>
<tr>
<td>mean</td>
<td></td>
<td>1.4*</td>
<td>3.7</td>
<td>2.8</td>
</tr>
<tr>
<td>80 mg</td>
<td>1</td>
<td>2.8</td>
<td>7.6</td>
<td>7.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.5</td>
<td>4.2</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.9</td>
<td>6.0</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2.3</td>
<td></td>
<td>4.8</td>
</tr>
<tr>
<td>mean</td>
<td></td>
<td>2.1*</td>
<td>5.9</td>
<td>5.5</td>
</tr>
</tbody>
</table>

Neutralized acid extracts of the three intestinal mucosa, equivalent to 20 and 80 mg of wet weight, were given intravenously to anesthetized rats. SN-like activity in chicken intestinal mucosa was half to one-third of that in dogs or rats.

*P<0.05

obviously lower than those in dogs and rats. From these data we supposed that the basal pancreatic secretion in chicken was maintained by a certain mechanism other than by the vagal tone and/or endogenous hormones. This mechanism is not clarified at present and further studies are required in the future.

Since the discovery of SN by Bayliss and Starling, it is clear that pancreatic secretion in the dog is mainly controlled by a humoral mechanism (FARELL and IVY, 1926; GROSSMAN, 1962). To establish the humoral control of pancreatic secretion the following conditions must be met: a) the existence of the hormone in the digestive tract (in the small intestine in this experiment), b) increase of pancreatic secretion by the exogenous hormone, and c) release of the endogenous hormone by intestinal stimulations (for example, 0.1 N HCl solution). These criteria have been met in many mammals.

SN has been extracted from the intestinal mucosa of a wide range of species including man and the common laboratory animals (GROSSMAN, 1953). Koschtojanz et al. reported the existence of SN in the chicken intestine (KOSCHTOJANZ et al., 1933). Recently, POLAK et al. (1974) demonstrated SN in the chicken duodenum and small intestine by means of immunohistochemical and ultrastructural techniques. In this study we also detected SN-like activity in the intestinal mucosa of chicken, even if it is obviously less than in dogs or rats (Table 3).

ANGELLUCII et al. (1970) reported that porcine SN did not stimulate the pancreatic secretion of chickens. According to HEATLEY et al. (1965) the response of the chicken pancreas to SN might be irregular or anomalous. In our experiments the pancreas was responsive to the exogenous SN, but, as shown in Fig. 2, the sensitivity is much lower than in the rat.
In order to examine the release of endogenous SN, we introduced well recognized intestinal releasers of SN into the intestine of conscious chickens and estimated their effectiveness in releasing SN from changes in the volume and enzyme output of the juice respectively. Acid, pepton, and olive oil were not constantly effective and, even when they were, they only slightly increased the volume and enzyme output (Table 1). In Table 2 the responses to duodenal hydrochloric acid in chickens, rats, and dogs are compared. It is clear that the juice volume increase in chickens is apparently less than in the rat or the dog.

![Graph showing differences in sensitivity of pancreatic secretory responses between six chickens and six rats to rapid intravenous injection of SN. The secretion was collected every 15 min. Volume response was indicated by the percent increase of two collection periods immediately after the treatment (control value: two samples before the treatment). Vertical lines indicate the standard deviation of the mean. Chicken pancreas was less sensitive to SN than that of rat.](image)

In brief, the data obtained in the present study indicate that, 1) SN activity in chicken was detected in the intestine but was very weak, 2) the apparatus of the exocrine pancreas is not so sensitive to the exogenous SN as those of dog and rat, 3) pancreatic secretion is increased by the instillation of intestinal releasers but the response was not so great as was observed in dog or rat. The first two results may support our former conclusion that the high basal secretion in the chicken pancreas is possibly spontaneous, not controlled by the hormone. Furthermore, all results in this paper suggest that the SN control may exist but play only a minor physiological role.

The protein concentration of the pancreatic juice in conscious chicken was increased slightly by the instillation of the pepton solution. Unfortunately we could not determine the action of cholecystokinin-pancreozymin (CCK-PZ) on the exocrine pancreas in chickens. It has been reported that caerulein which is similar to CCK-PZ chemically and biologically (ANASTAS et al., 1968; BERTACCINI et al., 1969) has a strong stimulating action on the pancreatic volume and protein secretions in chickens (ANGELLUCII et al., 1970). The participation of CCK-PZ in the mechanism of the exocrine pancreas in chicken awaits further study.
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REFERENCES


