Intravenous injection of caerulein or cholecystokinin increases the parotid-type and pancreatic-type amylase in the serum of rats

Sachiko Saikatsu, Kumiko Ikeno, Yasuhiro Hanada and Takeyuki Ikeno

Department of Oral Biochemistry, School of Dentistry, Ohu University, Tomita-machi, Koriyama 963, Japan

[Accepted for publication: May 11, 1989]

Key words: Serum amylase / caerulein / CCK

Abstract: Caerulein and cholecystokinin (CCK) injection (i.v.) into rats caused about a 2.3- and 1.9-fold increase in serum amylase activity within 2 h, respectively. The increase of amylase activity in the serum caused by pilocarpine or isoproterenol injection (i.p.) was only of parotid-type amylase. Caerulein or CCK markedly increased pancreatic-type amylase, and significantly increased parotid-type amylase in the serum.

Introduction

Our previous studies indicated that pilocarpine and isoproterenol affected rat parotid amylase in two ways; the stimulation of amylase secretion into the secretory ducts and the acceleration of amylase release into the blood. It is well established that amylase secretion from pancreatic acinar cells is stimulated by the intestinal hormone, cholecystokinin (CCK). Some evidence supports a role for secretin in the release of proteins secreted by the pancreas. Caerulein, a synthetic decapetide analogue of CCK, also causes pancreatic secretion. Infusion of caerulein via a jugular vein catheter at 5 μg/kg/h induced a 10-fold elevation in rat serum amylase after 3, 6 or 12 h. No increase in pancreatic-type amylase was observed in rat serum after administration of pilocarpine or isoproterenol after the separation of amylase isozymes by electrophoresis. In this study, we investigate the changes in amylase activity in the parotid gland, pancreas and serum after administration of caerulein or CCK in rats, compared the results with the effects of pilocarpine and isoproterenol.

Materials and Methods

Male Wistar rats weighing 200-250 g were fed a commercial pelleted diet (Oriental MF, Oriental Yeast Co. Ltd., Tokyo, Japan) and water ad libitum. All animals were made to fast for 16 h and then anaesthetized with sodium pentobarbital (25 mg/kg, Abbott Laboratories, North Chicago, U.S.A.). They received an intraperitoneal injection of pilocarpine hydrochloride (10 mg/kg, Wako Pure Chemical Industries Ltd., Osaka, Japan) or isoproterenol (5 mg/kg, Sooner solution, Kaken Kagaku Co. Ltd., Tokyo, Japan). CCK (12 μg/kg, CCK-8, Sigma, St. Louis., U.S.A.) or caerulein (8 μg/kg, Ceosunin Inj, Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan) was injected via the femoral vein. Control rats received an intravenous injection of saline. Whole saliva from rats was collected on
S. Saikatsu et al.: Rat serum amylase after caerulein or CCK injection

Table 1 Salivary volume and amylase activity in the saliva, parotid gland, pancreas and serum after administration of drugs

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Volume of whole saliva (g)</th>
<th>Whole Saliva (unit/total)</th>
<th>Parotid gland (unit/mg tissue)</th>
<th>Pancreas (unit/mg tissue)</th>
<th>Serum 0h (unit/ml serum)</th>
<th>Serum 2h (unit/ml serum)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (4)</td>
<td>0.026±0.004</td>
<td>318±78</td>
<td>1,186±84</td>
<td>526±38</td>
<td>202±30</td>
<td>209±30</td>
<td>(1.03)</td>
</tr>
<tr>
<td>Caerulein (8)</td>
<td>0.010±0.004**</td>
<td>1,385±141*</td>
<td>1,124±103</td>
<td>438±58</td>
<td>186±9</td>
<td>420±37*</td>
<td>(2.26)</td>
</tr>
<tr>
<td>CCK (7)</td>
<td>0.014±0.011</td>
<td>161±138</td>
<td>1,151±96</td>
<td>425±34**</td>
<td>201±7</td>
<td>383±15*</td>
<td>(1.91)</td>
</tr>
<tr>
<td>Pilocarpine (7)</td>
<td>3.666±0.417*</td>
<td>426,336±28,291*</td>
<td>794±141**</td>
<td>573±166</td>
<td>265±20</td>
<td>645±64*</td>
<td>(2.43)</td>
</tr>
<tr>
<td>Isoproterenol (8)</td>
<td>0.308±0.057*</td>
<td>724,516±96,454*</td>
<td>116±37*</td>
<td>897±76*</td>
<td>253±7</td>
<td>342±16*</td>
<td>(1.35)</td>
</tr>
</tbody>
</table>

A value of 1.0 for the absorbance at 620 nm per 1.0 mg of the tissue or 1.0 ml of the serum incubated at 37°C was defined as 1 unit.
Values represent the mean±SEM with the number of animals in parentheses.
Significantly different from control values (injection of saline) with * p<0.01 or ** p<0.05

Cotton wool for 2 h and eluted with saline. Blood was collected from the femoral vein and serum was prepared by centrifugation. The parotid gland and pancreas were separately dissected out 2 h after the injection, rinsed with saline, weighed, and homogenized with saline. The supernatant fluid was used for study. Amylase activity in the serum and tissues was assayed photometrically by using blue-starch as the substrate (Neo-Amylase Test, Daiichi Pure Chemical Co. Ltd., Tokyo, Japan) as described previously11). Rat parotid amylase was purified by the method of Kaczmarek and Rosenmund12). Antiparotid-amylase serum was prepared from white rabbits according to the method described by Baum et al.13). Total amylase activity in rat serum was assayed without anti-parotid-amylase serum. Ten µl of anti-parotid-amylase serum was preincubated at 37°C for 5 min with 10 µl of rat serum before or after injection of each drug. After preincubation of the serum with anti-parotid-amylase serum, the substrate (blue-starch) was added and amylase activity was assayed by incubation at 37°C for an additional 5 min. The amylase activity of the antiserum itself was assayed. The activity of the pancreatic-type amylase in rat serum remained after the addition of anti-parotid-amylase serum. Parotid-type amylase activity was obtained by subtracting that of pancreatic-type amylase from the total amylase activity. Ten µl of anti-parotid-amylase serum inhibited rat parotid-type amylase (0-500 units) completely, and did not inhibit rat pancreatic-type amylase (0-500 units) (99 % recovery). Amylase isozymes in serum were separated by electrophoresis in Cellogel (Chemetron, Milano, Italy).

Results

The total volume of secreted whole saliva was the highest after pilocarpine injection. Administration of isoproterenol strongly stimulated amylase secretion into saliva and depleted the enzyme content in the parotid gland. Caerulein and CCK caused a decrease in amylase activity in the pancreas. Serum amylase activity increased 2.26-, 1.91-, 2.43-, and 1.35-fold 2 h after the injection of caerulein, CCK, pilocarpine, and isoproterenol, respectively (Table 1).

Parotid-type and pancreatic-type amylase in the serum was analyzed by the addition of anti-parotid amylase serum. Only the parotid-type amylase was increased in the serum for 2 h after the injection of pilocarpine or isoproterenol (data not shown). Caerulein and CCK markedly increased pancreatic-type amylase, and significantly increased parotid-type amylase (Table 2 and 3). The rate of increase in both types was almost the same after either caerulein or CCK injection. Electrophoretic zymograms of serum amylase after caerulein or CCK injection are shown in Figure 1. Isoamylase of the pancreatic-type
was detected within 10 min, and markedly increased up to 2 h after either caerulein or CCK injection. Parotid-type amylase also increased significantly.

**Discussion**

Secretion of digestive enzymes from pancreas is regulated by hormones and neurotransmitters. Amylase secretion from the parotid gland is regulated by adrenergic and cholinergic stimulation\(^1\). Lampel and Kern\(^10\) reported that excessive doses of a pancreatic secretagogue (caerulein) led to severe disturbances of the secretory process and finally destroyed the exocrine pancreas. The primary event leading to this destruction was a premature fusion of condensing vacuoles and zymogen granules in the cytoplasm resulting in large vacuoles. The appearance of pancreatic-type amylase in serum after administration of caerulein or CCK is thought to be caused by excessive secretory stimulation of the pancreas leading to cellular destruction in acinar cells. Both caerulein and CCK increases pancreatic-type amylase more than the parotid-type in the serum (Table 2 and 3). We speculated that the large elevation of intraductal pressure might be the primary mechanism for the release of amylase from the parotid gland into the blood after administration of pilocarpine or isoproterenol\(^3\). With caerulein and CCK, the volume of whole saliva secreted from the parotid gland was very low (Table 1). The mechanism of increase in parotid-
Type amylase activity in the serum caused by either caerulein or CCK injection seems to be different from that by either pilocarpine or isoproterenol injection into rats.

Amylase isozymes in human serum were of pancreatic and salivary origin. Serum amylase isozymes of normal adult persons can be separated into two major isozymes (pancreatic isoamylase and salivary isoamylase) with high amylase activity and two or three minor ones with low amylase activity. In patients with acute pancreatitis, increased amylase activities were observed on those of pancreatic origin. While, amylase isozymes in rat serum were separated into one major band (parotid-type) and four minor bands. No pancreatic-type amylase was seen in normal rat serum (before CCK or caerulein injection). Pancreatic-type one was detected after either CCK or caerulein injection (Fig. 1).

Some data indicate that amylase is produced in rat liver and that the perfused rat liver secretes amylase into the perfusion medium. Further, Hammerton and Messer suggest that rat liver and serum amylase are identical and the major isozymes of liver amylase are electrophoretically identical with the major ones of the parotid. In addition, rat liver and serum amylase are identical and both are very similar to parotid amylase by using the antisera in immunodiffusion, immunoelectrophoresis and immunoinhibition experiments. We reported that amylase isozymes in the saliva, parotid gland, serum and liver were identical in C57 BR/cdJ and M.m. molossinus (Kor) mice. But amylase activity in the liver was lower than that in the serum and liver amylase almost disappeared by perfusion. Histochemical observation of the parotid gland and liver after the injection of caerulein and/or CCK remains to be further elucidated.
References


10) Lampel, M. and Kern, H. F.: Acute inti-