Interaction of Caerulein, Glucose, and Amino Acids on Insulin Secretion from the Perfused Rat Pancreas

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Abstract

The effect of caerulein on insulin response to graded amounts of glucose from the isolated perfused rat pancreas was investigated in the presence or absence of an amino acids mixture. Caerulein at a concentration of 0.1 ng/ml which is a submaximal concentration for an effect on exocrine pancreatic secretion potentiated insulin responses to glucose concentrations less than 200 mg/dl, but produced no further increase when added to a glucose stimulus over a 200 mg/dl. However, in the presence of amino acids the insulin response to 200 mg/dl glucose was significantly potentiated by the stimulation of 0.1 ng/ml caerulein. The effectiveness of caerulein as an insulinotropic agent depended on the glucose concentration only when amino acids were present. These results indicate that caerulein, at a concentration which stimulate pancreatic exocrine secretion, has a synergistic effect on insulin response to glucose and amino acids and therefore raises the possibility that endogenously released CCK may contribute to the entero-insular axis.

The findings of a significantly greater rise in plasma insulin levels after intraduodenal administration of glucose or an amino acids mixture compared to a comparable intravenous administration suggested an intestinal mechanism for the control of insulin release (McIntyre et al., 1965; Rapitis et al., 1973). Porcine gastric inhibitory polypeptide (GIP) has been shown to stimulate insulin release in vivo when administered at doses which mimic physiologically obtainable serum concentrations (Cataland et al., 1974; Pederson et al., 1975). In addition to in vivo studies, GIP has been demonstrated to augment glucose- or arginine-stimulated insulin release from the isolated perfused rat pancreas (Pederson and Brown, 1976). These results have given rise to speculation that GIP is the most important intestinal mediator of the identified gastrointestinal hormones for insulin release.

Recently, it has also been shown that pure natural porcine cholecystokinin (CCK), its COOH-terminal octapeptide of CCK or its chemical analogue caerulein has an insulinotropic action in vivo and in vitro (Frame et al., 1975; Otsuki et al., 1979). In our previous study using isolated perfused rat pancreas, a glucose-dependent insulinotropic action of CCK or caerulein was clearly demonstrated (Sakamoto et al., 1982). However, endogenous release of CCK seems to be related to the ingestion of amino acids or protein rather than glucose, judging from

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the pancreatic exocrine secretion induced by nutrients (Go et al., 1970). This raises the question that CCK may interact with other plasma nutrients in addition to glucose on insulin secretion.

We therefore conducted the present study to find out whether an amino acids mixture and caerulein exert a synergistic effect on the pancreatic β cell in isolated perfused rat pancreas.

Materials and Methods

Pancreas from male Wistar rats, weighing 250–300 g, fed ad libitum were isolated and perfused by the technique reported previously (Otsuki et al., 1979). The perfusate was Krebs Ringer bicarbonate buffer solutions; it contained 50 mg/dl glucose, 4.6% Dextran T-70 (Pharmacia Fine Chemicals, Uppsala, Sweden), and 0.25% bovine serum albumin (fraction V, Armour Pharmaceutical Co., Phoenix, AZ) and was gassed constantly with a 95% O₂, 5% CO₂ mixture to obtain a pH of 7.4. The flow rate through the pancreas was kept constant at 2 ml/min with a roller pump. The experiments were performed after a 30 min equilibration period. Ten min after the initiation of the experiments (at a period of 11 min), glucose at a dose of 100, 150 or 200 mg/dl was added and perfused for 40 min (a period from 11 min to 50 min) in the presence or absence of an amino acids mixture from Eagle minimum essential medium (MEM) (Eagle, 1959). Each amino acid concentration in a MEM mixture is shown in Table 1. Maximal IRI response to glucose or glucose plus amino acids during the period from 11 min to 20 min was evaluated as an initial phase of IRI secretion and total IRI output during the period from 21 min to 40 min as a late phase. Synthetic caerulein (Kyowa Hakko Kogyo, Ltd., Tokyo, Japan) was then added at a concentration of 0.1 ng/ml for 20 min, 10 min after the stimulation of glucose and amino acids (a period from 21 min to 40 min). 0.1 ng/ml caerulein is a submaximal concentration for an effect on exocrine secretion and cannot stimulate insulin secretion by itself unless 100 mg/dl or more glucose is present, as previously reported (Sakamoto et al., 1982).

Amylase output and immunoreactive insulin (IRI) response were determined simultaneously to evaluate the effect of caerulein in the presence or absence of an amino acids mixture on endocrine and exocrine pancreatic function. The portal effluent was collected in chilled tubes at 1 min intervals, and IRI levels were measured by polyethylene glycol radioimmunoassay (Desbuquois and Aurbach, 1971). Amylase activity in the pancreatic juice was determined by a chromogenic method with blue-dyed starch polymer (Ceska et al., 1969).

Data are expressed as the mean±SE. Statistical analysis was performed with Students’ t-test. Differences with P values less than 0.05 were considered statistically significant.

Table 1. Profile of amino acids mixture.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mM)</td>
</tr>
<tr>
<td>L-amino acids</td>
<td></td>
</tr>
<tr>
<td>arginine</td>
<td>0.6</td>
</tr>
<tr>
<td>cystine</td>
<td>0.1</td>
</tr>
<tr>
<td>glutamine</td>
<td>2.0</td>
</tr>
<tr>
<td>histidine</td>
<td>0.2</td>
</tr>
<tr>
<td>isoleucine</td>
<td>0.4</td>
</tr>
<tr>
<td>leucine</td>
<td>0.4</td>
</tr>
<tr>
<td>lysine</td>
<td>0.4</td>
</tr>
<tr>
<td>methionine</td>
<td>0.1</td>
</tr>
<tr>
<td>phenylalanine</td>
<td>0.2</td>
</tr>
<tr>
<td>threonine</td>
<td>0.4</td>
</tr>
<tr>
<td>tryptophan</td>
<td>0.05</td>
</tr>
<tr>
<td>tyrosine</td>
<td>0.2</td>
</tr>
<tr>
<td>valine</td>
<td>0.4</td>
</tr>
<tr>
<td>Total</td>
<td>5.45</td>
</tr>
</tbody>
</table>

Results

Effect of amino acid on glucose-induced IRI secretion.

After the perfusion with 50 mg/dl glucose, the addition of an amino acids mixture and glucose more than 50 mg/dl was simultaneously started. An initial rapid rise in IRI secretion in response to 50, 100, 150, or 200 mg/dl glucose was strongly potentiated in the presence of an amino acids mixture (Fig. 1, left panel). On the other hand, the late phase IRI responses to graded
Fig. 1. Effect of simultaneous perfusion with an amino acids mixture and various concentrations of glucose on the initial phase or the late phase of IRI secretion. The initial phase of IRI secretion was expressed as the mean values of initial peak responses and the late phase was expressed as the mean values of total IRI outputs during the period from 21 to 40 min. Each point represents the mean ± SE of 8 experiments.

Fig. 2. Effect of caerulein on insulin response to 150 mg/dl or 200 mg/dl glucose in the presence or absence of an amino acids mixture. Each point represents the mean ± SE of 8 experiments.

Table 2. Initial peak IRI response to caerulein in the presence of 150 mg/dl or 200 mg/dl glucose and amino acids.

<table>
<thead>
<tr>
<th>Caerulein (ng/ml)</th>
<th>Glucose 150 mg/dl</th>
<th>Glucose 200 mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+MEM</td>
<td>−MEM</td>
</tr>
<tr>
<td>Control</td>
<td>18.7±1.2a</td>
<td>15.7±1.1c</td>
</tr>
<tr>
<td>0.1</td>
<td>62.7±7.5b</td>
<td>36.4±8.9d</td>
</tr>
</tbody>
</table>

Initial peak IRI response means the peak IRI response to caerulein in samples during the period from 21 to 25 min.

All values are mean ± SE of 8 experiments.

b, P<0.01 compared with a  
b, P<0.05 compared with d  
d, P<0.05 compared with c  
y, P<0.05 compared with x, z
amounts of glucose were not enhanced by the presence of amino acids (Fig. 1, right panel).

**Effect of caerulein on IRI response to 150 mg/dl glucose in the presence or absence of amino acids.**

The effect of caerulein at a concentration of 0.1 ng/ml on a late phase of glucose-stimulated IRI release was studied in the presence or absence of an amino acids mixture. 0.1 ng/ml caerulein superimposed on 150 mg/dl glucose potentiated IRI response to glucose stimulation biphasically. A sharp rise in IRI secretion was observed just after the addition of caerulein and then a late phase of IRI secretion due to stimulation by caerulein followed. In the presence of an amino acids mixture, caerulein superimposed on 150 mg/dl glucose stimulated a further increase in IRI secretion, as shown in Fig. 2 (left panel). The mean IRI levels in all samples during the period from 21 to 40 min were about 1.5 times higher in the presence of an amino acids mixture than in the absence of amino acids. Table 2 shows caerulein-stimulated initial peak IRI secretion observed just after the addition of caerulein. Initial peak IRI response was 62.7 ± 7.5 ng/ml in the presence of an amino acids mixture, significantly higher than that obtained in the absence of amino acids.

**Effect of caerulein on IRI response to 200 mg/dl glucose in the presence or absence of amino acids.**

IRI response to 200 mg/dl glucose was not affected by the addition of caerulein. As shown in Fig. 2 right panel, IRI responses to glucose stimulation alone, glucose plus amino acids or glucose plus caerulein were nearly the same in all samples during the period from 21 to 40 min. However, in the presence of an amino acids mixture a sharp rise in IRI response to 200 mg/dl glucose was observed just after the stimulation of caerulein. As long as caerulein and amino acids were present, a biphasic IRI secretion was observed on the late phase of IRI responses to 200 mg/dl glucose concentration. Caerulein-stimulated initial peak IRI response observed at 22 min was 85.5 ± 17.4 ng/ml in the presence of an amino acids mixture, significantly higher than the respective control values (Table 2).

**Effect of caerulein on IRI response to graded glucose concentrations in the presence or amino acids.**

Total IRI outputs during 20 min stimu-
lation of 0.1 ng/ml caerulein in the presence of varying glucose concentrations with or without an amino acids mixture are shown in Fig. 3. The effectiveness of 0.1 ng/ml caerulein as an insulinotropic agent depended on the glucose concentration so far as amino acids were present; it was more effective at higher concentrations of glucose in the presence of an amino acids mixture.

Discussion

Natural porcine CCK, its COOH-terminal octapeptide or its synthetic chemical analogue caerulein which contains a COOH-terminal pentapeptide identical to CCK is a well known stimulator of pancreatic exocrine secretion.

Furthermore, submaximal concentrations of CCK or caerulein for an effect on exocrine secretion from perfused rat pancreas also potentiated insulin secretion induced by glucose. The effectiveness of CCK peptides as insulinotropic agents depends on the glucose concentrations, as previously reported (Sakamoto et al., 1982). Glucose ingestion, however, is not yet known to induce endogenous release of CCK. In contrast, certain amino acids which are important physiological determinants of insulin secretion have been shown to stimulate release of endogenous CCK (Go et al., 1970). Amino acids also potentiate the effect of glucose on insulin secretion. Nevertheless, almost no study concerning the effect of CCK on amino acids-induced insulin secretion is available. Our purpose was, therefore, to examine the possibility that caerulein and amino acids may interact on insulin secretion from perfused rat pancreas.

The present study clearly demonstrated a synergistic effect of caerulein and an amino acids mixture on insulin response to glucose concentrations more than 100 mg/dl, though these nutrients did not affect caerulein stimulated amylase secretion. Most strikingly, insulin secretion induced by 200 mg/dl glucose was enhanced by 0.1 ng/ml caerulein only when amino acids were present. Without amino acids, however, IRI response to 200 mg/dl glucose was not enhanced by the addition of caerulein, suggesting that the responsiveness of β-cells to glucose was not affected by the addition of caerulein alone, though dose related IRI response to glucose was shifted to the left. In contrast, GIP at a concentration of 1 ng/ml potentiated insulin secretion in response to graded glucose concentrations from 5.5 to 15 mM without amino acids (Pederson and Brown, 1978). Even in the presence of 20 mM arginine, GIP produced no further increase in insulin response to higher concentrations of glucose (Pederson and Brown, 1978). Therefore these results suggest that the interaction of amino acids may be more important for caerulein than for GIP in their action on beta cells, although the precise mechanism of interaction among glucose, amino acids and gastrointestinal hormones is not yet known.

In studying the interaction of peptides with amino acids, differences in experimental design may exist between our study and those of others. We feel this kind of experiment should be done under conditions in which the concentrations not only of peptides but also of amino acids and glucose used mimic levels to be achievable under considered physiological events. Nevertheless almost all previous experiments have been carried out in the presence of supraphysiological concentration of arginine or without estimation of plasma levels of amino acids. In those studies (Pederson and Brown, 1978; Yovos et al., 1982), arginine or an amino acids mixture stimulated insulin secretion by itself. However the MEM amino acids used in the present study which contain only 0.4 mM leucine and 0.6 mM arginine had no effect on the late phase of insulin secretion even in the presence of more than 100 mg/dl glucose. MEM amino acids (total
5.45 mM) seem to be roughly equal to the concentration of amino acids in normal rat plasma which is about 3 mM in the fasting state and increases to 4.5–5 mM after food ingestion (Scharff and Wool, 1964).

In spite of the fact that the concentrations of glucose and amino acids were considered to be within physiological range, caerulein is not identical to natural CCK with respect to its amino acids sequences and the ability to stimulate amylase secretion. However, the circulating levels and major form of CCK in plasma are not yet known. There is even the possibility that the COOH-terminal peptides may be released into the blood rather than CCK$_{33}$ (Rehfeld, 1978). In the present study, therefore, the concentration of caerulein was chosen on the grounds that the submaximal dose for an effect on exocrine secretion is within the physiological range. Under these conditions, 0.1 ng/ml caerulein, a submaximal concentration for an effect on exocrine secretion seems to have a potent insulinotropic action. Thus our results raise the possibility that CCK released endogenously to an extent which stimulates exocrine secretion may augment insulin secretion in response to glucose and amino acids in the plasma, though accurate evaluation of the physiological role of CCK has to await specific RIA for CCK.

References


