

Cosecretion of amylin and insulin from isolated rat pancreas

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Amylin, a 37 amino acid C-terminal amidated peptide is an integral part of secretory granules of pancreatic β -cells. Utilizing a specific radioimmunoassay system we demonstrate in the present study a cosecretion of amylin and insulin from the isolated rat pancreas. The secretion pattern of both peptides during glucose or glucose plus arginine stimulation is identical. The molar ratio of amylin amounts to 10% of that of insulin. The biological significance of amylin is still unknown, but a paracrine/endocrine role in glucose homeostasis is speculated.

Amylin; Insulin; Cosecretion; Rat pancreas

1. INTRODUCTION

Amylin, a 37 amino acid peptide processed from a 89 (human, [1]) or 93 (rat, [2]) amino acid precursor, was originally isolated from the pancreatic amyloid obtained from type II diabetics [3,4] and from human insulinoma [5]. Recent studies demonstrated amylin, also termed islet amyloid polypeptide (IAPP, [3]) and diabetes-associated peptide (DAB, 4), as a normal product of pancreatic β , but not of A-, D- or PP-cells colocalized with insulin into secretory granules [5,6]. These findings and the demonstrated inhibition of insulin-stimulated glycogen synthesis in skeletal muscle [7,8] suggested a possible role for amylin in the pathogenesis of type II diabetes [9,10]. The present study was performed to establish the pattern of amylin secretion in combination with insulin and amylin from rat pancreas. This was possible since a radioimmunoassay system is now available.

2. MATERIALS AND METHODS

2.1. Reagents

Bovine serum albumin (fraction V) was from Serva (Heidelberg, FRG) and aprotinin (Trasylol) from Bayer (Leverkusen, FRG). All other chemicals were of analytical grade and were purchased from Merck (Darmstadt, FRG).

2.2. Animals

Male albino Wistar rats (180–240 g) kept in a light- and temperature controlled room were fed a standard diet (Altromin, Lage, FRG) and had free access to water.

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2.3. Perfusion experiments

Rats were anesthetized by an i.p. injection of sodium pentobarbitone (45 mg/kg b.w.). The pancreas, spleen, stomach, and proximal part of the duodenum were perfused through cannulated abdominal aorta and coeliac axis as described before [11] and detailed previously [12]. The entire preparation was removed from the cadaver and placed into a perfusion chamber (37°C). The perfusion media consisted of a Krebs–Henseleit bicarbonate buffer (pH 7.4 when gassed with 95% O₂ and 5% CO₂). The venous effluent was collected in intervals of 1 and 5 min, respectively, by a cannula inserted into the portal vein. The flow rate was constant at 4 ml/min. One perfusion experiment lasted 40 min. After a basal period of 10 min for equilibration (2.8 mmol/l glucose) the buffer was changed to medium supplemented with 10 mmol/l glucose or 10 mmol/l glucose plus 10 mmol/l arginine.

2.4. Analytical determinations

Insulin in perfusate was measured by radioimmunoassay [13]. The standard was a mixture of rat insulin I and II and was obtained from Novo (Mainz, FRG). Amylin in perfusate was determined by radioimmunoassay kit for rat amylin (Peninsula, Merseyside, UK) using a rabbit antiserum specific for rat amylin. The antiserum cross-reacts with rat amylin 100%, human amylin 13%, rat CGRP <0.01%, rat CGRP II <0.01% and porcine NPY 0%. Standard was synthetic rat amylin (1–128 pg/ml). Integrated insulin and amylin secretion was determined as secretion rate of the first (0–10 min) and the second phase (11–20 min) of the biphasic insulin response from the isolated perfused rat pancreas.

Data are given as mean \pm S.E.M. of $n=6$ experiments in each group.

3. RESULTS

After perfusion of isolated pancreata by media containing 10 mmol/l glucose or 10 mmol/l glucose plus 10 mmol/l arginine a typical biphasic insulin secretion pattern was observed (figs 1 and 2). Arginine potentiated the insulinotropic action of glucose (fig.2).

Amylin was also released in a biphasic manner. The pattern of amylin release was identical with insulin (figs 1 and 2). The molar amount of amylin secreted was about 10% of that of insulin during glucose (phase 1: 2975 ± 322 fmol insulin, 434 ± 126 fmol amylin

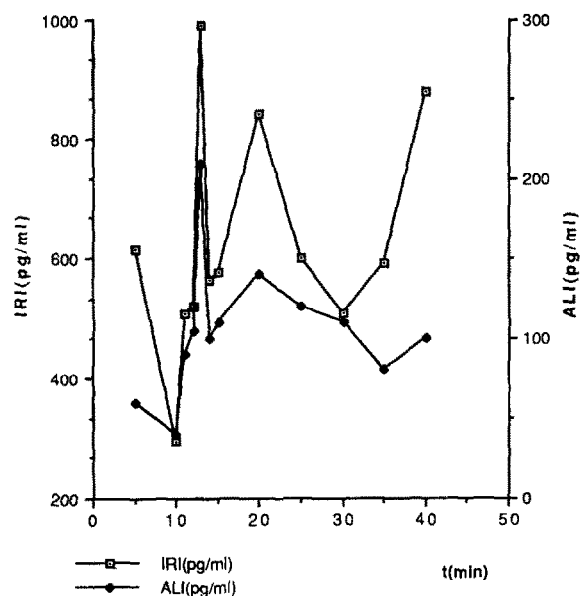


Fig.1. Secretion of insulin and amylin-like immunoreactivity from the isolated perfused rat pancreas under basal conditions (0–10 min) and during stimulation with 10 mmol/l glucose (11–40 min). IRI, insulin-like immunoreactivity; ALI, amylin-like immunoreactivity.

(14.5%); phase 2: 17235 ± 2718 fmol insulin, 2135 ± 392 fmol amylin (12.4%) and glucose plus arginine stimulation (phase 1: 13975 ± 808 fmol insulin, 1417 ± 218 fmol/l amylin (10.1%); phase 2: 92270 ± 2484 fmol insulin, 8804 ± 1678 fmol amylin (9.5%)). There was no change of the secreted insulin/amylin ratio during both phases of stimulated hormone release.

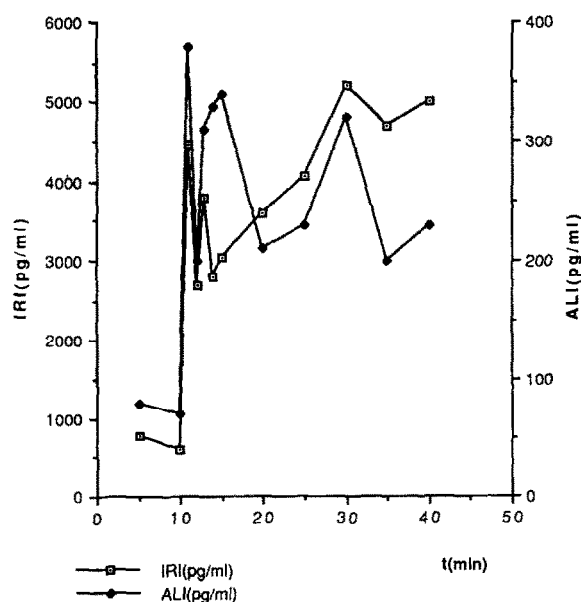


Fig.2. Secretion of insulin and amylin-like immunoreactivity from the isolated perfused rat pancreas under basal conditions (0–10 min) and during stimulation with 10 mmol/l glucose plus 10 mmol/l arginine (11–40 min). IRI, insulin-like immunoreactivity; ALI, amylin-like immunoreactivity.

4. DISCUSSION

Recent studies demonstrated amylin, the major component of pancreatic amyloid found in type II diabetes, as a normal product of pancreatic β -cells [5,6]. There is evidence that amylin represents a biological active peptide: the carboxy-terminal end of amylin is amidated [14] and it inhibits the insulin-stimulated glycogen synthesis of skeletal muscle [7,8]. Recently, we observed an inhibition of somatostatin secretion from isolated rat pancreas (Fehmann, M.C., unpublished observation). Therefore, endocrine and paracrine functions of amylin can be speculated in regulation of glucose homeostasis.

Using polymerase chain reactions Ferrier et al. found in islets an amylin hybridisation signal intensity of approximately 10% of that of insulin [15]. The present study now shows for the first time that amylin is released from the isolated rat pancreas revealing an identical secretion pattern like insulin (figs 1 and 2). The molar amount of secreted amylin was about 10% of that of insulin. This suggests amylin as a hormone produced by pancreatic β -cells and coreleased with insulin into circulation. The physiological role of amylin is still unknown.

In conclusion our data suggest amylin as a new secretory product of rat pancreatic β -cells which is coreleased with insulin.

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