Amylin Secretion from the Rat Pancreas and Its Selective Loss after Streptozotocin Treatment

Atsushi Ogawa, Virginia Harris, Sara K. McCorkle, Roger H. Unger, and Kenneth L. Luskey

Center for Diabetes Research, Gifford Laboratories, Department of Internal Medicine, University of Texas Southwestern Medical Center at Dallas, and Veteran’s Administration Medical Center, Dallas, Texas 75235

Abstract

Amylin, a peptide copackaged with insulin in \( \beta \)-cell granules, was measured in the effluent of the perfused rat pancreas by means of a newly developed specific radioimmunoassay. Its secretion parallels that of insulin in response to 20 mM glucose, 10 mM arginine, or the combination thereof. The relative molar amount of secreted amylin was estimated to be 25–37% that of insulin. Treatment with a borderline diabeticogenic dose of streptozotocin reduced amylin response without significantly changing the insulin response. A severely diabeticogenic dose of streptozotocin totally abolished amylin release and markedly reduced insulin release. The selective impairment of amylin secretion in streptozotocin-treated rats could represent an early manifestation of \( \beta \)-cell depletion or injury. (J. Clin. Invest. 1990. 85:973–976.)

Introduction

Amylin (or islet amyloid polypeptide) is a 37-amino acid peptide that was initially identified as the main constituent of islet amyloid in subjects with noninsulin-dependent diabetes mellitus (1–4) and has been shown to modify insulin’s effects in skeletal muscle (5–7). It is derived from a 93-amino acid precursor molecule in rat (8) or an 89-amino acid precursor in man (9, 10). Amylin mRNA is found in rat pancreatic islets and is not present in other tissues (8). Amylin mRNA content is \( \approx \) 10% that of insulin mRNA content in the pancreas. Immunolocalization studies at the electron microscopic level by Johnson et al. and Lukinius et al. have shown that amylin is located within secretory granules of the \( \beta \) cell and is presumably copackaged with insulin (11, 12). If this is the case, this polypeptide must be cosecreted with insulin. To test this possibility in normal and \( \beta \)-cell–depleted rats we have developed a radioimmunoassay capable of measuring amylin and quantitated amylin secretion from the perfused pancreas.

Methods

Radioimmunoassay of rat amylin. Synthetic rat amylin (Peninsula Laboratories, Belmont, CA) was iodinated by the method of Greenwood et al. (13) and had a specific activity of 50–53 \( \mu \)Ci/\( \mu \)g. The \(^{125}\)I-amylin was purified by HPLC on a LiChrosorb C18 reverse phase column and eluted with a gradient of 0–60% acetonitrile/0.1% trifluoroacetic acid. Purified fractions were collected in the assay diluent [0.05 M KH2PO4, pH 7.5, containing 0.25% Na2EDTA, 0.1% bovine serum albumin and 100,000 kallikrein units of Trasylol (Mobay Pharmaceuticals, Piscataway, NJ)]. Rat amylin or rat calcitonin gene-related peptide (CGRP)* (Peninsula Laboratories) standards were made up in modified KRB [1.5 mM KH2PO4, pH 7.5, 1.2 mM MgSO4, 2.4 mM CaCl2, 4.4 mM KCl, and 100 mM NaCl] containing 4.5% dextran T70 (Pharmacia Fine Chemicals, Piscataway, NJ) to mimic the perfusate samples. A volume of 0.2 ml of standard or perfusate fractions was mixed with 0.3 ml of the foregoing tracer-containing solution (5,000–7,000 ppm/sample) and 0.1 ml of a 1:40,000 dilution of an antibody directed against synthetic human amylin (Peninsula Laboratories). Rat amylin has 31 of 37 residues identical to human amylin (8). After incubation at 4°C for 4 d, bound and free \(^{125}\)I-amylin were separated by the dextran-coated charcoal method of Herbert et al. (14). Under these conditions, the \(^{125}\)I rat amylin was not degraded during the incubation, 90–95% of the input counts were precipitated by trichloroacetic acid in samples incubated for 0–96 h. Insulin was measured by the method of Yalow and Berson (15) as modified by Herbert et al. (14).

Secretion from perfused rat pancreas. Pancreata of Wistar rats were isolated and perfused by the method of Grodsky and Fanska (16), as modified by Hisatomi et al. (17). The perfusate was KRB buffer containing 4.5% Dextran T70, 5.6 mM glucose, 1% bovine serum albumin, and 5 mM each of sodium pyruvate, sodium glutamate, and sodium fumarate. The flow rate was 2.7 ml/min. After a 10–15-min equilibration period, the pancreas was perfused for 10 min with 5.6 mM glucose, 10-min stimulatory periods with 20 mM glucose, 10 mM arginine, or the combination of 20 mM glucose and 10 mM arginine were separated by 5-min "rest periods" during which the perfusate was returned to the baseline. Samples (2.7 ml each) were collected every minute for determination of amylin and insulin concentrations in chilled tubes containing 0.3 ml of 0.15 M NaCl, 0.05 M Na2EDTA, and 0.3 M benzamidine.

Streptozotocin treatment of rats. Male Wistar rats were given a single dose of either 30 or 65 mg/kg of streptozotocin dissolved in phosphate-buffered saline via the tail vein. Animals that received 65 mg/kg of streptozotocin were treated with insulin (2 U of isophane insulin twice daily) to prevent ketonuria and minimize hyperglycemia. Six to ten days later insulin and amylin secretion were studied by pancreatic perfusion.

Results

As shown in Fig. 1A, the amylin assay could detect rat amylin over a range of 300 to 10,000 pg/ml. This assay was specific for amylin in that CGRP, a neuropeptide that is 50% identical to

1. Abbreviations used in this paper: CGRP, calcitonin gene-related peptide.
In inscribed perfusates values are rat mg/ml (+), U/ml.

974 total insulin no zotocin, was 41-50 were had animals Streptozotocin Streptozotocin (30 mg/kg) (65 mg/kg) were performed in Methods. The values are the mean of triplicate determinations. (B) Serial dilutions of three samples (A, V, M) from pancreatic perfusates were compared to the displacement curve of synthetic rat amylin. The assay was performed as described in Methods. The values are the mean of duplicate determinations.

**Figure 1.** (A) Radioimmunoassay for rat amylin. Competition of binding of 125I-amylin to anti-amylin antisera was performed as described in Methods. The percent bound values in the presence of increasing concentrations of rat amylin (●) or rat CGRP (▲), insulin at 200 μU/ml (●), somatostatin-14 at 2 mg/ml (●) or glucagon at 2 mg/ml (+) are shown. The values are the mean of triplicate determinations. (B) Serial dilutions of three samples (A, V, M) from pancreatic perfusates were compared to the displacement curve of synthetic rat amylin. The assay was performed as described in Methods. The values are the mean of duplicate determinations.

**Table 1. Secretion Rates from the Perfused Rat Pancreas in Response to Secretogogues**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glucose</th>
<th>Glucose plus arginine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Insulin</td>
<td>Amylin</td>
</tr>
<tr>
<td></td>
<td>pmol/min</td>
<td>pmol/min</td>
</tr>
<tr>
<td>Controls</td>
<td>1.71±0.19</td>
<td>0.43±0.09</td>
</tr>
<tr>
<td>Streptozotocin</td>
<td>1.41±0.17</td>
<td>0.08±0.08</td>
</tr>
<tr>
<td>(30 mg/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptozotocin</td>
<td>0.23±0.01</td>
<td>ND</td>
</tr>
<tr>
<td>(65 mg/kg)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The total insulin and amylin secretion in response to glucose (fractions 11–20), arginine (fractions 26–35) and glucose plus arginine (fractions 41–50) were summed and converted to picomoles per minute per pancreas. Amylin concentrations were estimated assuming the 37 amino acid peptide was the only molecular species of amylin secreted from the pancreas. In rats treated with 30 mg/kg of streptozotocin only one of three animals had measurable amounts of amylin secreted in response to either glucose or arginine. In all the rats that received 65 mg/kg of streptozotocin, no amylin could be detected in response to any of the secretagogue challenges. ND, not detected.
all the stimuli (Fig. 2 B). Glucose or arginine alone resulted in
detectable amylin secretion in only one out of three rats. All
rats responded to the combination of glucose plus arginine, but
the peak amylin response was much lower than in the control
animals (740±104 pg/ml vs. 2,775±385 pg/ml).

Table I compares the estimated secretion rates for both
insulin and amylin in response to the various stimuli. In nor-
amal rats the amylin secretion rate in response to glucose or
arginine was 25–28% that of insulin. When the combined regi-
men was used, the secretion rate of amylin increased to 37%
that of insulin. However, in borderline diabetes induced by
pretreatment with 30 mg/kg of streptozotocin the amylin re-
sponse to glucose plus arginine perfusion fell to 7.2% of the
insulin response.

Discussion

These studies show that amylin and insulin are secreted simulta-
neously from the β cell, consistent with the earlier demon-
stration that amylin is present in β cell secretory granules (11,
12). Glucose and arginine stimulated the secretion of both
insulin and amylin in a parallel fashion and with similar rela-
tive potencies. However, with the more potent combined glu-
cose-arginine stimulus the relative amount of amylin to insu-
lin increased. This suggests either that there may be cell-to-cell
variation in the relative content of these two peptides or that β
cells can selectively release granules containing differing am-
mounts of insulin and amylin. In response to the more potent
secretory stimulus either amylin-rich cells are preferentially
stimulated to secrete, more amylin-rich β-granules are released
or a non-β cell source of amylin is responsive to the greater
challenge.

In rats with mild diabetes from streptozotocin treatment, a
selective loss in the ability to secrete amylin was observed. This
suggests that streptozotocin treatment may either be selec-
tively killing amylin-rich cells or selectively interfering with
the expression and/or secretion of amylin within cells that can
still secrete insulin in response to arginine.

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In autoimmune diabetes impairment of insulin secretion is one of the first detectable manifestations, being present before the onset of clinical symptoms (18, 19). The reduced secretion of amylin in the streptozotocin-diabetic rats raises the possibility that impaired amylin secretion is likely to be present and may actually precede the insulin abnormality in instances of β cell injury or depletion, such as human type 1 diabetes. The possible consequences of such amylin deficiency remain to be established.

Acknowledgments

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References