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

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Abstract

Amylin, a peptide copackaged with insulin in β-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 diabetogenic dose of streptozotocin reduced amylin response without significantly changing the insulin response. A severely diabetogenic 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 β-cell depletion or injury. (*J. Clin. Invest.* 1990. 85:973–976.) islets • radioimmunoassay • amyloid • diabetes • insulin

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 ~ 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 β 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 β 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 μCi/μg. The ^{125}I-amylin was purified by HPLC on a LiChrosorb C_{18} 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 KH_2PO_4, pH 7.5, containing 0.25% Na_2EDTA, 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)[1] (Peninsula Laboratories) standards were made up in modified KRB [1.5 mM KH_2PO_4, pH 7.5, 1.2 mM $MgSO_4$, 2.4 mM $CaCl_2$, 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 cpm/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 Na_2EDTA, 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. 1 *A*, 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

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1. *Abbreviations used in this paper:* CGRP, calcitonin gene-related peptide.

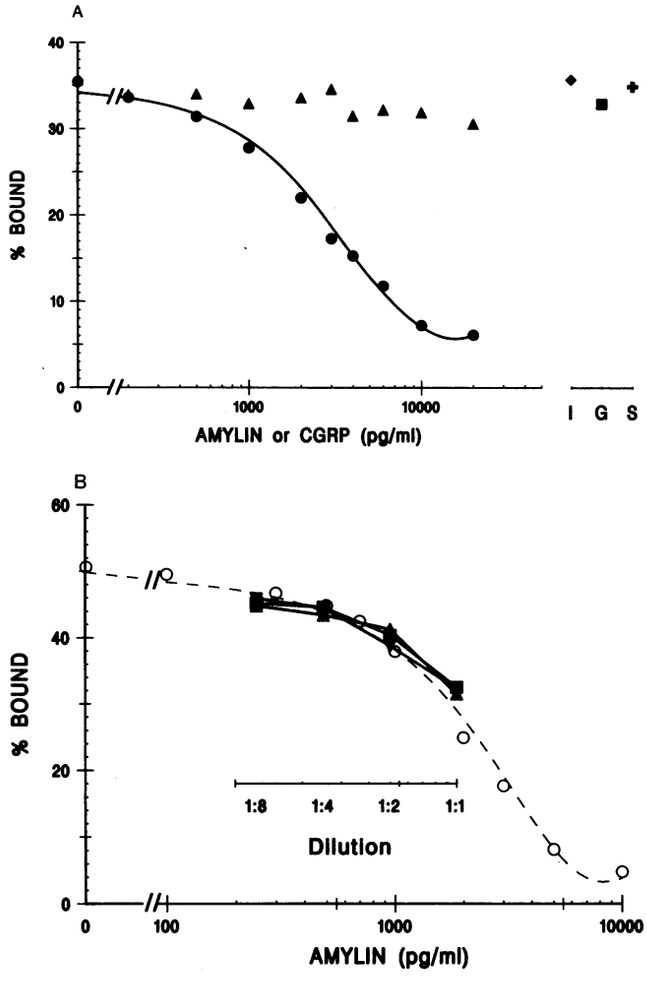

Figure 1. (*A*) Radioimmunoassay for rat amylin. Competition of binding of ^{125}I-amylin to anti-amylin antisera was performed as described in Methods. The percent bound values in the presence of increasing concentrations of rat amylin (●) or rat CGRP (▲), insulin at 200 μU/ml (◆), somatostatin-14 at 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 (▲, ▼, ■) 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.

the amylin sequence, and other pancreatic hormones including insulin, somatostatin-14, and glucagon did not crossreact in the assay. Furthermore the addition of human insulin and C-peptide to the assay did not inhibit the displacement of ^{125}I-rat amylin (data not shown).

With this assay amylin was measured in the effluent of perfused pancreata of normal rats under conditions known to stimulate the secretion of insulin. As shown in Fig. 2 *A*, insulin secretion rose in typical biphasic fashion in response to glucose, arginine, and the combination of the two. The insulin response was least with glucose and most with glucose plus arginine. Baseline amylin levels in the perfusate averaged 359±88 pg/ml, slightly above the approximate limits of detection of this assay (300 pg/ml). After secretogogue challenges amylin secretion rose to easily detectable levels. During the 20-mM glucose perfusion amylin rose to a maximum level of 882±138 pg/ml and in response to 10 mM arginine peaked at 1,247±308 pg/ml. The most potent stimulus for amylin secretion was the perfusion of glucose plus arginine, when the amylin concentration rose to a peak of 2,775±385 pg/ml. As shown in Fig. 1 *B*, the immunoreactive amylin species in the perfusate displaced the radioactive amylin tracer from the antibody in parallel to the amylin standard curve.

To determine the effects of minimally diabetogenic and fully diabetogenic doses of the β-cytotoxin streptozotocin on amylin secretion, rats that had received streptozotocin at either 30 or 65 mg/kg of body weight were studied using the same perfusion protocol. Rats that received the high dose of streptozotocin developed severe diabetes with hyperglycemia and ketosis (plasma glucose 450±37 mg/dl). The pancreata from these animals had a marked impairment in both insulin and amylin secretion (Fig. 2 *C*). Insulin did not rise above the baseline in response to glucose. The peak of the response to arginine alone or arginine plus glucose was only 60.3±3.2 μU/ml and 49±6.1 μU/ml, respectively, compared with a peak of 132.3±21.8 μU/ml or 224.5±41.7 μU/ml in response to these stimuli in normal animals. However, amylin was not detected in the perfusate of any of the high-dose streptozotocin-treated animals at any time.

Rats treated with the 30-mg/kg dose of streptozotocin exhibited only minimal hyperglycemia (plasma glucose 145±20 mg/dl), no ketosis, and did not require insulin therapy. Insulin secretion did not differ dramatically from that of control rats, whereas amylin secretion was markedly reduced in response to

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

	Glucose		Arginine		Glucose plus arginine	
Treatment	Insulin	Amylin	Insulin	Amylin	Insulin	Amylin
	pmol/min		*pmol/min*		*pmol/min*	
Controls	1.71±0.19	0.43±0.09	1.86±0.25	0.52±0.08	3.91±0.58	1.48±0.20
Streptozotocin (30 mg/kg)	1.41±0.17	0.08±0.08	2.77±0.10	0.12±0.12	5.66±0.62	0.41±0.02
Streptozotocin (65 mg/kg)	0.23±0.01	ND	0.76±0.11	ND	0.78±0.05	ND

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 secretogogue challenges. ND, not detected.

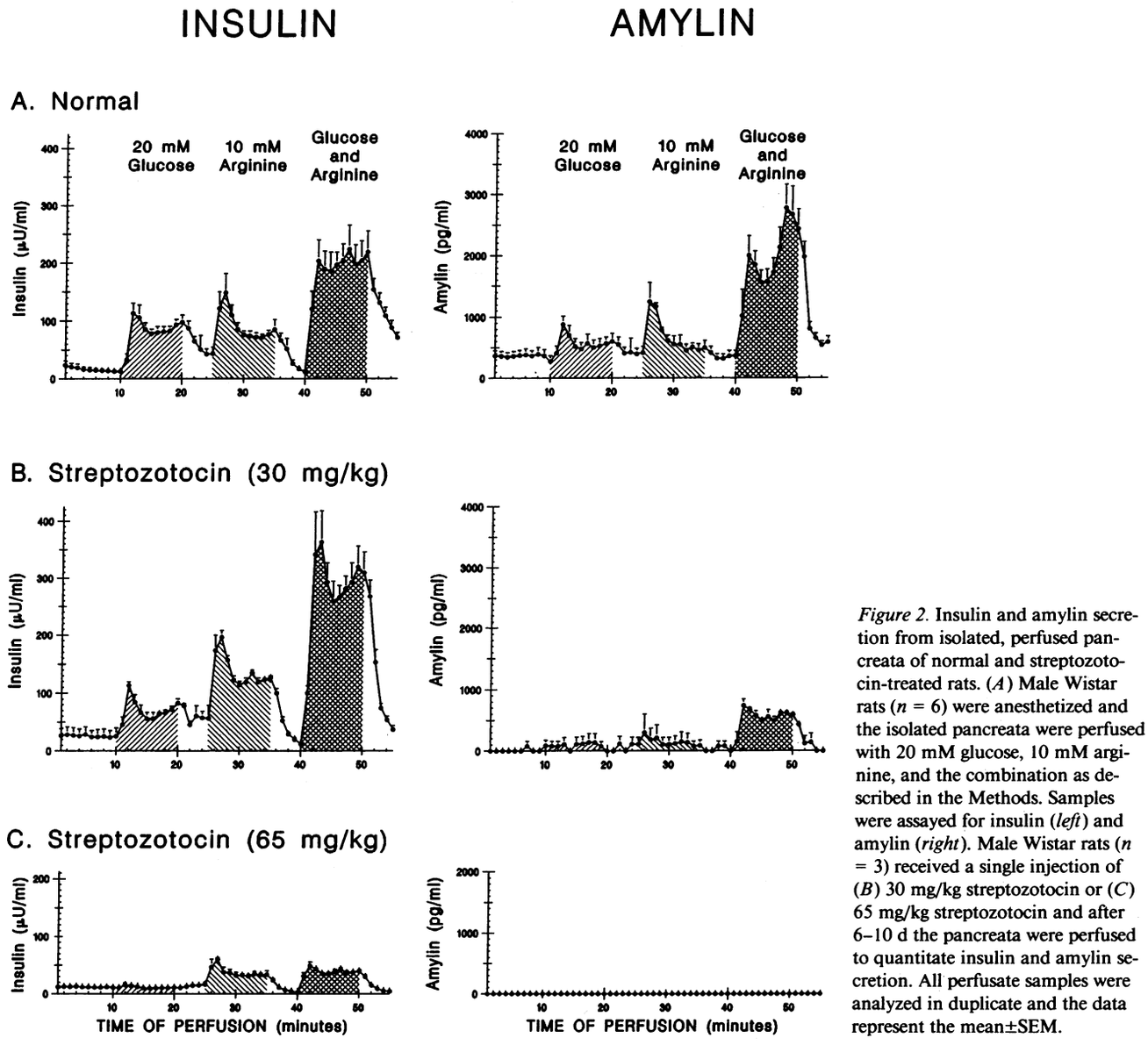

Figure 2. Insulin and amylin secretion from isolated, perfused pancreata of normal and streptozotocin-treated rats. (*A*) Male Wistar rats (n = 6) were anesthetized and the isolated pancreata were perfused with 20 mM glucose, 10 mM arginine, and the combination as described in the Methods. Samples were assayed for insulin (*left*) and amylin (*right*). Male Wistar rats (n = 3) received a single injection of (*B*) 30 mg/kg streptozotocin or (*C*) 65 mg/kg streptozotocin and after 6–10 d the pancreata were perfused to quantitate insulin and amylin secretion. All perfusate samples were analyzed in duplicate and the data represent the mean±SEM.

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 normal rats the amylin secretion rate in response to glucose or arginine was 25–28% that of insulin. When the combined regimen 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 response to glucose plus arginine perfusion fell to 7.2% of the insulin response.

Discussion

These studies show that amylin and insulin are secreted simultaneously from the β cell, consistent with the earlier demonstration 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 relative potencies. However, with the more potent combined glucose-arginine stimulus the relative amount of amylin to insulin 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 amounts 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 selectively 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.

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.

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