# Release of Amylin From Perfused Rat Pancreas in Response to Glucose, Arginine, β-Hydroxybutyrate, and Gliclazide

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**Amylin is a 37–amino acid peptide isolated from the islet amyloid of patients with non-insulin-dependent diabetes mellitus. The isolated perfused normal rat pancreas was used to evaluate the effects of glucose and insulin secretagogues, such as arginine, β-hydroxybutyrate, and gliclazide, on amylin secretion. Glucose and the other stimulants tested elicited a significant release of amylin from the rat pancreas in a biphasic pattern, similar to that of insulin. Dose-response studies of the glucose-induced release of amylin and insulin revealed that they possessed a similar dependency on glucose. However, the release of amylin induced by high concentrations of glucose was partially dissociated from that of insulin; that is, the amylin-insulin molar ratios induced by 22.2 and 33.3 mM glucose (1.11 ± 0.05 and 1.05 ± 0.04%, respectively) were significantly higher than those induced by 16.7 mM glucose (0.90 ± 0.04%, $P < 0.01$ vs. 22.2 mM glucose, $P < 0.05$ vs. 33.3 mM glucose). Additionally, when the basal concentration of glucose in the perfusate was increased from 5.6 to 11.1 mM, the response of amylin was unchanged. These data suggest that amylin may be an islet hormone whose abundant response to high concentrations of glucose might contribute to the oversecretion of amylin in the hyperglycemia that accompanies diabetes mellitus.**
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The pancreatic islets of patients with non-insulin-dependent diabetes mellitus (NIDDM) have been found to contain interstitial deposits of amyloid (1,2), a finding considered relatively specific for this disease (3). The peptide amylin has been purified and characterized from amyloid deposits in human insulinomas (4) and from the pancreatic islets of patients with NIDDM (5). Amylin is a 37–amino acid peptide that has been reported to exert a restraining action on the insulin-induced synthesis of glycogen in skeletal muscle (6,7). However, these experiments involved high concentrations of amylin, and it has not been established whether this is a physiological action of amylin. From the analysis of the cDNA sequence encoding preproamylin, which contains a typical signal peptide, it appears that human and rat amylin is probably generated by proteolytic processing from 89– and 93–amino acid precursors (8–12). An RNA hybridization study has shown that amylin mRNA is selectively expressed in the pancreatic islets (11). Immunocytochemical studies have demonstrated that amylin is colocalized with insulin in the secretory granules of the β-cells (13,14). These findings suggest that amylin may be an endocrine hormone synthesized by and secreted from the β-cells. However, the secretion of amylin has not been characterized in detail. This study was undertaken to evaluate the effects of physiological or higher concentrations of glucose on the secretion of amylin by the isolated perfused rat pancreas.

## RESEARCH DESIGN AND METHODS

For isolation of rat pancreas perfusions, we used the pancreas glands of male Wistar-King albino rats (350–400 g body wt, Inst. of Experimental Animals, Kyushu Univ., Fukuoka, Japan). The pancreases were isolated and perfused according to the system of Grodsky and Fanska (15), with minor modifications (16). After an overnight fast, the rats were anesthetized with pentobarbital sodium (50 mg/kg i.p.). The pancreas and the adjacent proximal portion of the duodenum were isolated and transferred to a thermostatically controlled plexiglass perfusion chamber in which the celiac trunk and the portal vein were cannulated. Nonrecirculating perfusion was begun at a constant flow rate of 3.6 ml/min maintained by a peristaltic pump (Harvard, Millis, MA) with a Krebs-Ringer bicarbonate buffer supplemented with 4.5% dextran T-70 (Pharmacia, Uppsala, Sweden), 1% bovine se-

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rum albumin (Sigma, St. Louis, MO), and 5 mM each of pyruvate, fumarate, and glutamate. Concentrations of glucose in the basal perfusates were 5.6 or 11.1 mM. The perfusion medium was oxygenated with a mixture of 95% $O_2$/5% $CO_2$ and maintained at 37°C. The perfusion pressure was monitored continuously. An equilibration period of 20 min preceded each experimental period. Glucose, arginine hydrochloride, β-hydroxybutyrate (βOHB; Sigma), and gliclazide (Dainippon, Osaka, Japan) were each dissolved in perfusate and added to the circulating perfusate through a side-arm infusion pump (model 975, Harvard). One-minute aliquots of the portal vein effluent were collected in chilled tubes containing 0.4 ml of an EDTA-benzamidine mixture (0.03 and 0.3 M, respectively) and stored until assay at −20°C.

The concentration of amylin in the effluent was determined by radioimmunoassay (RIA) (17). Specific antisera to rat amylin and $^{125}$I-labeled rat amylin were purchased from Peninsula (Belmont, CA). RIA was performed by a double-antibody technique. Antiserum to rat amylin was produced in rabbits immunized with synthetic rat amylin. This antiserum exhibited 13.3% cross-reactivity with human amylin but <0.01% cross-reactivity with human calcitonin gene–related peptides (CGRPs) and rat CGRPs. The minimum sensitivity of the assay was 2.6 pM. The dilution curve of the perfusate sample paralleled the standard curve.

The concentration of insulin in the effluent was measured by RIA with a kit (Insulin RIA Bead) purchased from the Dainabot Isotope Laboratory (Tokyo; 18). Rat insulin standards (Novo, Copenhagen) diluted with perfusion medium were employed as the insulin standards. The serial dilution curve of the perfusate sample paralleled the standard curve.

Data are expressed as means ± SE. Statistical evaluation was performed with Student's *t* test ($P = 0.05$).

## RESULTS

We studied the effects of high glucose on pancreatic amylin release. The release of amylin and insulin from the pancreas was stimulated by administering glucose in excess of the physiological levels of 8.3–33.3 mM (Fig. 1). Glucose elicited a biphasic release of both amylin and insulin in a dose-dependent fashion. At each concentration of glucose, the maximum release of amylin occurred at 2 min, followed by a less-prominent second-phase release. The maximum release of amylin in the first phase apparently increased in a glucose-dose–dependent manner and continued to increase even at 22.2 mM glucose. A dose of 33.3 mM glucose elicited a significantly higher maximum release of amylin (259 ± 18 pM, $P < 0.05$) than did 16.7 mM glucose (197 ± 10 pM). However, the maximum release of insulin (21,900 ± 1800 pM) induced by 33.3 mM glucose was not significantly increased compared with that induced by 16.7 mM glucose (18,500 ± 1200 pM). The cumulative output of both amylin and insulin during the high-dose glucose infusions increased significantly in a dose-dependent manner up to 33.3 mM glucose (Table 1). In the supraphysiological range of glucose from 8.3 to 33.3 mM, the amylin-insulin molar ratios were ~1%. However, the amylin-insulin molar ratios at 22.2 and 33.3 mM glucose were significantly increased compared with that induced by 16.7 mM glucose ($P < 0.01$ vs. 22.2 mM glucose, $P < 0.05$ vs. 33.3 mM glucose).

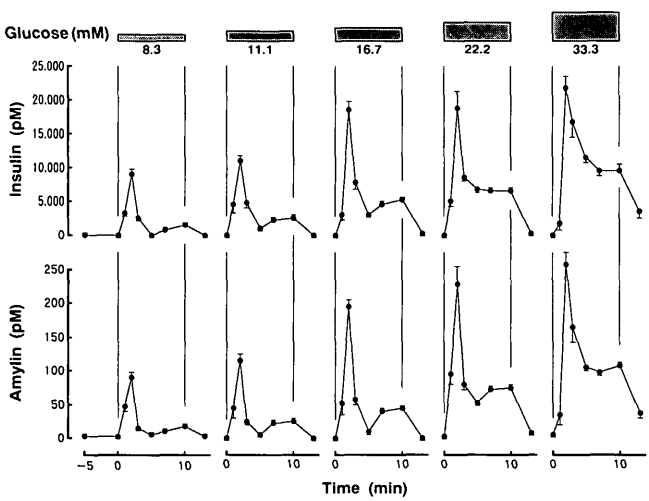


**FIG. 1. Release of amylin and insulin from isolated perfused rat pancreas in response to various concentrations of glucose varying from 8.3 to 33.3 mM. Concentration of glucose in basal perfusates was 5.6 mM. Data are expressed as means ± SE of 6 experiments.**

TABLE 1
Cumulative output of amylin and insulin in response to various concentrations of glucose in perfused rat pancreas

| Glucose (mM) | Amylin (pmol/10 min) | Insulin (pmol/10 min) | Amylin/insulin (%) |
|---|---|---|---|
| 8.3 | 0.924 ± 0.074 | 84.4 ± 7.7 | 1.12 ± 0.11 |
| 11.1 | 1.271 ± 0.198 | 141.9 ± 17.4* | 0.89 ± 0.07 |
| 16.7 | 2.179 ± 0.186† | 243.6 ± 19.4† | 0.90 ± 0.04 |
| 22.2 | 3.488 ± 0.255‡ | 313.5 ± 18.0§ | 1.11 ± 0.05‡ |
| 33.3 | 4.877 ± 0.245‖ | 466.0 ± 20.6¶ | 1.05 ± 0.04§ |

Values are means ± SE of 6 experiments. Cumulative output is calculated as the area under the curve. Amylin-insulin molar ratio is calculated as the ratio of cumulative output of amylin to that of insulin.
*$P < 0.05$ vs. 8.3 mM glucose.
†$P < 0.01$ vs. 11.1 mM glucose.
‡$P < 0.01$, §$P < 0.05$, vs. 16.7 mM glucose.
‖$P < 0.01$, ¶$P < 0.001$, vs. 22.2 mM glucose.

Next, to evaluate any differences between amylin and insulin in the glucose-induced response under conditions of ambient high glucose levels, we examined the effect of an increase from a high basal glucose level (11.1 mM) in the isolated perfused pancreas. In response to the elevation of glucose from 11.1 to 16.7 mM, the level of amylin rose from the baseline of 33 ± 6 pM to a peak of 74 ± 10 pM at 1 min (increase of 224%; Fig. 2). In response to the same glucose increment, the level of insulin rose from 4500 ± 600 pM to a peak of 10,800 ± 600 pM at 2 min (increase of 240%). Similarly, in response to the elevation of glucose from 11.1 to 22.2 mM, the first peak of amylin secretion was increased by 215%, and the first peak of insulin secretion was increased by 217%. To confirm the increase in amylin secretion stimulated by the high glucose levels, we compared the integrated incremental output of these peptides stimulated by the elevation of glucose from a high level of 11.1 mM with that from a physiological level of 5.6 mM in the basal perfusates. No significant difference was found between the incremental output of amylin in response to the same glucose elevation at 5.6 and 11.1 mM (Table 2). Similarly, the response of insulin to the glucose elevation from 11.1 mM was

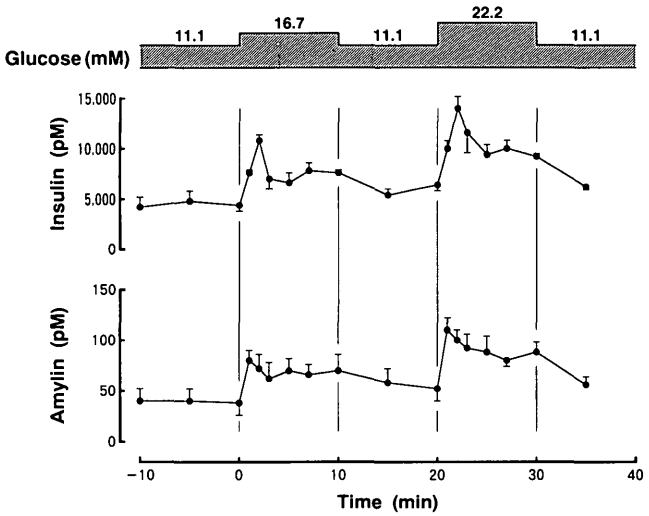


**FIG. 2. Release of amylin and insulin from isolated perfused rat pancreas in response to glucose elevation starting from 11.1 mM in basal perfusates. Data are expressed as means ± SE of 4 experiments.**

not significantly reduced compared with that produced by 5.6 mM glucose in the basal perfusates. Although the baseline level of amylin release was elevated by the high level of basal glucose, the response of amylin to the increase of glucose from a high level was equal to that from a physiological level. This finding was reproducible at the two different levels of glucose tested.

We also studied the effects of arginine, βOHB, and gliclazide on pancreatic amylin release. Ten millimolar arginine, 10 mM βOHB, and 0.28 mM gliclazide in the presence of 5.6 mM glucose stimulated a biphasic release of amylin and insulin (Fig. 3). The maximum release of amylin induced by respective infusion of 10 mM arginine, 10 mM βOHB, and 0.28 mM gliclazide, was 197 ± 12 pM at 2 min, 222 ± 32 pM at 2 min, and 188 ± 18 pM at 1 min. These substances consistently induced a maximum release of amylin and insulin simultaneously. As a result, a similar pattern of release for these peptides followed each infusion of secretagogue. The cumulative output of amylin and insulin is shown in Table 3. The amylin-insulin molar ratios induced by these secretagogues were ~1% and did not differ significantly. Concerning stimulation with arginine, the cumulative output of amylin during 10 mM arginine infusion was significantly increased compared with that induced by 5 mM arginine ($P < 0.01$). Thus, the response to arginine was dose dependent.

TABLE 2
Effects of glucose increment from 2 different baseline glucose levels on release of amylin and insulin from perfused rat pancreas

| Glucose increment | Change in glucose concentration (mM) | ΔAmylin (pmol/10 min) | ΔInsulin (pmol/10 min) |
|---|---|---|---|
| 5.6 mM | 5.6 → 11.1 | 1.261 ± 0.192 | 135.5 ± 16.9 |
| | 11.1 → 16.7 | 1.160 ± 0.152 | 107.6 ± 20.4 |
| 11.1 mM | 5.6 → 16.7 | 2.168 ± 0.189* | 237.0 ± 19.6* |
| | 11.1 → 22.2 | 1.996 ± 0.238† | 205.1 ± 30.8† |

Values are means ± SE of 6 experiments in the 5.6-mM basal glucose perfusion and of 4 experiments in the 11.1-mM basal glucose perfusions. ΔAmylin and Δinsulin, cumulative amylin and insulin output during 10-min glucose infusion − cumulative amylin and insulin output during original basal period (10 min).
*$P < 0.01$ vs. 5.6 → 11.1 mM glucose.
†$P < 0.05$ vs. 11.1 → 16.7 mM glucose.

## DISCUSSION

This study demonstrates that glucose and other insulin secretagogues stimulate a significant release of amylin from the perfused normal rat pancreas. At each glucose concentration tested, amylin and insulin simultaneously reached a peak release at 2 min; then, after cessation of the infusion, both peptides immediately returned to baseline levels. The similar pattern of release for these islet peptides is consistent with previous ultrastructural findings showing a colocalization of amylin and insulin within the pancreatic β-cell secretory granules (13,14). When considered together with the morphological evidence, our observations indicate that amylin is secreted by the pancreatic β-cells through a process that is stimulated and regulated by levels of glucose that are physiological or higher. In addition to the close relationship previously documented between amylin and insulin in terms of the islet cell–specific expression of their mRNA (11), post-translational proteolytic processing (19), intramolecular integration of their disulfide bonds (12,19), and colocalization in the secretory granules of β-cells (13,14), this study provides additional evidence for a similar dependency on the dose of glucose for the stimulation of the release of amylin and insulin.

This study also indicates some dissociation between the responses of amylin and insulin release to high concentrations of glucose, i.e., the significant increase of the amylin-insulin molar ratios at high glucose levels. This abundant response of amylin release to high levels of glucose is also supported by the finding that the glucose-stimulated amylin

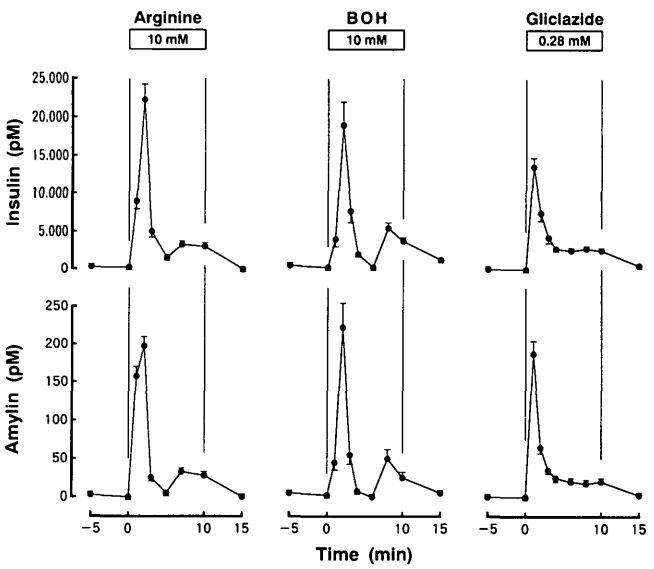


**FIG. 3. Release of amylin and insulin from isolated perfused rat pancreas in response to 10 mM arginine, 10 mM β-hydroxybutyrate (BOH), and 0.28 mM gliclazide. Concentration of glucose in basal perfusates was 5.6 mM. Data are expressed as means ± SE of 6 experiments.**

TABLE 3
Cumulative output of amylin and insulin in response to secretagogues arginine, β-hydroxybutyrate (βOHB), and gliclazide in perfused rat pancreas

| Secretagogue | *n* | Amylin (pmol/10 min) | Insulin (pmol/10 min) | Amylin/insulin (%) |
|---|---|---|---|---|
| 5 mM arginine | 4 | 0.923 ± 0.088 | 76.0 ± 2.4 | 1.22 ± 0.12 |
| 10 mM arginine | 6 | 2.164 ± 0.209* | 216.2 ± 16.9† | 1.00 ± 0.04 |
| 10 mM βOHB | 6 | 1.876 ± 0.255 | 197.9 ± 31.9 | 1.00 ± 0.18 |
| 10 mM gliclazide | 6 | 1.722 ± 0.175 | 173.6 ± 18.3 | 1.01 ± 0.09 |

Values are means ± SE of 4 or 6 experiments. Cumulative output is calculated as the area under the curve. Amylin-insulin molar ratio is calculated as the ratio of cumulative output of amylin to that of insulin.
*$P < 0.01$, †$P < 0.001$, vs. 5 mM arginine.

response remained unchanged even though the basal glucose concentration was elevated. However, the precise mechanism of this persistently high response of amylin in the hyperglycemic range was not clarified by this study. Perhaps a molecular genetic approach would resolve this question, and in fact, Northern-blot analysis has shown the dissociated gene expression of amylin and insulin in diabetic rats after the administration of dexamethasone and streptozocin (20).

This study also demonstrates that insulin secretagogues, such as arginine (21,22), βOHB (21,23), and gliclazide (24), stimulate the release of amylin. The concentration of arginine increases in the peripheral blood after a protein meal. βOHB is a ketone body whose concentration is elevated in the peripheral blood of patients with insulin-deficient diabetes. Also, an elevated βOHB level is observed in NIDDM patients with poor control of their hyperglycemia. Gliclazide, also used in this study, is an oral hypoglycemic that is widely used in treating patients with NIDDM.

Although the physiological or pathophysiological implications of this study are not clear-cut, the concomitant secretion of amylin and insulin induced by the glucose stimulus suggests that amylin may be a hormone that contributes to glucose homeostasis by opposing the action of insulin in skeletal muscle, e.g., by inhibiting the insulin-stimulated synthesis of glycogen, which was originally demonstrated in isolated skeletal muscle in vitro (6,7) and recently reconfirmed in vivo (25,26). Additional evidence provided by this study is the persistently high response of amylin to glucose administered in the hyperglycemic range. This characteristic of amylin secretion might contribute to its possible oversecretion in the hyperglycemia of diabetes and could lead to an excessive deposition of amyloid within the islets and an increase in the peripheral insulin resistance. Johnson et al. (27), studying the spontaneously diabetic cat, reported morphological evidence indicating that, before the development of overt diabetes, there may be an overproduction of amylin. Further investigations are required to investigate the role of amylin as a factor in the pathogenesis of NIDDM.

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