*Rapid communication***Evidence for selective release of rodent islet amyloid polypeptide through the constitutive secretory pathway****S. E. Kahn^{1,2}, C. B. Verchere^{1,2}, D. A. D'Alessio¹, D. L. Cook^{1,2}, W. Y. Fujimoto¹**¹ Division of Metabolism, Endocrinology and Nutrition, Department of Medicine, University of Washington, Seattle, Washington, USA² Veterans Affairs Medical Center, Seattle, Washington, USA

Summary. To determine the potential for differential release of islet amyloid polypeptide and insulin, we performed studies in rat islet monolayer cultures under conditions known to impair regulated beta-cell secretion. In inhibiting concentrations of epinephrine or the absence of calcium, islet amyloid polypeptide was secreted through a constitutive pathway while insulin was not. These findings suggest a

mechanism for persistent islet amyloid polypeptide secretion and amyloid accumulation when regulated insulin release is impaired as in Type 2 (non-insulin-dependent) diabetes mellitus and insulinomas.

Key words: Insulin, secretion, pancreas, amyloid, insulinomas.

Islet amyloid polypeptide (IAPP) and insulin are co-localized to beta-cell secretory granules [1] and are co-released by pancreatic beta cells. In early studies with glucose, we found that the molar proportion of IAPP to insulin released into the medium to be fixed at approximately 1% [2]. Subsequently, using combinations of glucose and non-glucose stimuli we found that the molar proportion of IAPP to insulin secreted could be reduced to below that seen in glucose alone suggesting that the release of these two peptides was not always proportionate [3], an observation also made by others [1]. However, it is unclear whether this differential release results from variability in secretory granule or beta-cell content of the two peptides or whether the secretory pathways responsible for IAPP and insulin release may be preferentially used by one of the peptides. Therefore, we have undertaken the present experiments to examine release of these two beta-cell peptides under conditions likely to differentially affect the regulated and constitutive secretory pathways.

Materials and methods*Pancreatic cell cultures*

Monolayer cultures were prepared from the pancreases of neonatal (3- to 5-day-old) Sprague-Dawley rats as previously described [2]. Studies were performed following 3 days of growth. Cultures were pre-incubated in Krebs-Ringer bicarbonate buffer containing 0.1%

dialysed bovine serum albumin (KRBB-BSA) supplemented with 1.67 mmol/l glucose for 2 h after which they were rinsed prior to incubation with test medium. Dialysed BSA was prepared by dissolving BSA in distilled water supplemented with 17 mmol/l EDTA and then dialysing exhaustively against distilled water. For the studies with epinephrine, the pre-incubation and rinse were performed with KRBB-BSA supplemented with 1.67 mmol/l glucose, while for the calcium studies, the pre-incubation was performed with KRBB-BSA and the rinse with KRBB-BSA supplemented with or without 2.6 mmol/l calcium depending on the subsequent test conditions. Cultures containing 0.5 pancreas equivalents per dish were incubated for 2 h with 1.0 ml of KRBB-BSA supplemented with either 1.67 or 16.7 mmol/l glucose with or without 0.1 to 100 μ mol/l epinephrine, while cultures containing 1.5 pancreas equivalents were incubated for 2 h with 1.25 ml test medium containing either 0 or 16.7 mmol/l glucose with or without 2.6 mmol/l calcium. No calcium chelating agent was added to the test medium. Medium was stored at -20°C until assayed.

Peptide measurements

Previously described radioimmunoassays were used to measure immunoreactive insulin (IRI) and IAPP-like immunoreactivity (IAPP-LI) [2].

Statistical analysis

Data are expressed as mean $\pm$ SEM. Differences in IAPP-LI and IRI between conditions were compared by the Mann-Whitney U test while changes over time were determined using ANOVA. $P < 0.05$ was taken as significant.

Fig. 1. Effect of epinephrine on the release of islet amyloid polypeptide-like immunoreactivity (IAPP; upper panel) and immunoreactive insulin (lower panel) by neonatal rat islet monolayer cultures ($n = 5$) following 120 min of incubation in 1 ml of test medium containing 1.67 mmol/l (■) or 16.7 mmol/l (▨) glucose. Data illustrated are mean $\pm$ SEM. Glucose-stimulated IAPP and insulin release was greater in 16.7 mmol/l than in 1.67 mmol/l glucose ($p < 0.01$). With epinephrine, insulin release was equally inhibited in 1.67 and 16.7 mmol/l glucose. In 16.7 mmol/l glucose with epinephrine, IAPP release was greater than in 1.67 mmol/l glucose with epinephrine ($p < 0.05$)

Results and discussion

In the first set of experiments, islet monolayer cultures were incubated for 120 min in low (1.67 mmol/l) or high (16.7 mmol/l) glucose with or without varying concentrations (0.1 to 100 μ mol/l) of epinephrine (Fig. 1). As expected, IAPP and insulin release were both stimulated by 16.7 mmol/l glucose ($p < 0.01$). In the presence of epinephrine, stimulation of insulin release by 16.7 mmol/l glucose did not occur and insulin concentrations were no different from those in 1.67 mmol/l glucose, whereas IAPP levels at 16.7 mmol/l glucose were suppressed by about 46 % by epinephrine, they were still 35 % higher than those in 1.67 mmol/l glucose ($p < 0.05$). Thus, when regulated secretion was impaired by epinephrine, glucose increased IAPP release independently of insulin. This dissociation of peptide release suggests that either various pools of secretory granules contain different quantities of IAPP and insulin, or that IAPP is sorted to a pathway not available to insulin and which is not suppressed by epinephrine [4].

Since withdrawal of extracellular calcium blocks regulated but not constitutive secretion [5] and does not impair

Fig. 2. Effect of calcium and glucose on islet amyloid polypeptide-like immunoreactivity (IAPP; ●) and immunoreactive insulin (□) release by neonatal rat islet monolayer cultures ($n = 4$) following 15, 60 and 120 min of incubation. Cultures were incubated without calcium and glucose (upper panel), in 16.7 mmol/l glucose without calcium (middle panel) or in 16.7 mmol/l glucose with 2.6 mmol/l calcium (lower panel). Data illustrated are mean $\pm$ SEM. Without both calcium and glucose, IAPP release increased with increasing duration of incubation ($p < 0.0001$) while insulin release did not. Similarly, in 16.7 mmol/l glucose without calcium, IAPP release increased with time ($p < 0.0001$) while insulin release did not exhibit a similar degree of change ($p < 0.05$). In 16.7 mmol/l glucose with 2.6 mmol/l calcium, both IAPP and insulin release increased with increasing duration of incubation ($p < 0.0001$)

beta-cell viability [6], in a second series of studies we incubated islet cultures in 0 or 16.7 mmol/l glucose with or without 2.6 mmol/l calcium for 15, 60 and 120 min in order to estimate regulated and constitutive secretion of IAPP and insulin. In the absence of calcium and glucose, a small amount of IRI was immediately released into the medium at 15 min but did not change over the next 105 min (15 min: 509 ± 24 , 60 min: 361 ± 65 , 120 min: 539 ± 105 fmol/ml; Fig. 2 upper panel). In contrast, IAPP release continually increased during the 120 min incubation ($p < 0.0001$; Fig. 2 upper panel). Secretion of IAPP through a constitutive pathway is therefore suggested.

When 16.7 mmol/l glucose was added in the absence of calcium (Fig. 2 middle panel), IAPP release increased more rapidly with time ($p < 0.0001$) resulting in a 23-fold increase in IAPP levels from 15 to 120 min while insulin release was similar to that without glucose or calcium being only 1.4-fold greater at 120 min than at 15 min ($p < 0.05$). To evaluate regulated secretion, cells were incubated in the presence of both glucose and calcium. In 16.7 mmol/l glucose plus 2.6 mmol/l calcium (Fig. 2 lower panel), both IAPP and insulin release increased with increasing duration of incubation ($p < 0.0001$). While the increase in output of both peptides was greater at all time points ($p < 0.05$) it is noteworthy that in the presence of calcium, insulin output increased 10-fold while IAPP release increased only 50% over that observed in 16.7 mmol/l glucose without calcium. Thus, regulated secretion accounted for only 34% of IAPP output in 16.7 mmol/l glucose plus calcium.

It is well recognized that beta cells continue to metabolize glucose and synthesize insulin when extracellular calcium is absent [7]. Since constitutive secretion is thought to depend on bulk flow [4] and to be regulated only by the rate of protein synthesis, our observation that without calcium, IAPP release was greater in 16.7 mmol/l glucose than in 0 mmol/l glucose suggests that the rate of synthesis of preproIAPP determines the rate of release of IAPP-related peptides by a constitutive pathway. Such a pathway also explains our findings with epinephrine. Epinephrine would appear to completely prevent glucose-stimulated secretion from the regulated pathway. IAPP however would still be released via a constitutive mechanism that is stimulated by glucose in a time-dependent manner, a finding consistent with the known ability of fasting and hypoglycaemia to change IAPP biosynthesis [8]. While IAPP biosynthesis is stimulated in the presence of glucose, epinephrine partially suppresses beta-cell peptide synthesis [9]. This reduction in biosynthesis would be expected to reduce bulk flow through the constitutive pathway and thereby could explain the apparently slightly greater degree of inhibition of 16.7 mmol/l glucose-stimulated IAPP release by epinephrine (46%) compared to that observed in the absence of calcium (34%). Thus, we describe two conditions where the release of IAPP and insulin are dissociated making it unlikely that IAPP is always co-released from beta-cell granules that contain both peptides [1] and participate only in regulated secretion. Further, these data suggest that under conditions of inhibition of beta-cell secretion with epinephrine, regulated secretion of insulin and IAPP is blocked while constitutive secretion of IAPP continues. These findings must be qualified by the fact that while in human islets IAPP has only been found in beta-cell secretory granules that contain insulin, rat delta cells do contain this peptide [10] and delta-cell release in the absence of calcium could explain the findings. However, since delta cells contain hormone in granules and therefore probably require calcium for regulated secretion, it seems unlikely that IAPP is coming solely from these cells in the absence of calcium.

These *in vitro* observations may be important to the pathological features of Type 2 diabetes and insulinomas,

both of which are characterized by failure of the beta cell to secrete insulin in response to glucose and the deposition of islet amyloid [11]. If secretion of IAPP by the constitutive pathway is possible when regulated secretion is impaired, then IAPP secretion would be expected to continue in hyperglycaemic Type 2 diabetes and in tumours. One may hypothesize that precipitation of islet amyloid in these conditions might be initiated by IAPP predominantly secreted from the constitutive pathway. Since this pathway is inefficient for peptide processing [4], proIAPP would be released and could participate in amyloid accumulation as suggested by positive immunostaining of islet amyloid with an antibody raised against the N-terminal region of proIAPP [1]. Since canine IAPP has an amyloidogenic sequence and diabetic dogs do not develop islet amyloid [1] it would appear that both an amyloidogenic sequence and some other factor underlie the development of islet amyloid. Defective regulated secretion and enhanced constitutive release provide a feasible explanation for the development of islet amyloid in Type 2 diabetes and insulinomas. Such a mechanism may also explain the development of amyloid in other polypeptide-secreting tumours.

Finally, the results of the present study could explain the often observed progressive loss of beta-cell function in Type 2 diabetes and the secondary treatment failure occurring with sulphonylureas. If enhanced secretion through the constitutive pathway is involved in amyloidogenesis and is maintained despite sulphonylurea treatment, then increased amyloid formation might be predicted to continue despite treatment. This mechanism would then have a feed forward effect such that islets replaced by amyloid would result in falling endogenous insulin release, increased hyperglycaemia, further IAPP release and the loss of beta cells.

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References

1. Westermark P, Johnson KH, O'Brien TD, Betsholtz C (1992) Islet amyloid polypeptide – a novel controversy in diabetes research. *Diabetologia* 35: 297–303
2. Kahn SE, D'Alessio DA, Schwartz MW et al. (1990) Evidence of cosecretion of islet amyloid polypeptide and insulin by β -cells. *Diabetes* 39: 634–638
3. Kahn SE, Fujimoto WY, D'Alessio DA, Ensink JW, Porte D Jr (1991) Glucose stimulates and potentiates islet amyloid polypeptide secretion by the B-cell. *Horm Metab Res* 23: 577–580
4. Halban PA (1991) Structural domains and molecular lifestyles of insulin and its precursors in the pancreatic beta cell. *Diabetologia* 34: 767–778
5. Wollheim CB, Sharp GWG (1981) Regulation of insulin release by calcium. *Physiol Rev* 61: 914–973
6. Hellman B, Idahl LA, Lernmark Å, Sehlin J, Täljedal IB (1974) The pancreatic beta-cell recognition of insulin secretagogues. Effects of calcium and sodium on glucose metabolism and insulin release. *Biochem J* 138: 33–45

7. Welsh M, Weber T, Wrange O, Nielsen DA, Matthieu M, Steiner DF (1988) Regulation of insulin gene expression by dexamethasone, Ca^{2+} and a phorbol ester. *Biomed Biochim Acta* 47: 299–303
8. Alam T, Chen L, Ogawa A, Leffert JD, Unger RH, Luskey KL (1992) Coordinate regulation of amylin and insulin expression in response to hypoglycemia and fasting. *Diabetes* 41: 508–514
9. Zhang H-J, Redmon JB, Andresen JM, Robertson RP (1991) Somatostatin and epinephrine decrease insulin messenger ribonucleic acid in HIT cells through a pertussis toxin-sensitive mechanism. *Endocrinology* 129: 2409–2414
10. De Vroede M, Foriers A, Van de Winkel M, Madsen O, Pipeleers D (1992) Presence of islet amyloid polypeptide in rat islet B and D cells determines parallelism and dissociation between rat pancreatic islet amyloid polypeptide and insulin content. *Biochem Biophys Res Commun* 182: 886–893
11. Porte D Jr, Kahn SE (1989) Hyperproinsulinemia and amyloid in NIDDM: clues to etiology of islet β -cell dysfunction? *Diabetes* 38: 1333–1336

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