Human amylin fibril-mediated killing of islet b-cells

'ETUATE L. SAAFI, MSc*

Introduction

The human amylin hormone is one of several novel peptides recently discovered to be released from pancreatic b-cells[1]. It has been isolated as the major component of extracellular amyloid deposits present in the isle of Langerhans of patients with non-insulin dependent diabetes mellitus (NIDDM)[2,3,4] and from insulinoma[5]. Amylin has also been isolated from a number of "non-diabetic" adults[6], although the extent of amyloid formation in "non-diabetics" is lesser compared to that found in NIDDM patients[7,8,9].

The amylin hormone is a 37 amino acid polypeptide, with a molecular weight of 3904 Da, which is synthesised and co-secreted with insulin from secretory granules in the b-cells of the isle of Langerhans in the pancreas[2,8,10,11,12]. A number of localisation studies have identified the presence of amylin mRNA in the pancreas of rat [13,14,15] and other animals[11]. Amylin presence has also been identified in both human diabetic and non-diabetic b-cells[1,11]. These studies have provided strong evidence regarding the origin of the amylin hormone from pancreatic b-cells.

" The exact mechanism through which amyloid fibrils elicit programmed cell death in b-cells relative to the role of the amyloid deposits in the pancreases of patients with NIDDM in the causation of NIDDM still remains elusive and is a subject of great interest. "

Structure of amylin

Structural similarities exist between human amylin, calcitonin, and calcitonin-gene related peptide (CGRP). Calcitonin is a peptide hormone secreted by the thyroid gland which lowers blood calcium levels and is antagonistic to the actions of the parathyroid hormone. CGRP is a potent vasodilator found in the nervous system[16,17].

Between different species there is a strong conservation of the *C*-terminal and the *N*-terminal sequences of the amylin peptide with a greater degree of variation within the central region. Human amylin has two post-translational modifications; a disulphide bond at positions 28 and 29 and amidation at the COOH terminal end [2]. The region between positions 20 to 29 of human amylin has been reported to form b-pleated sheets held in place by hydrogen bonding and shows a strong self-aggregating property which is a likely necessary factor in the early stages of pancreatic amyloid plaque formation[18]. Other studies have reported region 7 to 20 of the primary sequence to assume an a-helical conformation and region 30 to 36 to form a b-turn, a seemingly important factor for amyloid fibril formation[19].

Production of amylin

Together with other metabolic and neural influences, amylin has been proposed as an endocrine hormone that regulates carbohydrate metabolism[24,15], a co-regulatory role amylin likely shares with insulin and other gluco-regulatory hormones.

The major regulatory role of insulin seems to be the promotion of the clearance and storage of glucose and dietary carbohydrates as glycogen in the liver and skeletal muscles. A process which involves glucose transportation by an insulin-stimulated glucose transporter, glycogen synthesis by glycogen synthase, and glycogenolysis which involves phosphorylase activity, with the ultimate end result of this insulin action being the prevention of hyperglycaemia[15].

*Developmental Biology and Cancer Research Group, School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand. Email: e.saafi@auckland.ac.nz.

Figure 1. The amino acid sequences of human amylin, human calcitonin, and human CGRP (I) and (II)

	1 10 20 30
Human Amylin	KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY-NH_2
Human Calcitonin	CGNLSTCMLGTYTQDFNKHTFPQTAIGVGAP- NH_2
Human CGRP (I)	ACDTATCVTHRLAGLLSRSGGVVKNNFVPTNVGSKAF- NH_2
Human CGRP (II)	ACNTATCVTHRLAGLLSRSGGMVKSNFVPTNVGSKAF- NH_2

Amylin and CGRP on the other hand have both been reported to stimulate glycogen breakdown in both the liver and skeletal muscle [3,4,20,21] by mainly affecting the rate-limiting phosphorylase and glycogen synthase enzymes in a manner reverse to that by insulin[22,23]. The inhibition of insulin action by amylin has been demonstrated in the perfused pancreas [23] the isolated b-cell [24,25] and the isolated islet [26].

Amylin has been proposed to exist as a monomer under "normal" physiological conditions and most likely adopts a different conformation altogether under amyloid-forming conditions[16]. Amylin's ability to adapt different conformations or polymorphic fibrillar assemblies of different mass, size and length *in vitro* under different conditions has also been reported[27].

Role of amylin

The role of amyloid in the causation of NIDDM is of great interest. NIDDM is a disorder most common in adults. It is normally characterised by (i) insulin resistance where there is a decreased sensitivity of peripheral tissues to insulin, a phenomena amylin most likely contributes to [4], (ii) impaired secretion of insulin, (iii) an increased basal hepatic glucose production and (iv) the presence of amyloid deposits in the extracellular spaces of the pancreatic islets of Langerhans, a characteristic that has been reported to be common in more than 90% of patients with NIDDM [2,12,28,29].

Despite early descriptions of hyaline, later known as amyloid, early in the century by the pathologist Opie[30,31], and Opie hypothesising a possible role for amyloid in the development of NIDDM, amyloid was never regarded as important in the development of NIDDM by the majority of investigators in the field until recently.

For years amyloid presence in the pancreas was generally regarded more as a feature associated with the general ageing process instead of playing any particular role in the pathology of NIDDM. However, amyloid deposits found in the extracellular spaces of the pancreases of diabetic patients is now receiving greater acceptance as most likely to play a possible role in the initiation and development of NIDDM [5,32,33].

Certain studies have shown a number of interesting observations including amyloid's observed juxtaposition next to the membranes of islet b-cells[27] and the cytotoxicity of amyloid to b-cells in the pancreatic islet[34] and to cultured b-cells from studies by our group (unpublished data). Evidence showing decreased islet cell DNA content, attributed to an amyloid-mediated loss in b-cell numbers, with increasing concentrations of amylin has also been reported[35].

Conclusion

The exact mechanism through which amyloid fibrils elicit programmed cell death in b-cells relative to the role of the amyloid deposits in the pancreases of patients with NIDDM in the causation of NIDDM still remains elusive and is a subject of great interest. Also of interest are the ultrastructural and biochemical changes that characterise islet amyloid-mediated b-cell death.

Our studies involve the use of electron fluorescent and laser-confocal microscopy techniques combined with various labelling and biochemical methods. These methods are used to study the ultrastructural and biochemical changes that reflect the early events occurring after amylin fibril-cell interaction and initiation of programmed cell death, and also the time-dependent changes that occur in cellular organelles after being exposed to amylin fibrils.

Results from this study is of great importance as it will help provide a better understanding of the nature of human amylin fibril-mediated programmed cell death in islet b-cells relative to its role in the initiation and development of NIDDM.

References

1. Cooper GJS, Willis AC, Leighton B. Amylin hormone. *Nature*, 1989 340: 272.

2. Cooper GJS, Willis AC, Clark A, et al. Purification and characterisation of a peptide from amyloid-rich pancreases of type 2 diabetic patients. *Proc Natl Acad. Sci* USA 1987, 84: 8628-8632.

3 Cooper GJS, Leighton B, Dimitriadis GD, et al. Amylin found in amyloid deposits in human type 2 diabetes mellitus may be a hormone that regulates glycogen metabolism in skeletal muscle. *Proc Natl Acad Sci* USA. 1988; 85: 7763-7766.

4 Leighton B, Cooper GJS. Pancreatic amylin and calcitonin gene-peptide cause resistance to insulin in skeletal muscle in vitro. *Nature* 1988; 335: 632-635.

5. Westermark P, Wernstedt C, Wilander E, Sletten K. A novel peptide in calcitonin gene related peptide family as an amyloid fibril protein in the endocrine pancreas. *Biochem Biophys Res Commun*, 1986; 140: 827-831.

6. Bell ET. Hyalinization of the islets of Langerhans in diabetes mellitus. *Diabetes*, 1952; 1: 341-344.

7. Bell ET. Hyalinization of the islets of Langerhans in non-diabetic individuals. *Am J Pathol*, 1959; 35: 801-805.

8. Erlrich JC, Ratner IM. (1961) Amyloidosis of the islets of Langerhans. *Am J Pathol*, 1991; 38: 49-59.

9. Clark A, Cooper GJS, Lewis CE. Islet amuloid formed from diabetes-ssociated peptide may be pathogenic in type-2 diabetes. *Lancet* 1987; 2: 231-234.

10. Johnson KH, O'Brian TD, Hayden DW, et al. Immuno-localization of islet amyloid polypeptide (IAPP) in pancreatic beta-cells by means of peroxidase (PAP) and protein A gold techniques. *AM J Pathol*, 1988; 130: 1-8.

11. Lukinius A, Wilander E, Westermark GT, et al. Co-localisation of islet amyloid polypeptide (IAPP) and insulin in the B-cell secretory granules of the human pancreatic islets. *Diabetologia* 1989; 32: 240-244.

12. Clark A, Edwards CA, Ostle LR, et al. Localization of islet amyloid peptide in lipofuscin bodies and secretory granules of human B-cells and in islets of type-2 diabetic subjects. *Cell Tissue Res*, 1989; 257: 179-185.

13. Ferrier GJM, Pierson AM, Jones PM, et al. Expression of the rat amylin (IAPP/DAP) gene. *J Mol Endocrinol*, 1989; 3: R1-R4. (1989)

14. Nishi M, Chan SJ, Nagamatsu S, Bell GI, Steiner DF. Conservation of the sequence of the islet amyloid polypeptide in five mammals is consistent with its putative role as an islet amyloid. *Proc Natl Acad Sci* USA, 1989; 86: 5738-5742.

15. Leffert JD, Newgard CB, Okamoto H, Milburn JL, Luskey KL. Rat amylin: cloning and tissue specific expression in pancreatic islets. *Proc Natl Acad Sci* USA, 1989; 86: 3127-3130.

16. Cooper GJS. Amylin compared with calcitonin gene-related peptide: Structure, biology, and relevance to metabolic disease. *Endocr Rev*, 1994; 15(2): 163-201.

17. Ishida-Yamamoto A, Tohyama M. Calcitonin gene-related peptide in the nervous tissue. *Progr Neurobiol*, 1989; 33: 335-386.

18. Glenner GG, Eanes D, Wiley CA. Amyloid fibrils formed from a segment of the pancreatic islet amyloid protein. *Biochem Biophys Res Commun*, 1988; 155: 608-614.

19. Pittner RA, Albrandt K, Beaumont K, et al. Molecular physiology of amylin. *Journal of Cellular Biochemistry*, 1994; 55S: 19-28.

20. Molina JM, Cooper GJS, Leighton B, Olefsky JM. Induction of insulin resistance in vivo by amylin and calcitonin gene-related peptide. *Diabetes*, 1990; 39: 260-265.

21. Young DA, Deems RO, Deacon RW, et al. Effects of amylin on glucose metabolism and glycogenolysis in vivo. *Am J Physiol*, 1990; 259: E457-461.

22. Young AA, Mott DM, Stone K, Cooper GJS. Amylin activates glycogen phosphorylase in the isolated soleus muscle of the rat. *FEBS Lett*, 1991; 281(1-2): 149-151.

23. Deems RO, Deacon RW, Young DA. Amylin activates glycogen phosphorylase and inactivates glycogen synthase via a Camp-independent mechanism. *Biochem Biophys Res Commun*, 1991; 174: 716-720.

24. Gudelin B, Cooper GJS, Young AA. Amylin secretion from the perfused pancreas: dissociation from insulin and abnormal elevation in insulin-rsistant diabetic rats. *Biochem Biophys Res Commun*, 1991; 180:782-789.

25. Wagoner PK, Chen C, Worley JF, et al. Amylin modulates beta-cells glucose sensing via effects on timulus-secretion coupling. *Proc Natl Acad Sci* USA, 1993; 90: 9145-9149.

26. Ohsawa H, Kanatsuka A, Yamaguchi T, et al. Islet amyloid polypeptide inhibits glucose-stimulated insulin secretion from isolated rat pancreatic islets *Biochem Biophys Res Commun*, 1989; 160: 961-967.

27. Goldsbury CS, Cooper GJS, Goldie KN, Muller SA, Saafi EL, et al. Polymorhic fibrillar assembly of human amylin. *Journal of Structural Biology*, 1997; 119: 17-27.

28. Westermark P, Grimelius L. The pancreatic islet cells in insular amyloidosis in human diabetic and non-diabetic adults. *Acta Path Microbiol Scand* 1973; sect A 81: 291-300.

29. Maloy AL, Longnecker DS, Greenberg ER. The relatin of islet amyloid to the clinical type of diabetes. *Human Pathol*, 1981; 12: 917-922.

30. Opie EL. The relation of diabetes mellitus to lesions of the pancreas. Hyalin degeneration of the islands of Langerhans. *J Exp Med*, 1901; 5: 527-540.

31. Opie EL. On the relation of chronic interstitial pancreatitis to the islands of Langerhans and to diabetes mellitus. *J Exp Med*, 1990; 5: 391-428.

32. Verchere CB, D'Alesio DA, Palmiter RD, et al. Islet amyloid formation associated with hyperglycaemia in transgenic mice with pancreatic beta cell expression of human islet amyloid polypeptide. *Proc Natl Acad Sci* USA, 1996; 93: 3492-3496.

33. de Koning EJP, Morris ER, Hofhuis FMA, et al. Intra- and extracelluar amyloid fibrils are formed in cultured pancreatic cells of transgenic mice expressing human islet amyloid polypeptide. *Proc. Natl Acad Sci* USA, 1994; 91: 8467-8471.

34. Lorenzo, A., Razzaboni, B., Weir, G. C., Yankner, B. A., Pancreatic islet cell toxicity of amylin associated with type-2 dibetes mellitus. *Nature*, 1994; 368: 756-760.

35. Sandler S, Stridsberg S. Chronic exposure of cultured rat pancreatic islets to elevated concentrations of islet amyloid polypeptide (IAPP) causes a decrease in islet DNA content and medium insulin accumulation. *Regulatory Peptides*, 1994; 53: 103-109. □