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Review

Pancreatic peptides, neuropeptides and neurotransmitters in diabetes mellitus: a review

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Abstract
Diabetes mellitus is due to defective secretion and/ or function of insulin. It is a common chronic disease affecting up to 6% of the world population. This prevalence increases to about 24% in the island of Nauru and some Middle Eastern countries. Diabetes mellitus is associated with profound changes in the endocrine pancreas. These changes include a significant decrease in the number of beta cells, the cell type that produces and secretes insulin. A decrease in the number of insulin producing cells is more evident in type I diabetes. The decrease in the number of insulin-secreting cells is associated with a concomitant increase in the number of glucagon, somatostatin and pancreatic-polypeptide producing cells. The increase in glucagon producing cells results in hyperglucagonaemia, which further exacerbates the hyperglycaemia induced by lack of insulin. In addition to the changes in the number and plasma levels of pancreatic hormones, the number of calcitonin-gene-related peptide- and galanin-positive cells in the islet of Langerhans decreases after the onset of diabetes. Pancreatic amino butyric acid is also decreased in diabetes. These abnormal changes in the pattern of distribution of peptides, neuropeptides and neurotransmitters may contribute to the pathogenesis of diabetes mellitus.

Key words: *peptides, diabetes mellitus, hormones, neuropeptides, neurotransmitters*

Introduction

Diabetes mellitus is a chronic metabolic disease, which is associated with hyperglycaemia. This hyperglycaemia is a result of defective insulin secretion and/or function. The prolonged hyperglycaemia accompanying diabetes causes tissue damage, which results in degenerative complications in many organs including the kidney, heart, muscles, eye and many other organs.[1] These degenerative complications arise partly because of the damage inflicted on the blood vessels. This review examines the pattern of distribution of insulin-, glucagon-, somatostatin- and pancreatic polypeptide-containing cells in the pancreas of streptozotocin (STZ)-induced diabetic rats. In addition, the topographical relation of calcitonin gene-related peptide (CGRP)-, galanin-, neuropeptide Y (NPY)- and γ-aminobutyric acid (GABA)-positive cells of the endocrine pancreas and their role in determining the pathogenesis of diabetes mellitus is discussed.

The pancreas

The pancreas is a mixed gland, with a large exocrine and a much smaller endocrine gland. The endocrine cells are arranged into small islands of cells called the islets of Langerhans. The interactive function of both the exocrine and the endocrine parts are particularly important for the normal functioning of the body. The endocrine cells produce indispensable hormones such as insulin, glucagon, somatostatin and pancreatic polypeptide, which are crucial to the optimum functioning of body metabolism. The pancreas is well innervated by autonomic nerves rich in different types of neuropeptides including vasoactive intestinal polypeptide (VIP) and NPY;[2] galanin, CGRP and cholecystokinin; [3] and leucine-enkephaline.[4] In addition to the presence of neuropeptides, neurotransmitters such as serotonin,[5] GABA[6] or neurotransmitter-regulating enzymes such as tyrosine hydroxylase[7] and dopamine hydroxylase.[8] have been identified in the pancreas. Many neuropeptides such as galanin co-localize with hormones of pancreatic beta cells. [9]

Islet morphology in diabetes mellitus

There is selective damage to the beta cells in STZ-induced experimental diabetes although macroscopically, the pancreas appears normal. Immunohistochemistry technique may be used to identify morphological abnormalities in the islet cells of diabetic pancreas. Briefly, normal and diabetic rats are anaesthetized with 7% chloral hydrate (given intraperitoneally) and the pancreas removed and cut into small fragments. The fragments are immersion-fixed in a solution containing formaldehyde and glutaraldehyde in picric acid for 48 h and processed for immunohistochemistry or immunofluorescence. [10]

Beta cells

Insulin-positive cells are the most numerous cell types in the normal pancreas. They are located in both the central and peripheral parts of the islet and account for about 60-

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70% of the total cell population in a given islet of Langerhans.[11,12] In experimental diabetes, however, this number decreases significantly due to selective damage of pancreatic beta cells by STZ (Fig. 1A and B; Figs 3A and B).

Alpha cells

Glucagon-containing cells constitute about 28% of the total number of endocrine cells in a normal pancreatic islet.[11,12] They are located in the periphery of the islets in normal animals. However, in STZ-diabetic animals many glucagon-positive cells are seen scattered within the central portion of the islets. Representative sections of glucagon immunostaining in both normal and diabetic pancreas are shown in figures 1C and D. The percentage of glucagon positive cells increased significantly in STZ-diabetic animals compared to controls.

Delta cells

The immunostaining of somatostatin is shown in figures 2A and B). Somatostatin-immunopositive cells are located mainly in the outer part of the islets of normal pancreas with a percentage distribution of about 6% (Fig. 2A and B). Previous studies have demonstrated significant increases in the number of somatostatin-positive cells in diabetic rats.[3, 11,12]

Pancreatic polypeptide (PP) cells

PP cells are located in the peripheral region of the islet of Langerhans in normal rat.[3,10,11] The percentage distribution of PP cells in normal pancreas is 6%. The percentage distribution of PP cells increases significantly after the onset of diabetes (Fig. 2C and D).

Pancreatic neuropeptides in diabetes mellitus

Neuropeptide-Y

NPY is present in the endocrine cells of the islets of Langerhans as well as in the nerves innervating the pancreas of both normal and diabetic rats.[2,13] NPY also co-localizes with PP in the mammalian pancreas.[14] After the onset of diabetes, the number of these NPY-positive cells changes, in a similar pattern as for PP.[2] NPY has a dose-dependent stimulatory effect on insulin secretion in normal rat pancreas.[2] However, it decreases plasma insulin level in mouse islet cells.[15] In addition NPY also inhibits both basal and glucose-stimulated insulin secretion in the *in vivo* normal pig pancreas.[16] The species differences may account for the differences in the effect of NPY on insulin release.

Calcitonin-gene-related-peptide

CGRP is a 37-amino acid generated from the alternate tissue-specific processing of calcitonin gene pre-mRNA.[17,18] It is synthesized predominantly in the central nervous system[19] and has been demonstrated in plasma and cerebrospinal fluid.[20] CGRP also co-localizes with many peptides, including calcitonin in para-follicular cells of thyroid gland.[21] CGRP is present in large numbers of cells in the islets of Langerhans of normal rat pancreas. The number of CGRP-immunoreactive cells decreased significantly in diabetes. CGRP co-localizes with insulin[3, 22] and somatostatin[23] in the islets of Langerhans. The presence of CGRP in the endocrine cells of the pancreas and the subsequent decrease of CGRP in the pancreas of diabetic rats demonstrates that CGRP may be involved in the synthesis and or release of insulin from secretory granules. The presence of CGRP may also indicate a neurotrophic role in the pancreas. Other studies have shown that CGRP has trophic effects on structures such as myotubules,[24] motoneurons[25] and peripheral nerves.[26] In addition to the endocrine pancreas CGRP has been demonstrated in the nerves innervating the pancreas and several other tissues.

CGRP can induce large increases in insulin release from the pancreatic tissue of normal rats[27]and dogs.[28] However, reports have shown that CGRP inhibits insulin release from human,[29] calves,[30] rat,[31] and mouse[31] pancreata. Species differences may account for the difference between the findings. CGRP has also been shown to induce hyperglycemia[32] and inhibits insulin-stimulated glucose uptake[33] through a different pathway than cyclic AMP.

Galanin

Galanin, a brain-gut peptide, is present in the nerves innervating the pancreas of normal rats.[9,34] It is also present in the normal porcine,[5] camel[12] and bovine endocrine pancreas,[35]where it co-localizes with insulin. The number of galanin-positive cells is significantly decreased in diabetes.[9] The co-localisation of galanin with insulin and the near absence of galanin in the pancreatic islets of diabetic rats suggest that galanin might indeed influence insulin secretion in an autocrine manner. Galanin inhibits insulin secretion from the pancreas of normal rat,[9] man[36] and dog.[37,38] This implies that galanin may be involved in the pathophysiology of diabetes.

Pancreatic neurotransmitters in diabetes

Gamma amino butyric acid

GABA is present in large quantities in inhibitory neurons of the central nervous system.[39] It is formed by the decarboxylation of L-glutamic acid by glutamic acid decarboxylase (GAD). GABA and GAD are present in the pancreatic beta cells of normal rat.[40] Moreover, GABA-metabolizing enzymes, such as GABA-Transaminase (GABA-T), has been shown to be present in the pancreatic beta cells.[41] Antibodies against GAD and GABA are present in the sera of patients suffering from Type I diabetes, epilepsy and stiff-man syndrome.[42] GAD, especially the GAD65 isoform has been demonstrated to be a target of early autoimmune T-cell response associated with beta cell destruction in the non-obese-diabetic mouse model of insulin-dependent diabetes.[43] GABA is present in a large number of islet cells in the pancreas of non-diabetic rats.[6,40] The number of GABA-positive cells is decreased significantly in the endocrine pancreas of diabetic rats.[6] The concentration of GABA in the endocrine pancreas is said to be comparable to that measured in the central nervous system. [44]

GABA increases insulin release from the pancreas of normal rat.[6,45] In contrast, inhibitory[46] and neutral[47] effects

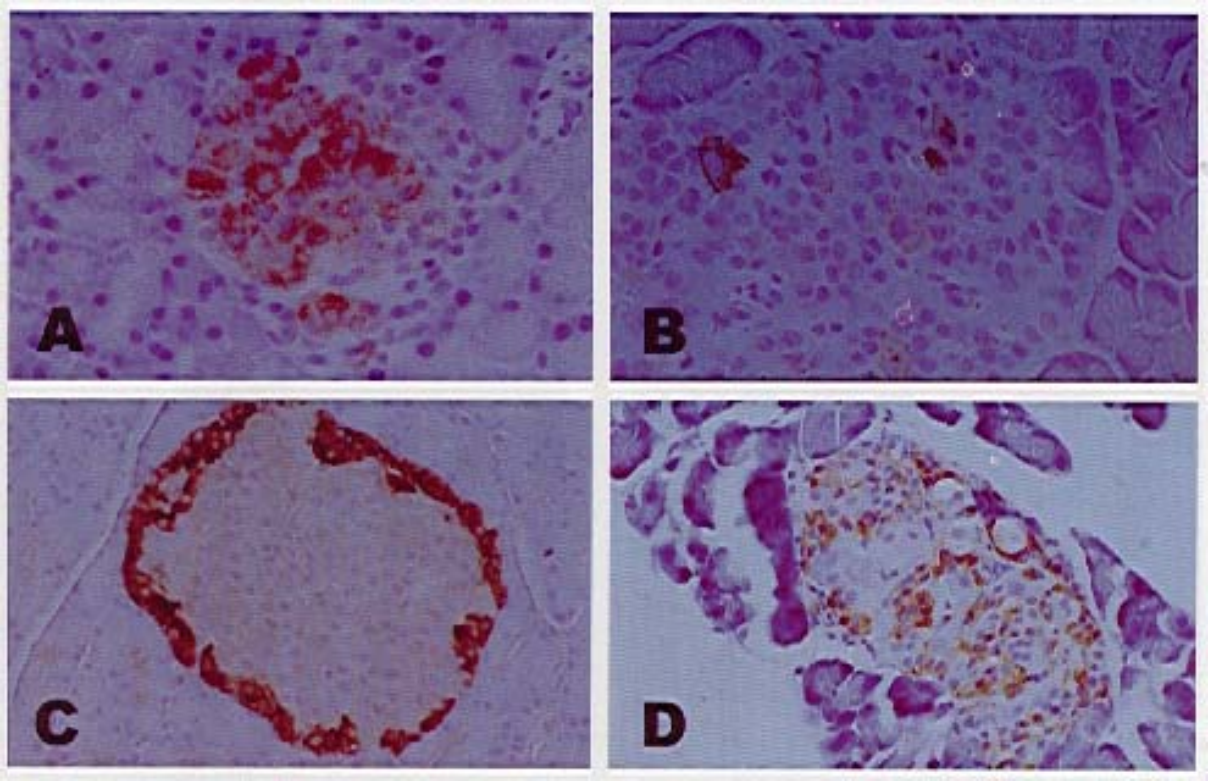

Figure 1: Micrographs showing the distribution of insulin (INS)-and glucagon (GLU)-positive cells in the pancreatic islet of normal and diabetic rats. (A) INS-positive cells in normal rat pancreas; (B) INS-positive cells in diabetic rats; (C) GLU-positive cells in normal rats and (D) GLU-Immunoreactive cells in the pancreas of diabetic rats. Magnification: X320

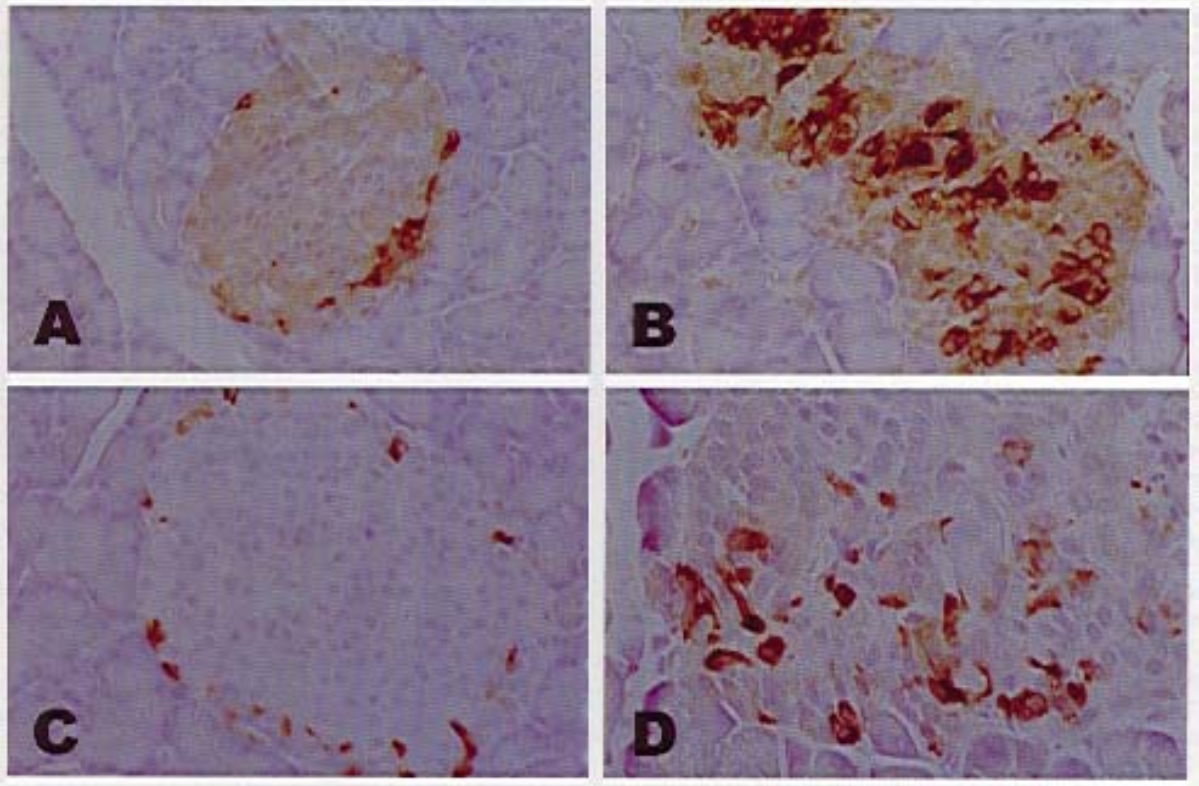

Figure 2: Micrographs showing the distribution of somatostatin (SOMA)- and pancreatic polypeptide (PP)-positive cells in the pancreatic islet of normal and diabetic rats. (A) SOMA positive cells in normal rat pancreas; (B) SOMA-positive cells in diabetic rats; (C) PP-positive cells in normal rat and (D) PP-immunoreactive cells in the pancreas of diabetic rats. Magnification X 320

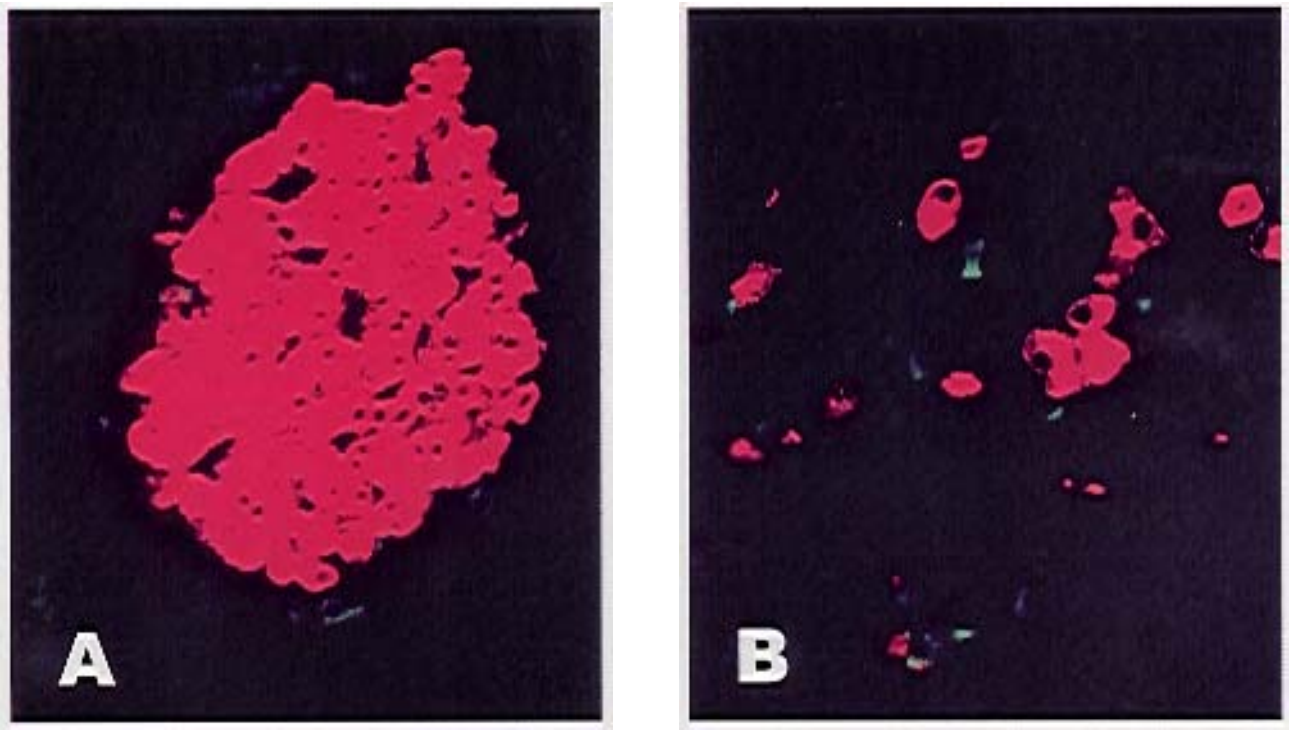

Figure 3: Micrographs showing the distribution of insulin (INS)- and neuropeptide Y (NPY)-positive cells in the pancreatic islet of normal and diabetic rats. (A) INS(red) and NPY (green)-positive cells in normal rat pancreas; (B) INS (red) and NPY (green)-positive cells in diabetic rats. Magnification: X320

of GABA on insulin release have also been reported. GABA-A receptors have been shown to be present on the plasma membrane of pancreatic alpha cells, some gastro-pancreatic neuroendocrine cells and rat insulinoma cell line RIN 38.[48] GABA-A receptors activate voltage-gated Ca^{2+} channels,[48] triggering an increase in intracellular Ca^{2+}. An increase in intracellular Ca^{2+} stimulates exocytosis and insulin release.

The co-localisation of GABA with pancreatic beta cells indicates that GABA may play a role in the regulation of insulin biosynthesis and or function as an alternative energy source for the beta cell through the GABA shunt. It may also stabilize the insulin molecule. GABA and its metabolizing enzyme GAD are also linked to the pathogenesis of Type I diabetes.[49]A high correlation of anti GAD titer and incidence of Type 1diabetes has also been reported.[50] The relationship of GAD and Type 1 diabetes is so important that the production of GAD autoantibodies precedes other autoantibodies such as, insulin autoantibodies and islet cell antibodies.[51]

Conclusion

Diabetes mellitus is associated with significant changes in the pattern of distribution of insulin-, glucagon-, somatostatin- and pancreatic polypeptide-positive cells in pancreatic tissue of diabetic animals compared to that of normal controls. Neuropeptides and neurotransmitters such as NPY, CGRP, galanin and GABA are present in the endocrine pancreas and play a role in the metabolism of the insulin molecule. These findings suggest that insulin metabolism may be regulated by many neuropeptides and neurotransmitters in a more complex manner than we currently understand.

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