Diabetes II and amylin: a modern perspective

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The diabetes type I results from an autoimmune mediated irreversible destruction of pancreatic B cells and type II is associated with hyperamylinaemia and hyperinsulinaemia in the early stages of the disease. Insulin levels are decreased in the later stages due to the secondary failure owing to the deposition of amylin in the B cells of pancreas. Amylin, a 37-amino-acid peptide, co-secreted with insulin from the B cells has 50% sequence identity to calcitonin gene-related peptide (CGRP). It inhibits insulin-stimulated glycogenesis in muscle and promotes the indirect pathway for glycogenesis via gluconeogenesis in liver, rather than the direct pathway. Amylin and CGRP act as hormone and neurotransmitter respectively. Hyperamylinaemia promotes lipogenesis from carbohydrates in adipocytes, consequently leading to obesity and other disorders.

The hormones, glucagon, insulin and somatostatin, secreted by alpha (A), beta (B) and delta (D) cells of the endocrine pancreas respectively, play a fundamental role in maintaining blood glucose level within the physiological range[1-6]. These islet cells constitute a *functional syncytium exercising a paracrine control over the coordinated secretion* of the pancreatic polypeptides[7]. In diabetes type I *(insulin-dependent* diabetes mellitus—IDDM), the *insulin-synthesizing* ability is irreversibly lost, leading to an absolute requirement of exogenous insulin for survival of the patients. The *IDDM* possibly results from molecular mimicry by a 64-kd autoantibody and consequent autoimmune-mediated destruction of B cells, involving interleukins, heat-shock proteins, pro-insulin, free radicals, MHC class II proteins and aspartic acid at 57th position[8-14]. The present article attempts to summarise the results of the current research on the role of the recently discovered *hormone, amylin, in the etiology of* diabetes type II.

Pathophysiology of diabetes type II

Diabetes type II (noninsulin-dependent diabetes *mellitus—NIDDM) is characterized by hyperinsulinaemia*[15,16] and defective sensitivity of carbohydrate metabolism to insulin. The euglycaemic/hyperinsulinaemic *clamp*[15,16] *and the incorporation of* ($1C^{13}$) *glucose load in healthy subjects*[17] showed that skeletal muscle was the major site of glucose disposal. NIDDM *begins in the third decade of life, sharply raising in people over 45 years of age*[18] *and is frequently associated with obesity and essential hypertension. Progress of* NIDDM state is followed by a relative decrease in *glucose-induced insulin secretion. However, fasting levels of insulin in* NIDDM *subjects are higher than the* levels in normal and non-obese people[15,16]. Studies with Pima Indians[19] *(an ethnic race with a high prevalence* of NIDDM) and a colony of primates, *Macaca mulatta*[20], *(with a spontaneous NIDDM-like syndrome)* indicated that insulin resistance was a major determinant of the decline of glucose tolerance. Insulin-mediated glucose disposal and glycogen synthase activity in skeletal muscle are considerably reduced in NIDDM patients[21].

Primary biochemical defect in NIDDM

Defective insulin binding and/or defective transduction of the insulin signal were earlier assumed to be responsible for NIDDM. If this assumption were to be true, both oxidative and nonoxidative mechanisms of intracellular insulin-mediated glucose disposal in muscle should be affected equally. But euglycaemic clamp studies[16,22] showed that oxidative and nonoxidative mechanisms were differentially reduced by 30% and 70% respectively in NIDDM subjects. Insulin binds to *the extracellular α-subunit of its plasma membrane*-bound receptor to trigger the signal transduction pathway[23]. *The β-subunit contains an intracellular domain* with tyrosine kinase activity. Insulin binding may induce signalling through initiation of a phosphorylation cascade, *via endogenous tyrosine kinase-mediated* phosphorylation of cytosolic substrates. Alternatively, insulin binding may generate a second messenger (perhaps derived from inositol phosphate glycans) by altering the confirmation of the *β*-subunit, which activates a GTP-binding protein (G-protein) and/or specific *phospholipases. Various* abnormalities of these elements in obesity and NIDDM were analysed completely. However, the pathogenic nature of any abnormality was not established so far. Hence it is likely that post-receptor defects in insulin signalling pathway are responsible for NIDDM and obesity[24].

Differential response to insulin

The differential response elicited by several insulin-*mediated glucose disposal mechanisms to hormone*

concentration was analysed in hyper-insulinaemic *models*[18,25–29] closely resembling the prediabetic state. The dose-related effects were studied *in vivo*[30] in perfused hind limb preparations or in isolated incubated *muscle preparations*[25–29]. The *in vivo*[30] and *in vitro*[31] measurements of insulin sensitivity were closely similar. In most insulin-resistant states, except obesity[25], glucose transport remains unaltered or only modestly decreased. Except in uraemia[28], in all other states the sensitivity of glycogenesis was dramatically decreased *(Table 1). These differential sensitivities* clearly indicate that insulin resistance is not due to a simple defect in insulin action. *The recent discovery of a new hormone,* amylin, *in 1987 opens up new avenues in the study of* the etiology of NIDDM.

Discovery of amylin

Majority of NIDDM subjects[32] and diabetic Pima *Indians*[33] *contain deposits of islet amyloid* in their pancreatic tissues. Amylin constitutes the major peptide *component of islet amyloid*[34,35]. It was originally named as 'diabetes-associated peptide' and was subsequently rechristened as amylin[36]. It was isolated in a pure form and was completely characterized as a 37-amino-acid peptide[35]. It is synthesized in B-cells[37] and located in the secretory granules of B-cells[38]. It is co-secreted with insulin from *isolated islets of Langer*hans[39] and perfused rat pancreas[40], in response to nutrient secretogogues such as glucose and arginine. The rate of amylin secretion is 10%–37% of the rate of insulin secretion in rat islets[40]. The concentration of amylin is nine times higher than that of glucagon[41]. Data on the levels of circulating amylin in glucose intolerant subjects are not yet available. The recent development of amylin-specific radioimmunoassay techniques[40] may accelerate the availability of information in the near future.

Table 1. Insulin resistance in skeletal muscle in various hyperinsulinaemic models

Condition	Glycogenesis	Glycolysis (representing glucose transport)	Reference
Ageing	(–––––)	No change	29
Endotoxaemia	(–)	No change	29
Glucocorticoid excess	(––––)	(–)	26
Hyperthyroidism	(–––––)	No change	27
Obesity	(––––)	(–––––)	25
Uraemia	(No change	(–)	28
NIDDM	(––––)	(–––––)	21

Each ( ) sign represents a decrease of insulin sensitivity of $100\,\mu U\,ml^{-1}$ in comparison with control muscle IDDM is characterized by absolute lack of insulin and absence of peripheral resistance (i.e. receptor defect).

Molecular analysis of amylin

Human and rat amylins are synthesized from a precursor, *pre-proamylin containing* a signal peptide and a prohormone-like sequence containing mature amylin[37,42]. The amino-acid sequence of amylin is well conserved among various species[43]. The sequence of amylin is 50% identical to the sequence of the 37-amino-acid neuropeptide, *calcitonin gene-related peptide* (CGRP)[35]. The sequences are different over residues 20–29 in amylin and CGRP. This differential region of human amylin causes the formation of amyloid fibrils[34] (twisted and paired helical filaments formed by peptides in an antiparallel β-pleated sheet configuration). A peptide similar to amylin was earlier isolated from insulinoma-associated amyloid. It was called 'insulinoma/islet amyloid polypeptide' and was incompletely sequenced[32].

Physiology of amylin

Amylin and CGRP exhibit functional similarity, since they have 50% sequence identity[35]. Exogenous CGRP stimulates secretion of insulin, gastric acid and amylase. It is a potent vasodilator and shows positive chronotrophic and ionotrophic effects in atrial tissue[44]. It promotes adenylate cyclase activity[45] and specifically binds to rat liver plasma membrane[46]. Amylin and CGRP inhibit insulin-stimulated glycogen synthesis in *the skeletal muscle*[47]. *However, the concentration of* CGRP in liver is well below detectable limits[48]. This shows that CGRP itself cannot control the hepatic metabolism effectively.

Amylin is available to liver since it is secreted into the portal veinous blood supply. The hormone probably regulates the hepatic metabolism by binding to the putative CGRP-binding sites in view of 50% sequence identity and increases the rate of hepatic glucose production. It promotes the indirect pathway for hepatic glycogenesis (glucose $\xrightarrow{\text{oxidation}}$ lactic acid $\xrightarrow{\text{gluconeogenesis}}$ *glucose* $\xrightarrow{\text{glucogenesis}}$ *glycogen) instead of the* direct pathway (glucose→glycogen)[49]. The elevated post-prandial level of blood glucose stimulates insulin *secretion and consequent hepatic glycogenesis. Insulin* inhibits post-prandial flux through gluconeogenic pathway. Interestingly, amylin decreases the sensitivity of the hepatic gluconeogenesis to insulin, albeit temporarily in healthy persons and enhances the hepatic glucose output and glycogenesis via the indirect pathway[49]. The role of amylin in the day to day changes of peripheral and hepatic insulin sensitivity was clearly established[31].

There is no innervation of the bulk of skeletal muscle fibre, even though CGRP is located in motor end plate in skeletal muscle[47]. However amylin is the blood-borne hormone agent, interacting at the membrane to decrease the effects of insulin. Consequently CGRP and

Table 2. An overview of endocrine pancreatic physiology in fuel metabolism[1-6, 36, 47, 49, 50, 52, 53]

Hormone/ structure	Fuel type	Target site	Physiological action	Control
Glucagon/ polypeptide with 29 amino-acid residues	Carbohydrate	Liver	(+) Glycogenolysis, Gluconeogenesis, glycaemic action (×) *Peripheral glucose utilisation*	(+) by CGK, gastrin, secretin, GIP, epinephrine, hypo-glycaemia, arginine, alanine
	Fat	Adipocyte	(+) Activation of triglyceride lipse by cAMP, lipolysis	(−) by insulin, somatostatin, circulatory levels of fatty acids
	Protein	Liver	(+) Proteolysis, amino-acid oxidation, urea formation (−) *Protein synthesis*	
Insulin· polypeptide with two disulphide bridges, A chain — 21 amino acids B Chain — 30 amino acids	Carbohydrate	Liver	(+) Glucokinase, glycogenesis (−) Phosphorylase, gluconeogenesis	(+) by metabolisable monosacc-harides (glucose, triose), sorbitol formation from glucose, GIP, gastrin, CGK, secretin, arginine lysine, phenyl alanine, medium chain triglycerides, glucagon, GH, corticosteroids, estrogens, progesterones, parathormone, obesity, Ach, epinephrine
		Muscle	(+) Glycogenesis, glycolysis, glucose transport	
		Adipocyte	(+) Glucose transport, glycerol synthesis	
	Fat	Liver	(+) Lipogenesis, (−) lipolysis, ketogenesis	
		Adipocyte	(+) Fatty acid formation, triglyceride, (−) *Lipolysis*	
	Protein	Liver	(−) Proteolysis (+) Amino-acid uptake, protein synthesis (−) Amino-acid oxidation	(−) by glucose metabolism inhibitors, somatostatin, epinephrine and nor epinephrine by α-adrenergic receptor activation and amylin
	Electrolyte metabolism	Liver Muscle	(+) K^+ uptake, (−) serum K^+ concentration, natriuretic effect	
Somatostatin' tetradecapeptide	*Carbohydrate*	*Intestine*	(−) Intestinal glucose absorption, secretion of gastrin and secretin	(+) by glucagon, glucose, GIP, gastrin, CGK, secretin
		Islet cells	(−) Secretion of insulin and glucagon	
Amylin' polypeptide with 37-amino acid-residues	Carbohydrate	Liver	(+) Indirect glycogenesis, gluconeogenesis, glucose output, insulin resistance	–
			(−) Direct glycogenesis, hepatic insulin sensitivity.	
		Muscle	(+) Insulin resistance (−) Insulin-stimulated glycogenesis	–
	Fat	Adipose tissue	(×) Insulin-stimulated glycogenesis	
		Adipose tissue	(+) Lipogenesis, triacylglycerol deposition	

(+) Stimulation; (−) Inhibition; (×) No effect.

amylin act as a pair of regulators, the former being a neurotransmitter and the latter being a hormone, in a way similar to noradrenaline and adrenaline[50]. These regulators are available to distinct responsive end organs and exhibit differential sensitivity to possible receptor subtypes. Even though they are functionally related, they are distinctly conserved ligands[51].

Amylin potentially inhibits insulin-stimulated glycogenesis in isolated incubated rat skeletal muscle preparation[52], but not in adipose tissue[36]. It causes both peripheral and hepatic insulin resistance in rats during hyperinsulinaemic/euglycaemic clamp conditions[53]. The CGRP also shows similar effects *in vitro*[47,52] and *in vivo*[53] as well. Table 2 gives an overview of endocrine pancreatic physiology in the disposal of biological fuel molecules based on modern information (Figure 1).

Pathophysiology of amylin in NIDDM

The following evidences clearly indicated that amylin was involved in the pathogenesis of NIDDM[34, 52]. (i) Sequence of amylin isolated from islet amyloid is identical to the sequence of amylin encoded by the normal gene[35,52]. (ii) Amylin causes peripheral insulin resistance both *in vivo*[53] and *in vitro*[52]. (iii) Amylin does not affect the binding of insulin to its receptor[50]. Therefore inhibition of insulin-stimulated glycogenesis in skeletal muscle by amylin *in vitro* must occur via modulation of post-insulin receptor responses[52], as occurring in NIDDM[50]. (iv) Amylin increases the rate of glucose output *in vivo*[53]. (v) Amylin decreases the rate of insulin secretion from isolated islets of Langerhans[34].

Amylin regulates the glycogenic activity of insulin in healthy subjects by modulating the post-insulin receptor-signalling pathways. Hyperamylinaemia decreases the sensitivity of insulin-stimulated glycogenesis in liver[49] and muscle[52] and increases peripheral insulin resistance[18,25,26,52], leading to increased glucose turnover. So larger quantity of insulin is required to cope up with the enhanced glucose turnover, resulting in hyperinsulinaemia during the initial stages of NIDDM. As the disease progresses, glucose-induced insulin secretion is relatively decreased, because of islet amyloid formation leading to secondary islet cell failure, even though fasting levels of insulin are higher in NIDDM patients. Thus hyperamylinaemia, peripheral insulin resistance, enhanced glucose turnover and hyperinsulinaemia cause NIDDM.

Role of amylin in obesity

Amylin does not inhibit insulin-stimulated glucose metabolism in adipocytes[36]. Hence hyperamylinaemia promotes lipogenesis from carbohydrates and enhances triacyl glycerol deposition in adipose tissue, leading to obesity. Presently it is believed that obesity is the effect of the metabolic disorder[50], rather than a predisposing factor for NIDDM, as was believed earlier. Varying degrees of hyperinsulinaemia and hyperamylinaemia

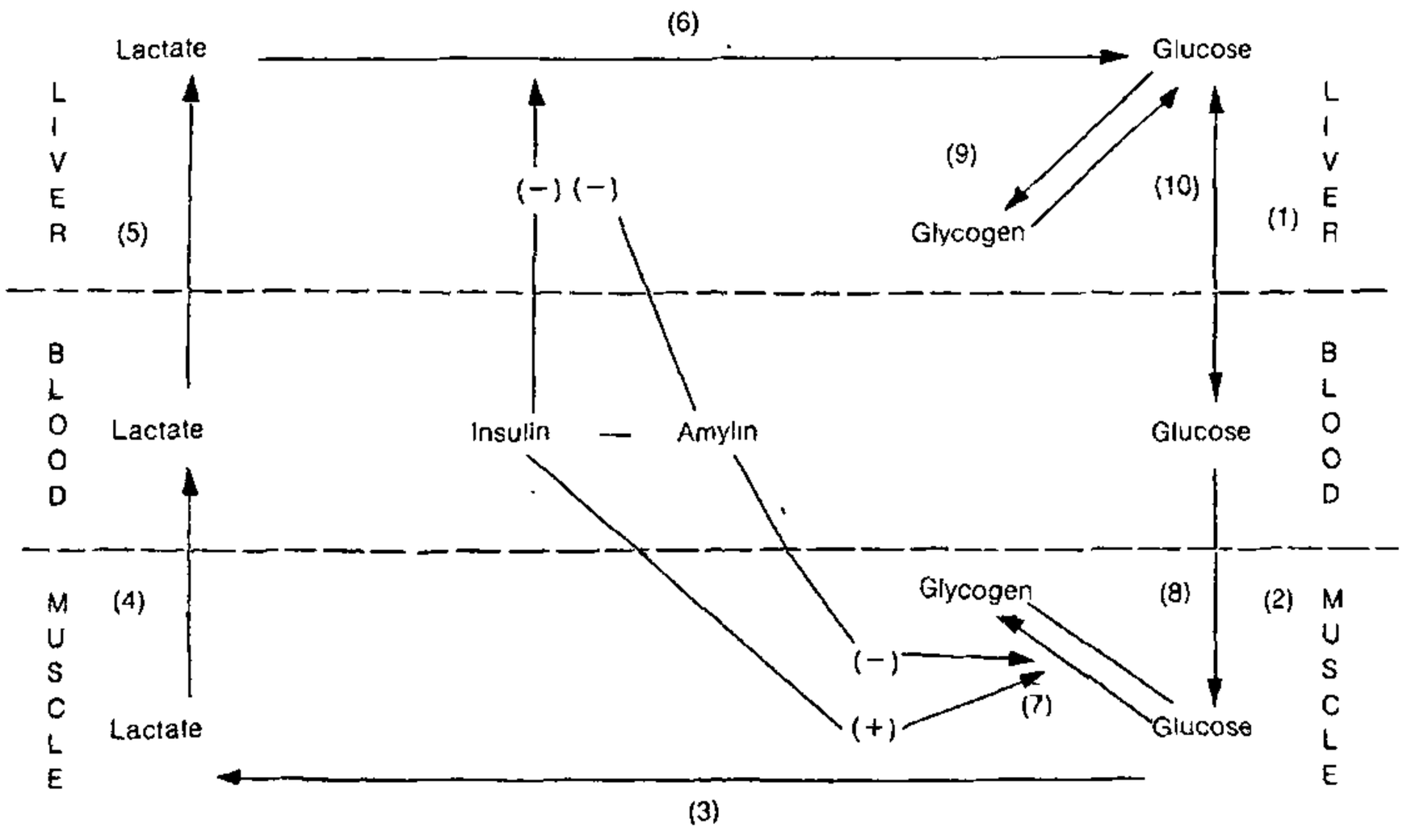

Figure 1. Effect of amylin on the action of insulin: (1), (2) Glucose transport; (3) glycolysis; (4), (5) lactate transport; (6) gluconeogenesis; (7), (9) direct glycogenesis; (8), (10) glycogenolysis. Amylin inhibits direct glycogenesis (7), (9) and promotes glyconeogenesis (6). (+), (−) and // indicate stimulation, inhibition and block respectively.

would possibly contribute to obesity in other disease states as well. Amylin inhibits insulin-mediated and noninsulin-mediated glucose disposal[52]. Amylin-incubated muscle preparations require 100-fold greater concentration of insulin than do control muscles, incubated with physiological concentration of insulin (100 μUml^{-1}). Amylin at 1 nM causes the same degree of insulin resistance in skeletal muscle, as found in obese[25] and ageing[18] animals. A comparison of the above results[18,25,52] suggests that increased circulating amylin is responsible for the peripheral insulin resistance found in ageing and obesity[50]. Amylin may act in collaboration with other catabolic hormones. Thus enhanced secretion of amylin and insulin, increased glucose turnover and decreased sensitivity of muscle glycogenesis to insulin are all features of endotoxaemia[29] and hyperthyroidism.

Conclusion

Thus a brief survey of the ongoing research clearly shows that amylin works in collaboration with insulin to regulate the disposal of fuel molecules in liver and skeletal muscle. Further work in this direction may explain some major disorders of fuel metabolism, including NIDDM.

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