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Landmarks in Our Understanding of Insulin Synthesis Secretion and Action

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Abstract

The discovery and the use of insulin to treat patients in January 1922 marked an important milestone in our quest for finding out the cause and cure for diabetes—a disease haunting humanity since ancient times. The pace of discovery accelerated since the discovery of the islets of langerhans. This review focuses on the advances since 1922 that have helped us understand the nature control secretion function and action of insulin. From its physical characterization in the mid-1920s to determination of its molecular structure, precursor and measurement in the 1950s through the discovery of its receptor tyrosine phosphorylation and gene in the eighties these advances have borrowed from and contributed richly to advances in protein genetics and molecular biology. Paralleling advances in purification modification and delivery of insulin, these advances form the basis of our understanding and the therapy of diabetes.

Keywords

Insulin, Discovery, Synthesis, Physicochemical Characterization, Proinsulin, Incretin, Tyrosine Kinase, KNCJ11, SNARE Complex

1. Introduction

Diabetes was known in the ancient world. Sushrusha had even classified it into two different kinds whose features are comparable to what we recognize as Type 1 and Type 2 diabetes. The Eber's papyrus documented a remedy used to treat diabetes in ancient Egypt [1].

The treatment of the child Leonard Thomson with a purified pancreatic extract on January 23 1922 was a culmination of work that began in the nineteenth century that led to the identification and clinical use of insulin. There are indeed

other claimants to the discovery of insulin; the Toronto four were the first to use it to save lives [2]. There are excellent reviews on the events that lead to the discovery of insulin. We will confine ourselves to landmarks that helped us understand the secretion and action of insulin since 1922 [1]. We will not also address the advances in insulin production, commercialization, modification and manufacture that are again well reviewed [3]. The review is not meant to be an exhaustive review of the many contributions that have shaped the science; it is intended to record the key events that have helped us understand insulin, its biology and action (**Figure 1**).

2. Characterization of Insulin

In 1925 John Abel at Johns Hopkins was able to crystallize insulin. Abel had other laurels to his credit including the isolation of adrenaline [4]. Working in John Abel's lab Vincent Du Vigneaud identified disulfide linked cysteine in insulin [5]. He also recognized that up to 30% of the insulin molecule was made up of cystine, tyrosine, arginine, histidine and lysine and speculated that the rest of the molecule was also made up of amino acids [6]. Du Vigenaud incidentally won the Nobel Prize in chemistry (1955) for the discovery of oxytocin. Despite this it was widely considered that insulin acted through a smaller molecule like thyroxine or adrenaline. Working in Abel's lab it was Hans Jensen and Earl A Evans Jr who established that the material isolated by acid extraction was indeed a protein. They also identified for the first time an N terminal phenylalanine end group [7]. Insulin secretion from isolated human islet cells was demonstrated in 1976 [8].

3. Sequencing and Assay

Frederik Sanger was working with protein chemistry; his attention turned to insulin simply because of its easy availability with reasonable purity. Sanger helped determine that insulin had two chains and he also sequenced both chains [9]. Insulin indeed was the first protein that was sequenced and resulted in one of the two Nobel prizes that Sanger received. The determination of the three dimensional structure of insulin was through a 34 year old effort of Dorothy Hodgkins. Her group first published an electronic density map of the structure of porcine zinc. [10] Insulin was the first protein to be synthesized. It was also the first hormone to be assayed. This was the work of Berson and Yalow who reported an immunoassay for plasma insulin based on the reaction of human insulin competing with beef I131 insulin in the sera of guinea pigs immunized with beef insulin [11].

4. Synthesis of Insulin

Based on work done initially on an islet cell adenoma (insulinoma), Donald Steiner and his colleagues working in the University of Chicago reported the discovery of the precursor of insulin in 1968 which they named proinsulin [12].

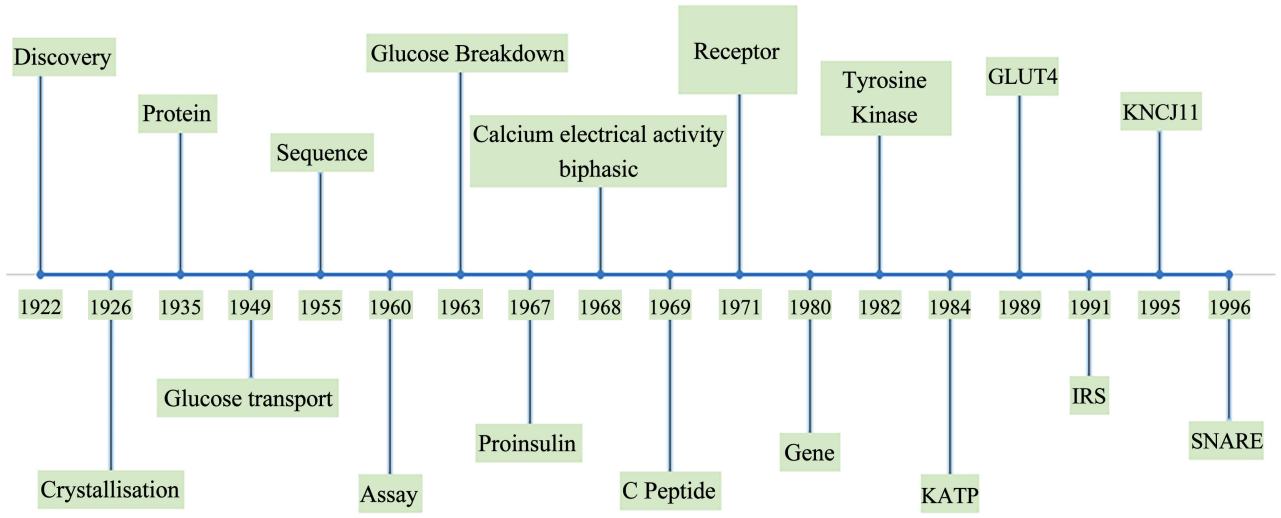

Figure 1. Landmarks in our understanding of insulin its secretion and action (see text for expansion of abbreviations and details).

Through pulse chase techniques they subsequently demonstrate the time lapse in accumulation of proinsulin and its conversion of insulin [13]. This led to the development of models predicting that newly synthesized proinsulin moved from the endoplasmic reticulum (ER) to the Golgi region within 15 - 20 min, a process requiring energy, and shortly thereafter was packaged into secretory granules and then converted to insulin. This was subsequently confirmed by Leilo Orci and colleagues in 1985 [14]. Steiner and his colleagues further identified connecting peptide (c-peptide) and also demonstrated that it was stored in mature granules and secreted along with insulin [15]. This was used to develop a C-peptide assay as an index of beta cell function by Arthur Rubenstein and Kenneth Polonsky.

5. Gene

It is commonly assumed that the human insulin gene was cloned and then introduced into *E. coli* to make the first recombinant Human Insulin that was introduced in 1981. Neither the mRNA nor the gene sequence of insulin was known. The genes were designed based on the known amino acid sequences and the codons preferred by *E. coli*. The genes were then chemically synthesized by organic chemical methods. It was sheer providence that *E. coli* synthesized human insulin [16] [17]. Subsequently David Owerbach, Graeme Bell and colleagues described the location of human insulin in short of chromosome 11 in 1980 [18] [19].

6. Control of Insulin Secretion

Work on stimulus secretion coupling that was started in the 1960s resulted in three seminal discoveries [20]. The first was that the insulin secretion required the metabolic breakdown of glucose [21] [22]. Thirty years later glucokinase was identified as the first glucose metabolizing enzyme [23]. The role of amino acids

in insulin secretion was also first understood around this time with the identification of the insulinotropic effects of leucine [24]. Demonstration of the effects of amino and keto acids further strengthened the view that the mitochondrial matrix is the hub of nutrient control of insulin synthesis [25]. Of note the differential effects of intravenous and oral glucose on insulin dynamics—the incretin effect was described by Perley *et al.* in 1967 [26].

The second was the discovery that glucose does not increase insulin signaling in the absence of calcium. Grodsky and Bennet (1966) demonstrated that calcium was necessary for the pancreas to secrete insulin [27]. Subsequently Milner and Hales (1967) demonstrated that a sodium pump was involved in insulin secretion. They also demonstrated that increase in extracellular potassium stimulated insulin secretion. Interestingly they demonstrated that the sulfonylurea tolbutamide increased insulin secretion independent of glucose concentration [28].

The third was the discovery that pancreatic beta cells are electrically excitable which was accomplished by recording the action potential [29]. A decrease in glucose induced potassium efflux as the triggering event for electrical activity of the beta cell was postulated in 1978 [30]. The demonstration that mitochondrial activation through increased oxidative phosphorylation and alteration in the ATP/ADP ratio results in electrical activation of the beta cell and the identification of the KATP channel by Cook and colleagues in 1984 provided a link between nutrient stimulus and electrical activity [31]. Its molecular composition—an inwardly rectifying K+ channel (Kir 6.2 = KCNJ11) as the pore-forming unit and a member of the ATP cassette-binding family (SUR1 = ABCC8) as the regulatory subunit was described in 1995 [32].

7. Mechanics of Insulin Secretion

The involvement of a cytoskeleton in the transport of synthesized insulin was proposed as early as 1968 [33]. These were confirmed by work done in the 70s [34]. Grodsky and colleagues demonstrated biphasic insulin secretion perfused pancreas in 1968 [27]. The availability of new techniques helped understand the segregation of insulin granules to different pools. The role of secretory machinery such as the SNARE complex were first identified in the 90s [35]. Work on refining our understanding of the secretory dynamics has continued well into the 21st century.

8. Insulin Action

That insulin exerted its action by transport of glucose across cell membranes in extrahepatic tissues was postulated by Rachmiel Levene in 1949 [36]. In 1960 glycogen synthase was identified as the first enzyme to be stimulated by insulin. The existence of a membrane receptor for insulin was first demonstrated by Jesse Roth and colleagues [37]. Working with patients with severe insulin resistance (type B) characterised by antibodies to the insulin receptor Ronald C Kahn

and his colleagues were able to identify tyrosine kinase activity and identify that the insulin receptor belonged to the tyrosine kinase family [38] [39]. That the receptor becomes phosphorylated on tyrosine residues in response to insulin binding was an unexpected finding, as tyrosine phosphorylation had until then been thought of as exclusive to oncogenes. This recognition was undoubtedly a major landmark in the understanding of insulin and its action and shifted the focus from the cell surface to intracellular pathways. This concept was strengthened by the cloning of the insulin receptor cDNA by two groups in 1985 [40].

Evidence for an intracellular transport mechanism for glucose came with the discovery by Tetsuro Kono and colleagues from Vanderbilt and Samuel Cushman and colleagues from the NIH that insulin promoted the translocation of a cellular transporter of glucose to the surface in insulin sensitive tissue [41] [42]. This transporter was further characterized, cloned and named—GLUT4 in 1989 [43]. The first of four insulin receptor substrate proteins were cloned by Kahn and White in 1991 [44]. These adaptor proteins were identified as responsible to convert the tyrosine phosphorylase signal into a lipid kinase signal by recruitment of PI3K [40]. Further understanding the distal actions of insulin signaling was accomplished by the identification of AKT as an insulin activated serine threonine Tyrosine Kinase signal by Kohn et al in 1995 [45]. Continued work on our understanding of insulin action has proceeded well into the 21st century

9. Miscellaneous

There are several other streams of work that have helped us further understand insulin and its secretion and action in health and disease. For instance neuronal control of insulin secretion was first proposed by Claude Bernard who demonstrated glucosuria by puncture of the floor of the fourth ventricle [1]. Work on glucagon by Roger Unger and colleagues' merits mention [46]. While the work on insulin has helped us understand other hormonal action, work on hormones such as somatostatin has been a forerunner for quite a volume of work on insulin.

Work on genetic conditions that influence insulin secretion and action have helped us understand insulin (and diabetes) better—an example is the discovery of the inheritance and characterization of Maturity Onset Diabetes in the Young [47]. An association between inflammation and diabetes was demonstrated as early as 1876. High doses of sodium salicylate were used to treat diabetes [48]. The relationship between inflammation insulin resistance and insulin secretion and action are areas of active interest at this time.

Advances in cellular and molecular biology that began with the pioneering work of Watson and Crick [49], techniques to interrogate single cells, the glucose clamp [50], and ability to directly image beta cells are but a few examples of the considerable impact of advances in the last century on the science of insulin. This article has not done justice to these contributions. Mention must be made of the tremendous advances in our ability to study insulin biology in animal

models (such as knock out or humanized mice) and cells (*in vitro*) in the past century. These require a tribute of their own. Lastly it must be emphasized that the contribution of patients with rare genetic syndromes and complex problems to the science and biology of insulin has been priceless

10. Conclusion

We know far more about the heaps of cells that were first identified by Paul Langerhans [1]. The scientists who have spent a lifetime in the pursuit of the biology of insulin have helped us understand not only the working of the endocrine system but also pursue therapies for diabetes. In retrospect the achievements of the last century are indeed a testimony to human quest for truth and perseverance. These achievements also epitomize in many ways the power of collaboration networks and mentorship.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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