**Defective regulation of insulin secretion in diabetes and insulinoma**

PETER R. FLATT

Department of Biological and Biomedical Sciences, University of Ulster, Coleraine, Co. Londonderry BT52 1SA, Northern Ireland, U.K.

In view of the general interest in disorders of insulin secretion and glucose homeostasis, and the cost of such diseases in terms of treatment and quality of life, much research has been devoted to understanding the physiology and pathophysiology of insulin secretion. Studies of insulin secretion in diabetes and insulinoma, which in their various forms affect up to 4% and 0.02%, respectively, of the population, have almost exclusively used islets or tumour B-cells from animal models. In the present paper, the results of such studies will be considered in the framework of our current understanding of the insulin secretory mechanism.

**Insulin secretory mechanism**

The mechanism by which glucose and other nutrient fuels provoke insulin secretion from normal pancreatic B-cells has been considered in detail elsewhere in this Colloquium. In brief, it is generally believed that glucose metabolism leading to the generation of cellular ATP provides the stimulus to insulin secretion through a sequence of ionic events triggered by the closure of ATP-sensitive K⁺ channels in the B-cell plasma membrane. This results in a decrease of K⁺ permeability which in turn leads to membrane depolarization, opening of voltage-dependent Ca²⁺ channels and Ca²⁺ influx. The resulting increase in cytoplasmic Ca²⁺ triggers exocytosis by affecting enzyme activities, electrostatic membrane charges, microtubules and microfilaments. Non-metabolizable secretagogues such as hormones and
neurotransmitters may potentiate insulin secretion by activation of adenylate cyclase or phospholipase C, leading to changes in intracellular handling and sensitivity to cytoplasmic Ca\(^{2+}\) [1].

**Defective insulin secretion in diabetes**

Nature has provided a wealth of animal models with spontaneous diabetes which may be classified into three major groups according to the severity of the islet defect and the coexistence of obesity [2]. Models with B-cell degeneration and ketosis include the BioBreeding (BB) rat and non-obese diabetic (NOD) mouse. Those with B-cell degeneration and possible ketosis are more numerous including the diabetes-obese C57BL/KsJ db/db mouse, Chinese hamster spiny mouse, Egyptian sand rat and djungarian hamster. Finally, there is a large group of models with B-cell hyperplasia, obesity and no ketosis which includes the obese hyperglycaemic (ob/ob) mouse, the New Zealand obese mouse, the yellow obese mouse, the KK mouse, the fatty Zucker (fa/fa) rat and many others.

Although no single animal model is an exact match for human diabetes, which itself is a highly heterogenous group of disorders, diligent investigation of the range of available animal diabetes syndromes continues to provide important information on the aetiology, pathogenesis and therapeutic approaches to diabetes in man [2].

In the various animal models, glucose stimulation of insulin secretion is defective in magnitude or kinetics, providing the opportunity for interesting studies to be conducted which are impossible to perform for both ethical and technical reasons using islets isolated from diabetic patients. Studies of insulin secretion in spontaneously diabetic animals have stressed that the nature and severity of defective insulin secretion depends on the mutation itself, the genetic background on which the mutation is carried, the age and sex of the animal in question, its diet and nutritional status, and the general environment where it is maintained [2]. Some caution must be applied therefore in the integration of individual pieces of knowledge from different laboratories which may not have defined such variables or characterized the diabetic status of the animals employed. The two best characterized and extensively studied models of defective insulin secretion are the diabetes-obese C57BL/KsJ db/db mouse and the diabetic Chinese hamster.

**Diabetes-obese C57BL/KsJ db/db mouse.** This syndrome which is inherited as an autosomal recessive trait is characterized by obesity, hyperphagia, hyperglycaemia, islet hypertrrophy and hyperinsulinenaemia [2]. Depending on the energy density and carbohydrate content of the diet, adult db/db mice exhibit a progressive age-dependent deterioration of B-cell function potentially culminating in extensive islet necrosis, severe insulin deficiency, marked hyperglycaemia and ketosis [2]. Studies utilizing the perfused pancreas or isolated islets of young adult C57BL/KsJ db/db mice have shown that both the dynamics and magnitude of glucose-induced insulin release are defective [3, 4]. C57BL/KsJ db/db mice exhibit marked and age-dependent alterations in the hormone content and cellular composition of the islets [5]. This undoubtedly interferes with the subtle paracrine interactions involved in insulin release. However, since glucagon secreted locally within the islet can be expected to be an important determinant of basal cyclic AMP formation, it is notable that the basal islet cyclic AMP content is similar to that of control mice [6]. This observation together with the established flow of blood through islet capillaries from the central B-cell core towards A-cells and D-cells in the islet periphery do not indicate a key role for alterations of cellular composition in the defective response to glucose.

Of greater significance in regard to alterations of islet composition is evaluation of defective insulin secretion in terms of metabolic and ionic events in islets which necessarily involves comparison of measurements with control islets containing a different proportion of B-cells. Thus the demonstration in islets of C57BL/KsJ db/db mice of small differences in any individual link in the chain of events thought to underlie glucose-stimulation of insulin secretion is difficult to interpret. Viewed in this context, it must be concluded from available evidence that the glucose-induced increase in islet glycolytic flux is not greatly altered in the mutant [7], and that islet cyclic AMP turnover is consistent with responsiveness to phosphodiesterase inhibitors [6].

In contrast to the lack of data regarding possible abnormalities of metabolic events in islets, there is compelling evidence that defective insulin secretion in C57BL/KsJ db/db mice is associated with deranged regulation of membrane potential, ion fluxes and cytoplasmic Ca\(^{2+}\) ions. Thus the islets of C57BL/KsJ db/db mice show depolarization and electrical activity at low glucose concentrations [8], abnormally low K\(^+\) permeability [9], glucose-insensitive efflux of K\(^+\) and Cl\(^-\) [9, 10], and a lack of effect of glucose on the inhibitory and stimulatory phases of Ca\(^{2+}\) efflux [11, 12]. Since K\(^+\) permeability is considered the major determinant of membrane potential which is linked to Ca\(^{2+}\) influx through the opening of voltage-dependent Ca\(^{2+}\) channels, these data point to a key role of K\(^+\) channels and their regulation in the secretion defect. Evaluation of ATP-sensitive K\(^+\) channels in C57BL/KsJ db/db mice has not been performed, but it is of interest that a closely related model, namely the Aston ob/ob mouse, exhibits disturbed electrical activity and unresponsiveness to glucacon and quinine [12, 14]. Both of these agents are known to close ATP-sensitive K\(^+\) channels in normal pancreatic B-cells [15].

**Diabetic Chinese hamster.** The inheritance of this syndrome is polygenic and therefore studies with diabetic Chinese hamsters rely on comparison with non-diabetic sublines of hamsters [2, 6]. Despite the involvement of differing genes, the manifestation and characteristics of the diabetes syndrome in Chinese hamsters is similar in many respects to C57BL/KsJ db/db mice [2]. The main difference between the two types of mutant appears to be the limited capacity in Chinese hamsters to temporarily offset the severity of diabetes by a compensatory increase in islet B-cell number [17]. Insulin is markedly decreased in the diabetic Chinese hamster pancreas in association with an increase of pancreatic glucagon and a decrease of somatostatin [18].

Studies of the perfused pancreas of diabetic Chinese hamsters have shown age-related defects in both the first and second phases of glucose-stimulated insulin release [19]. Observations by Matschinsky (cited in [20]) indicating that the activities of glucokinase, hexokinase and phosphofructokinase are not compromised in diabetic Chinese hamster islets do not favour the idea that abnormalities in the sequence of metabolic events leading to secretion are responsible for defective insulin secretion. However, further studies examining parameters of islet metabolism, including glucose-induced changes in redox state and cellular ATP content, are clearly required before such an attractive possibility is discounted.

The cyclic AMP system appears relatively normal in diabetic Chinese hamster islets as assessed from the ability of theophylline to enhance insulin release [19]. However, as with C57BL/KsJ db/db mice, the participation of Ca\(^{2+}\) ions in the stimulus–secretion coupling process is severely compromised. Thus, diabetic Chinese hamster islets do not respond to glucose with normal changes in the uptake and efflux of Ca\(^{2+}\) ions [21, 22]. Recent studies indicate that these disturbances are associated with abnormalities in the regulation of membrane K\(^+\) permeability, possibly attribut-
Defective insulin secretion in insulinoma

Insulinomas arise sporadically in many species in addition to man, including the cat, cow, dog, hamster, hogfish, mouse and rat [23]. Several useful models of heritable or serially transplantable experimentally induced insulinomas have been produced in laboratory animals [24–28]. These provide an excellent opportunity for detailed functional studies of defective insulin secretion which are otherwise difficult to perform because of the unpredictable and low incidence of spontaneous insulinomas in man.

The five most popular models include spontaneous and BK virus-induced transplantable Syrian hamster insulinomas [24, 26], the transplantable radiation-induced NEDH rat insulinoma [25], the transplantable streptozotocin-nicotinamide-induced Lewis rat insulinoma [27] and the heritable insulinoma induced in transgenic mice through expression of recombinant insulin/simian virus 40 oncogenes [28]. Each syndrome is characterized by defective glucose regulation of insulin secretion, which leads to the development of marked hyperinsulinaemia and severe life-threatening hypoglycaemia. The serially transplantable NEDH rat insulinoma has been extensively characterized in terms of its metabolic effects and the nature of the underlying insulin secretion defect.

Serially transplantable NEDH rat insulinoma. Transplantation of small insulinoma fragments into subcapsular, pancreatic or hepatic sites of NEDH rats results in hyperphagia, progressive hyperinsulinaemia, defective regulation of insulin secretion and hypoglycaemia, culminating in neuroglycopenic coma within 1 month [29]. Excised tumours contain large amounts of insulin with almost negligible content of other well-known islet peptides [30]. The altered hormonal milieu compared with that in normal islets, loss of neural input, lack of integration with surrounding pancreatic tissue, abnormalities in cellular environment and loss of cell–cell contact may contribute to disturbed insulin secretion in vivo [29].

Studies with isolated rat insulinoma cells indicate fundamental disturbances in the normal relationship between the regulation of cytoplasmic Ca²⁺ ions and insulin secretion. Thus, whereas the secretory responsiveness to cyclic AMP modulation is retained [31], glucose and a range of other agents believed to trigger secretion through elevation of cytoplasmic Ca²⁺ do not affect transmembrane Ca²⁺ fluxes or insulin release [31, 32].

The failure of glucose to elicit changes of cytoplasmic Ca²⁺ and insulin secretion in rat insulinoma cells is likely to partly reflect a deficiency of glucokinase [33] and the lack of effect of glucose on membrane potential as assessed with bisoxonol [31]. However, as witnessed by the independence of insulin release on variations of extracellular Ca²⁺ ions leading to a 4-fold increase of cytoplasmic Ca²⁺ concentration, rat insulinoma cells also display grossly abnormal sensitivity to the stimulatory effects of Ca²⁺ on exocytosis [31]. These observations indicate that rat insulinoma cells exhibit multiple abnormalities in the normal stimulus–secretion coupling mechanism. Regulation of ATP-sensitive K⁺ channels has not been assessed in rat insulinoma cells, but it is notable that established chemical probes for such channels, namely sulphonylureas and diazoxide, failed to exert normal effects on transmembrane Ca²⁺ fluxes or insulin release [32].

Concluding remarks

Studies using animal models of diabetes and insulinoma have highlighted the involvement of abnormalities of membrane K⁺ permeability and the regulation of cytoplasmic Ca²⁺ ions in defective insulin secretion. Insufficient information is available to judge whether abnormalities of B-cell glucose metabolism contribute to the impaired sequence of ionic events through inept regulation of ATP-sensitive K⁺ channels. No pertinent data exist concerning diabetic human islets, but the few studies in vitro recently performed with human non-responsive medullary-type insulinomas [34] indicate that defects in the insulin secretion pathway similar to those considered above occur in man.

Part of this research was supported by a grant from the Cancer Research Campaign (SP 1630).


Received 24 July 1989