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CONGENITAL HYPERINSULINISM: FROM BENCH TO BEDSIDE

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Abstract Congenital Hyperinsulinism in Infancy (HI) is a potentially-lethal condition of neonates and during early childhood. For many years the pathophysiology of this disorder was unknown. Recent advances in genetics, histopathology and molecule physiology have now revealed the causes of HI in a large cohort of patients. From defects in ion channel subunit genes to lesions in the control of pancreatic B-cell metabolism and anaplerosis, the causes of HI are both varied and numerous. However, in all cases they appear to share a common target protein - the ATP-sensitive K-channel. The function of these channels is not only critical to the control of healthy normal insulin-secreting cell function, but "activating" defects in these channels lead to permanent neonatal diabetes and type 2 diabetes. HI can therefore arise through "channelopathies" of K-ATP channels: HI-KATP through gene defects in ABCC8 and KCNJ11 (Ch11.p15); or as a result of "metabolopathies" through defects in the genes encoding glucokinase HI-GK (GCK, Ch.7p15-p13), glutamate dehydrogenase HI-GDH (GLUD1, Ch.10q23.3) and Short-chain L-3-hydroxyacyl-CoA dehydrogenase HI-SCHAD (HADHSC, Ch.4q22-q26). Advances in the integration of genetic medicine and cell biology have provided key insights into the causes of HI, and this has been of key importance to the definition of pathogenesis. However, medical therapy for HI remains largely unchanged due to the availability of limited agents that are selective and specific for the termination of insulin release from β-cells. CHI can be a devastating disease, and in this review focuses upon the relationship between the basis of HI and current / future therapies, including stem cells.

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Background

Congenital Hyperinsulinism in Infancy (CHI) is a disease that principally affects pancreatic β-cells of the islets of Langerhans. CHI can be a devastating condition, and one of the most difficult medical problems to face the pædiatric endocrinologist. Hyperinsulinism in neonates/infants represents a group of clinically, genetically, morphologically and functionally heterogeneous disorders and recent follow-up studies have indicated that complications of hyperinsulinism are found in up to 50% of survivors, a figure which has changed little during the past 20 years[1,2,3]. The term "hyperinsulinism" is somewhat misleading since most patients present with moderately elevated serum levels of insulin which are, however, inappropriate for the level of blood glucose. The condition is therefore most accurately described as "inappropriate insulin release for the level of glycæmia" rather than "hypersecretion" per se. CHI has not been detected in utero and there are no characteristic visual, auscultatory or tactile observations to indicate the onset of hyperinsulinism. At birth, infants may have a characteristic appearance of macrosomia, however, many are born with appropriate or even low birth weights and yet others are pre-term children. The first clinical manifestations of the disorder are mainly experienced shortly following birth. These may include respiratory distress, sweating, hypothermia, irritability, poor feeding, hunger, jitteriness, lethargy and apnoea. Symptoms can progress to vomiting, seizures, tachycardia and, in the most severe cases, death. In older children and adults, symptoms tend to be typical of those of hypoglycæmia including

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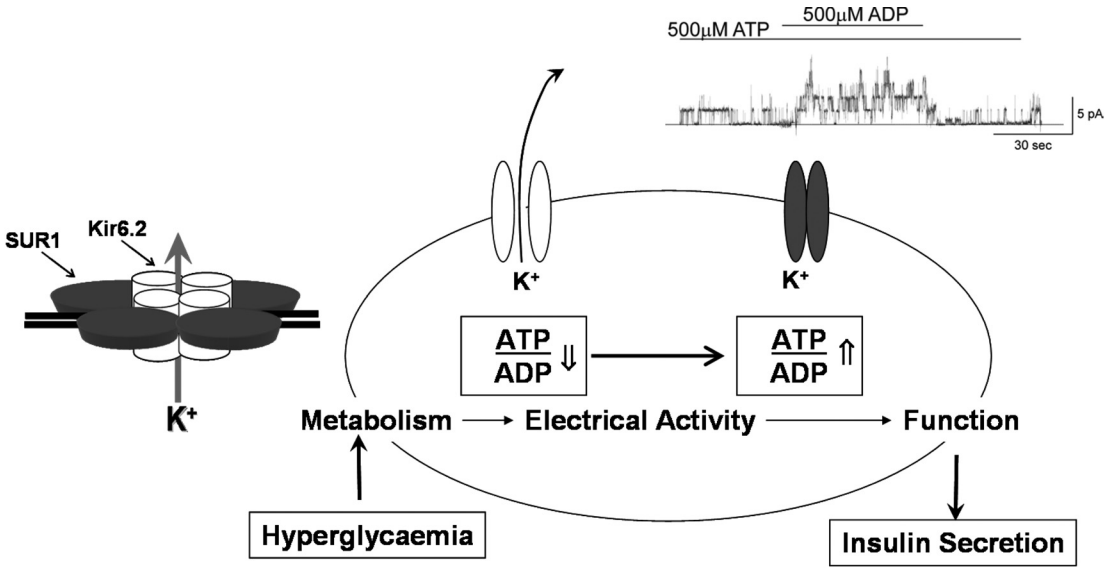

Figure 1 Schematic representations of the structure and the role of K_{ATP} channels in pancreatic β-cells. These channels couple metabolism to electrical activity through the intracellular ATP:ADP ratio. In resting β-cells open K_{ATP} channels prevent voltage gated Ca^{2+} influx, whilst in stimulated β-cells K_{ATP} channel closure depolarises the membrane and triggers Ca^{2+} dependent insulin release. The electrophysiological recording illustrates ADP-dependent activation of ATP-inhibited channels.

confusion, headaches, dizziness, syncope and, when severe, loss of consciousness. Clinical diagnosis of CHI is therefore based on evidence of inappropriately-elevated serum insulin for the level of glycæmia, inappropriate suppression of lipolysis and ketogenesis and upon positive glycæmic responses after the administration of glucagon when hypoglycæmic[1,3].

This review focuses upon the relationship between genes, β-cell function and current treatment options following recent advances in the genetics, histopathology and molecular physiology of CHI. The causes of CHI are varied and range from defects in ion channel subunit genes to mutations in the control of β-cell metabolism and anaplerosis. Yet, in all cases they appear to share a common target protein -the ATP-sensitive K^+ channel. "Channelopathies" of K_{ATP} channels - CHI-KATP are caused by gene defects in ABCC8 and KCNJ11 (Ch11.p15), whilst "metabolopathies" arise through defects in the genes encoding glucokinase CHI-GK (GCK, Ch.7p15-p13), glutamate dehydrogenase CHI-GDH (GLUD1, Ch.10q23.3) and short-chain L-3-hydroxyacyl-CoA dehydrogenase CHI-SCHAD (HADHSC, Ch.4q22-q26). In recent years, advances in the integration of genetic medicine and cell biology have been impressive, yet medical therapy for CHI remains largely unchanged due to the limited availability of agents that are selective and specific for the termination of insulin release from β-cells.

The Ionic And Metabolic Control Of Insulin Release

ATP-sensitive potassium (K_{ATP}) channels determine metabolic-ionic sensing in β-cells and co-ordinate stimulus-secretion coupling events[4] (Figure 1).

These channels are open in unstimulated β-cells, and establish the resting membrane potential. Following the uptake and metabolism of glucose, the intracellular ATP:ADP ratio increases causing channel closure and membrane

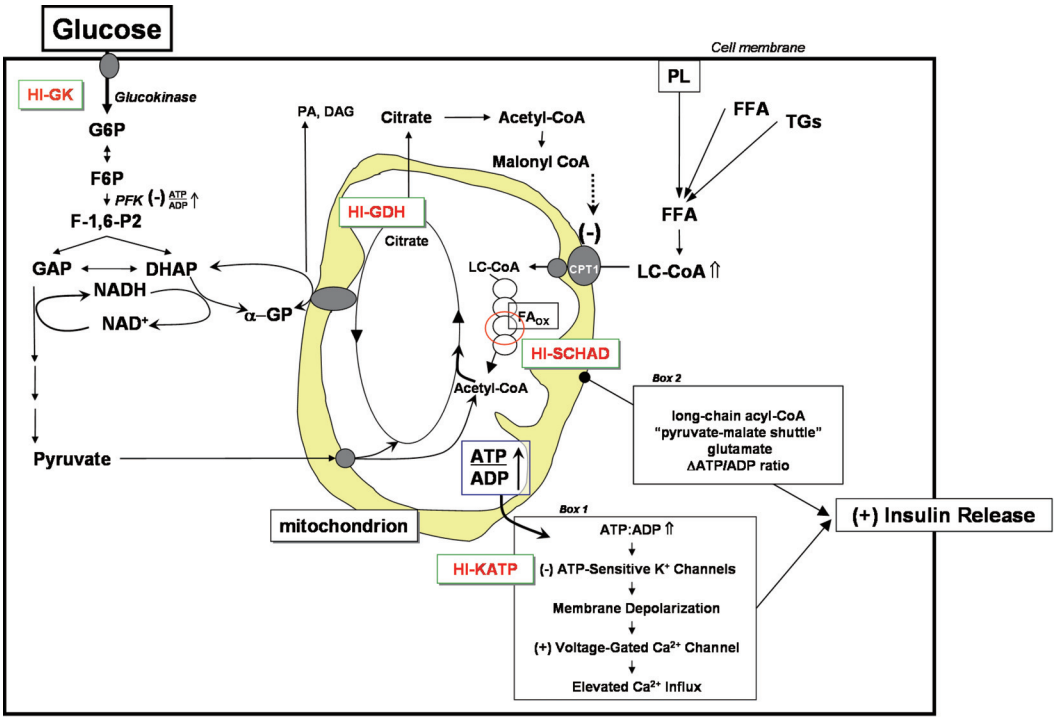

Figure 2 Overview. Metabolism-response coupling in pancreatic β-cells. Glucose metabolism, through changes in the availability of ATP:ADP, causes K_{ATP} channel closure and Ca^{2+} influx leading to insulin release in normal insulin-secreting cells. This is the triggering pathology of regulated secretion, Box 1. Mitochondrial metabolism is also coupled to exocytosis but independently of K_{ATP} channels, thereby amplifying the effects of glucose on insulin release, Box 2. In patients with HI-KATP, K_{ATP} channels are defective and under basal conditions channel closure is linked to Ca^{2+} influx. Also illustrated, sites of pathology for HI-GK, HI-GDH and HI-SCHAD.

depolarisation. The opening of voltage-dependent Ca^{2+} channels follows depolarisation of the membrane potential, leading to a rise in the cytosolic Ca^{2+} concentration close to the plasma membrane. Insulin release is then initiated by Ca^{2+}-dependent exocytosis (Figure 2).

As K_{ATP} channels are central to this process, channel inhibitors such as sulphonylureas (e.g. glibenclamide, tolbutamide, etc.) and glinides (e.g. repaglinide and nateglinide) function in vivo as anti-diabetic compounds, whilst agonists or "openers", such as diazoxide, have hyperglycæmia-inducing capabilities and inhibit secretion by preventing membrane depolarisation[1].

β-cells express a K_{ATP} channel complex formed by subunits belonging to two distinct families of proteins[5] (Figure 1). The K^+-selective pore is formed by the weak inward rectifier K^+ channel, Kir6.2. This protein has a predicted membrane topology with two α-helical transmembrane domains linked by a highly conserved sequence of amino acids that determines K^+ selectivity. The other subunit is an ATP-Binding Cassette (ABC) protein and receptor with high affinity for sulphonylureas, designated SUR1. ABC proteins generally function as transporters, ion channels or channel regulators in both prokaryotes and eukaryotes. SUR1 has 17 predicted transmembrane regions organised into three discrete transmembrane domains (TMD), 9 cytoplasmic loops and two intracellularly-disposed nucleotide-binding domains (NBD). NBDs are generally involved in nucleotide binding and hydrolysis[5]. In prokaryotes, these domains are usually discrete subunits that co-assemble to produce a functional

ABC protein, whereas in eukaryotes, a single gene usually encodes both NBDs and TMDs[4].

In β-cells, K_{ATP} channels are thought to be hetero-octameric complexes (SUR1: Kir6.2)$_4$, formed by 4 inward rectifiers and 4 SUR1s that physically associate in a 1:1 stoichiometry[4,5]. This requirement is tightly regulated by the presence of endoplasmic reticulum retention and exit signals that ensure that only correctly assembled, full-length K_{ATP} channels reach the plasma membrane[6]. In addition, TMD0 of SUR1 can enhance surface expression of Kir6.2 (if the retention signal is removed) since these subunits will form a channel with properties similar to the K_{ATP} channel[7]. In other tissues, K_{ATP} channels are heteromultimeric complexes of different Kir6.x and SURx proteins; e.g. cardiac K_{ATP} channels: Kir6.2 + SUR2A, smooth muscle K_{ATP} channels: Kir6.2 + SUR2B and the smooth muscle nucleotide-activated K^+ channel: Kir6.1 + SUR2B[5].

For all K_{ATP} channels, subunit specificity is known to be involved in the differential regulation of channel activity and to control discrete functions of the channel complex. For example, Kir6.2 determines ATP-induced channel inhibition and biophysical characteristics such as K^+ selectivity, kinetics and rectification. SUR1 acts as a conductance regulator of Kir6.2, determines the sensitivity of the complex to changes in the ATP / ADP ratio by binding Mg^{2+}ADP and regulates the sensitivity of channels to pharmacological agents. Nucleotide-binding studies indicate that ATP and ADP bind with high affinity to NBD1 of SUR1 in a Mg^{2+}-independent fashion, whereas both nucleotides bind with lower affinity to NBD2 in a Mg^{2+}-dependent fashion[8]. ATP hydrolysis at NBD1 is much less frequent than at NBD2[8].

To date, there are 5 known genetic causes of CHI that involve either defects in K_{ATP} channel genes -"channelopathies", or defects in K_{ATP} channel function as a result of altered metabolism- "metabolopathies". It is also evident that other key control points in the process of glucose-induced insulin release are associated with both hyperinsulinism and a reduced insulin output leading to diabetes, see Table 1. The most severe type of CHI involves defects in ABCC8 (encoding SUR1) and KCNJ11 (encoding Kir6.2), termed CHI-KATP.

Most cases of CHI-KATP are sporadic but familial forms - although rare, are also well documented in the literature. Sporadic CHI has an estimated incidence of between 1 in 27,000 to 1 in 50,000 live births compared to an estimated incidence of 1 in 2,500 within certain population groups i.e. Ashkenazi Jewish, Central Finnish, etc.[3,4]. ABCC8 comprises 39 exon boundaries and is clustered with KCNJ11, a single open reading frame lying immediately 3' downstream of ABCC8 on the short-arm of chromosome 11 - Ch.11p15. This is a genetic locus linked to both diffuse (Di-) and focal (Fo-) CHI.

Di-CHI predominantly arises from autosomal recessive inheritance (although autosomal dominant mutations have also been described) (see[3,4] for review). This condition affects all of the islets of Langerhans of the CHI pancreas. Until 1997 Di-CHI was widely believed to be the main cause of congenital hyperinsulinism, even though "focal hyperinsulinism" was first described more than 25 years ago[9,10]. Fo-CHI by contrast, has a non-Mendelian mode of inheritance of both K_{ATP} channel dysfunction and β-cell hyperplasia. In this condition, epigenetic phenomena involving gene silencing lead to loss of heterozygosity of a region of the maternal chromosome 11p15 and a reduction to homozygosity of paternally-derived genes. When SUR1 gene defects are present in the paternal copy of ABCC8, this results in a somatic lesion of defective β-cells within the pancreas with hyperplasia that develops through imbalanced expression of maternally-imprinted tumour suppressor genes H19 and $p57^{kip2}$, and the paternally-derived, insulin-like growth factor II

Table 1. Ionic and functional determinants of abnormalities in glucose metabolism in β-cells and their relationship to the onset of disease.

Condition	Disorder	Identified Gene Defect	β -cell Disorder
Hypoglycæmia	HI-KATP	ABCC8 (SUR1)	K_{ATP} channelopathy
Hypoglycæmia	HI-KATP	KCNJ11 (Kir6.2)	K_{ATP} channelopathy
Hypoglycæmia	HI-GK	GCK (Glucokinase)	Metabolic
Hypoglycæmia	HI-GDH	GLUD1 (Glutamate dehydrogenase)	Metabolic
Hypoglycæmia	HI-SCHAD	HADHSC (SCHAD)	Anaplerotic
Hypoglycæmia	HI-EI	Undetermined	Undetermined
Hypoglycæmia	Insulinoma	Undetermined	K_{ATP} channelopathy?
Hyperglycæmia & Deafness	MIDD	M. tRNALeu(UUR) (Mito. tRNALeu (UUR))	Metabolic
Hyperglycæmia	MODY1	HNF4A (HNF4α)	HNF1α expression
Hyperglycæmia	MODY2	GCK (Glucokinase)	Metabolic
Hyperglycæmia	MODY3	TCF1 (HNF1α)	Metabolic
Hyperglycæmia	MODY4	IPF1 (PDX1)	Insulin gene transcription
Hyperglycæmia	NDM	GCK (Glucokinase)	Metabolic
Hyperglycæmia	T2DM	KCNJ11 (Kir6.2)	K_{ATP} channelopathy
Hyperglycæmia	PNDM	KCNJ11 (Kir6.2)	K_{ATP} channelopathy
Hyperglycæmia	PNDM	ABCC8 (SUR1)	K_{ATP} channelopathy
Hyperglycæmia	T2DM	M.GPDH (Mito. Glycerol-3-phosphate dehydrogenase 2)	Metabolic
Hyperglycæmia	T2DM	SERCA3 (SERCA Ca^{2+}-pump)	Cytosolic Ca^{2+}

Abbreviations: T2DM - Type 2 Diabetes, NIDDM; MODY - Maturity Onset Diabetes of the Young; HI - Hyperinsulinism in Infancy; MIDD - Maternally Inherited Diabetes and Deafness; NDM - Neonatal Diabetes Mellitus; SCHAD - short-chain L-3-hydroxyacyl-CoA dehydrogenase; HNF - hepatocyte nuclear factor; HI-EI - Exercise-Induced Hyperinsulinism in Infancy; PNDM – Permenant Neonatal Diabetes Mellitus.

gene. Recent estimates from France, Israel and the USA suggest that 40-65% of all patients with CHI have focal disease[11].

More than 100 mutations in the ABCC8 and KCNJ11 genes have been described. Some of these mutations have been shown to result in abnormalities of recombinant K_{ATP} channels producing defects in protein folding, assembly or trafficking, and alterations in either nucleotide regulation or open-state frequency (Figure 2). However, in >50% of Di-CHI and ～30% of Fo-CHI patients, the genetic basis of the disease has yet to be defined[3,12].

In patients with the most severe K_{ATP} channelopathy, functional channel currents are ablated. Affecting around 10% of all Di-CHI patients and approximately 55% of Fo-CHI patients, this condition is typified by abnormalities in gene expression, protein synthesis, maturation, assembly or trafficking. Many patient β-cells- approximately 60% of Di-CHI and ～45% of Fo-Hi patients have K_{ATP} channel defects that are more diverse in their presentation. These channelopathies have K_{ATP} channel currents that are recordable in β-cells, but contain defects in function or are present in limited numbers.

The causal relationship between CHI-KATP gene defects and β-cell function has been defined by studying tissues isolated from patients with CHI. Defects in channel density, kinetics or regulation, lead to a depolarised membrane potential in CHI β-cells, resulting in the generation of action potentials and elevated concentrations of cytosolic Ca^{2+} under basal conditions[4].

So far, mutations in three other genes, each associated with glucose homeostasis and acquired K_{ATP} channel abnormalities in β-cells have been described. Each of these

metabolopathies gives rise to a clinically distinct form of hyperinsulinism: CHI-GK, CHI-GDH and CHI-SCHAD, (Figure 2, Table 1). In addition, hyperinsulinism induced by exercise has also recently been described.

CHI-GDH is caused by dominantly-expressed, gain-of-function mutations of the mitochondrial enzyme, glutamate dehydrogenase (GDH)[13]. CHI-GK results from the inheritance of autosomal dominantly-expressed gain-of-function mutations in the glucokinase enzyme[14]. CHI-SCHAD results from gene defects in short-chain L-3-hydroxyacyl-CoA dehydrogenase -the the penultimate enzyme in fatty acid β-oxidation[15].

Treatment For Chi

Most infants with hyperinsulinism present during the first postnatal days; others during the first year. It is rare for older children to present de novo with symptoms of hyperinsulinism-induced hypoglycæmia. Medical regimens for CHI address the inhibition of insulin release (below), the use of glucagon to promote mobilization of hepatic glucose and the administration of a constant glucose infusion to protect against hypoglycæmia-induced neurological damage[16]. The first choice of drugs to inhibit insulin release is of those that can be administered orally, followed by agents that are delivered intravenously or subcutaneously. Unfortunately, none of the agents that are currently available or administered are specific for the inhibition of insulin release, and this can lead to complications. When medical therapy fails or is inappropriate, surgery to remove part or the entire pancreas is undertaken.

Glucagon has a powerful effect on mobilising glucose from hepatic glycogen by increasing the rates of glycogenolysis and gluconeogenesis. Somatostatin analogues such as Octeotride and Sandostatin are important clinical agents in CHI treatment providing both short- and long-term benefits through the inhibition of insulin release. Diazoxide is an effective inhibitor of insulin secretion and, since it can be administered orally, is the cornerstone of medical treatment for CHI. Treatment is complicated by the fact that the agent is poorly tolerated by a number of patients due, mainly, to adverse side effects. Diazoxide is generally used in combination with chlorothiazide (7 to 10 mg/kg/day), a diuretic which overcomes the fluid-retaining actions of diazoxide and inhibits insulin release. Nifedipine. As the link between loss of K_{ATP} channels and uncontrolled insulin release was established, this led to the suggestion that clinically-relevant inhibitors of voltage-gated Ca^{2+} channels such as nifedipine may be of therapeutic value[17]. Corticosteroids. Agents such as prednisone, prednisolone and methylprednisolone are used in CHI treatment since they increase gluconeogenesis. The action of these compounds is not immediate, but is useful in the short term to maintain adequate blood glucose levels. Adverse effects of prolonged corticosteroid administration limit their long-term application and these include a reduced immune responsiveness, a tendency towards obesity, cataracts and decreased bone mineral density.

Concluding Remarks.

Since the late 1990s there has been a considerable volume of knowledge and information concerning the molecular genetics, cell biology, histology and physiology of hyperinsulinism syndromes. This has provided unparalleled insights into the pathogenesis and diagnosis of CHI, and has informed contemporary management and treatment strategies. A marked heterogeneity in genes leading to hyperinsulinism is already apparent and, since many patients with CHI have yet to have the genetic basis of their condition defined, the next few years are likely to yield further insights into the genetic causes of hyperinsulinism in infancy and childhood.

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