Therapy for Persistent Hyperinsulinemic Hypoglycemia of Infancy

Understanding the Responsiveness of β Cells to Diazoxide and Somatostatin

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Abstract

The neonatal disorder persistent hyperinsulinemic hypoglycemia of infancy (PHHI) arises as the result of mutations in the subunits that form the ATP-sensitive potassium (K_{ATP}) channel in pancreatic β cells, leading to insulin hypersecretion. Diazoxide (a specific K_{ATP} channel agonist in normal β cells) and somatostatin (octreotide) are the mainstay of medical treatment for the condition. To investigate the mechanism of action of these agents in PHHI β cells that lack K_{ATP} currents, we applied patch clamp techniques to insulin-secreting cells isolated from seven patients with PHHI. Five patients showed favorable responses to medical therapy, and two were refractory. Our data reveal, in drug-responsive patients, that a novel ion channel is modulated by diazoxide and somatostatin, leading to termination of the spontaneous electrical events that underlie insulin hypersecretion. The drug-resistant patients, both of whom carried a mutation in one of the genes that encode K_{ATP} channel subunits, also lacked this novel K^+ channel. There were no effects of diazoxide and somatostatin on β cell function in vitro. These findings elucidate for the first time the mechanisms of action of diazoxide and somatostatin in infants with PHHI in whom K_{ATP} channels are absent, and provide a rationale for development of new therapeutic opportunities by K^+ channel manipulation in PHHI treatment. (*J. Clin. Invest.* 1997. 100:1888–1893.) Key words: PHHI • nesidioblastosis • K^+ channel • hyperinsulinism • sulphonylurea

Introduction

Hyperinsulinism is the most common cause of persistent or recurrent hypoglycemia in infancy (persistent hyperinsulinemic hypoglycemia of infancy [PHHI][1]) (1). The disorder is characterized by inappropriate insulin secretion in the face of hypoglycemia, and is diagnosed by demonstrating hypoketotic hypofatty acidemic hypoglycemia in association with hyperinsulinism and an elevated glucose requirement (1). While PHHI has variable clinical phenotypes, it most commonly presents within the first few hours or days of birth. The combination of hypoketotic hypoglycemia carries with it a substantial risk of neurological damage if not recognized and treated promptly and adequately. Short-term medical therapy for the disorder involves provision of an increased carbohydrate intake to meet the elevated requirement, and usually one or more drugs that inhibit insulin secretion (2). One such agent is diazoxide, which was first introduced to treat hyperinsulinism in the 1960s (3) and is usually administered in conjunction with a thiazide diuretic (chlorothiazide) that both potentiates its hyperglycemic action and inhibits the associated fluid retention. The somatostatin analogue octreotide is also of proven benefit in PHHI therapy by inhibiting insulin secretion (4, 5). In normal pancreatic β cells, both diazoxide and somatostatin are specific agonists of the ATP-sensitive K^+ (K_{ATP}) channels (6). In normal β cells, these channels regulate the cell membrane potential, and their closure after glucose metabolism leads to depolarization of the membrane, generation of action potentials as a result of Ca^{2+} influx, and initiation of insulin secretion (6). Thus, activation of these channels by either diazoxide or somatostatin in normal β cells reverses the events in association with hyperpolarization of the membrane potential and inhibition of insulin release.

In the clinical situation the sensitivity of children with PHHI to medical therapy is highly variable, with a spectrum from extreme sensitivity to total drug resistance. Patients who fail to achieve stable normoglycaemia despite treatment with diazoxide and somatostatin (alone or in combination with other agents) usually require a partial or subtotal pancreatectomy to prevent recurrent hypoglycemia (7). The mechanisms of action of these agents in patients with PHHI as well as the varying sensitivity of different patients to the agents remains unexplained. An understanding of the pathogenesis of this disorder has advanced considerably over the last 24 months. First, a *PHHI-susceptibility* gene locus on the short arm of chromosome 11 was identified (8), and then it was demonstrated that nucleotide base mutations in both genes that encode the subunits of K_{ATP} channels (9, 10) at this locus were associated with familial forms of PHHI (11, for review see references 12 and 13). Second, it has been shown that β cells isolated from patients with PHHI lack functional ATP-sensitive K^+ channels, leading to

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1. *Abbreviations used in this paper:* K_{ATP}, ATP-sensitive potassium channel; PHHI, persistent hyperinsulinemic hypoglycemia of infancy.

the loss of normal regulated insulin secretion (14, 15). In this study, we describe both the mechanisms of action of diazoxide and somatostatin on β cells from children with PHHI who lack functional K_{ATP} channels, and explain the pathological basis for drug resistance.

Methods

Over a 15-mo period, surgical specimens of pancreatic tissue were obtained from seven patients with PHHI undergoing subtotal pancreatectomy (Ethical permission was granted by the Great Ormond Street Hospital for Children National Health Service Trust Ethics Committee, United Kingdom). Six of the patients presented within 30 min of birth with severe hypoglycemia (blood glucose < 2.5 mM/liter), and one presented with hypoglycemic seizures 36 h after birth. All required very high rates of glucose infusion to achieve normoglycemia, and all were subsequently shown to have hyperinsulinemia during spontaneous episodes of hypoglycemia (Table I).

Medical therapy with diazoxide and/or somatostatin (octreotide) was only partially effective in one group of patients. In none of these cases was there a family history of PHHI. All five of the patients in this partially drug-responsive group were treated with diazoxide (15–20 mg/kg/d) together with hydrochlorothiazide and/or octreotide (25–100 μg/kg/d) during their hospitalization. In each of the children there were periods when this treatment resulted in either an increase in median blood glucose concentration and/or a reduction in glucose administration rate necessary to maintain normoglycemia (Fig. 1 *A*). Despite the fact that these criteria were used to establish a positive response, none of the patients could maintain a truly normal feeding regimen without intermittent episodes of severe hypoglycemia and without an intravenous glucose supplement. In each case this necessitated treatment by subtotal pancreatectomy. Two other patients formed the drug-resistant group. Both children were of Saudi Arabian origin, both were from families with a known history of familial PHHI, and both were found to have a mutation in the *SUR1* gene known to be associated with defects in the function of K_{ATP} channels (14). These patients were totally diazoxide- and somatostatin-insensitive, as were their siblings with PHHI, and both underwent early subtotal pancreatectomy (Fig. 1 *B*). The median age of the children at the time of pancreatectomy was 50 d, and the excised specimens of pancreas had histological features typical of diffuse ($n = 6$) or focal ($n = 1$) nesidioblastosis with nuclear gigantism of endocrine cells (16).

To screen for K_{ATP} channel gene defects in the PHHI patients, genomic DNA was extracted from blood (Wizard purification kit; Promega Corp., Madison, WI). SUR1 and $K_{IR}6.2$ intron primers were used to amplify each of the exons on which we have identified mutations that either cause or are associated with PHHI (see Table II) to be analyzed by PCR. In all cases PCR products were directly sequenced, analyzed by single-stranded conformational polymorphism analysis, and where there was either a loss or gain of a restriction site, by digestion with the corresponding enzyme (14).

To address the molecular basis for drug responsiveness in PHHI

Table I. Clinical Information

	Nonfamilial					Familial		
Child	1	2	3	4	5	6	7	Normal
Glucose (mM/liter)	2.1	2.1	2.3	1.8	1.6	2.1	2.1	3.5–5.5
Insulin (mU/liter)	20.8	12.9	9.7	24.3	11.9	52.3	17.2	< 5
Glucose infusion (mg/kg/min)	15	16.5	16.5	18	22.5	18	17	< 12.5

Median blood glucose concentrations measured during numerous episodes of spontaneous hypoglycemia (blood glucose < 2.5 mM/liter) in the seven infants with the corresponding data for insulin, and typical intravenous glucose infusion. The glucose requirements for patients 1, 3, and 4 were calculated when the infants were not receiving any medication, and in patients 2, 5, 6, and 7 while the infants were receiving glucagon infusions. For direct comparison we include typical normal fasting plasma glucose levels (3.5–5.5 mM) and normal serum insulin levels recorded under hypoglycemic conditions from age-matched controls.

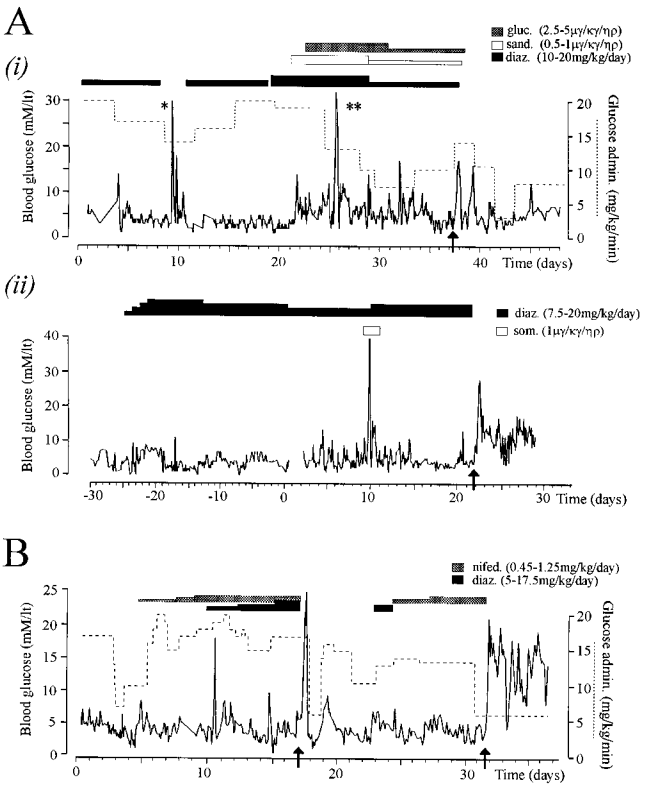

Figure 1. Details of blood glucose concentrations and clinical management strategies of two drug-responsive infants (*A*) and one drug-resistant infant (*B*) with the exon 35 *SUR1* gene mutation. (*A*) (*i*) Preterm (34 wk gestation) infant who responded to combination therapy with octreotide (Sandostatin®), diazoxide, and glucagon with an increase in blood glucose concentration from 3.5 mM/liter to 4.5 mM/liter despite a reduction in glucose administration rate from 18 to 8 mg/kg/min (from days 21 to 34). The *arrow* indicates the time of 95% pancreatectomy. *Ligation of patent ductus arteriosus; **episode of septicaemia. (*ii*) Term infant who demonstrated extreme sensitivity to somatostatin infusion (day 10). Full glucose administration rate data is unavailable for this infant, but a hyperglycaemic response to diazoxide was reported by the referring hospital when the agent was commenced (days −25 to −20). Time = 0 represents the day of referral to tertiary care at Great Ormond Street Hospital for Children, London. The *arrow* indicates the time of 95% pancreatectomy. (*B*) Term Saudi Arabian infant with familial PHHI (third affected child) documenting a drug-resistant phenotype. The infant had failed to respond to somatostatin at the referring hospital, and failed to respond to diazoxide or the L-type calcium channel blocker nifedepine at this hospital. Note how the glucose infusion rate remained fairly constant during drug therapy. The *first arrow* indicates the time of 95% pancreatectomy. This surgery did not result in satisfactory resolution of hypoglycaemia, and a total pancreatectomy was subsequently performed (*second arrow*).

Table II. Mutations in SUR1 and Kir6.2 Genes Identified in Patients with PHHI

	Treatment	Zygosity	Reference
SUR1			
Intron 11 & 18	Surgical	Compound heterozygote	see 12, 13
Exon 16-G716V	Surgical	Homozygote	21
Exon 34/Intron 32	Octreotide	Compound heterozygote	see 12, 13
Intron 32	Surgical	Homozygote	see 12, 13
Intron 32	Diazoxide	Compound heterozygote	see 12, 13
Exon 34-ΔF1388		Compound heterozygote Homozygote	20
Exon 35-R1437Q	Surgical	Homozygote	see 12, 13
Exon 37-G1479R	Diazoxide	Compound heterozygote	19
KIR6.2			
Y12X	Surgical	Homozygote	22
L147P	Surgical	Homozygote	23

The table documents published mutations in K_{ATP} channel genes, the original treatment regime associated with the cases and patient genotype. All PHHI patients upon which this study is based have been screened for each of the nine mutations listed.

β cells, in vitro experiments of stimulus-response coupling were carried out using islets of Langerhans isolated from the pancreatic tissue after surgery. As controls, islets were also isolated from normoglycemic infantile and adult human pancreata using previously described procedures (14, 15, 17). In brief, after collagenase digestion, cleaved intact islets were hand-picked. Once isolated, these intact islets were maintained for up to 8 d under standard tissue culture conditions at 37°C. Exocrine cells and ductal epithelial cells are not maintained in a differentiated state under these conditions. Finally, before experimentation islets were disrupted by mechanical aspiration, liberating individual cells and isolated cell clusters. At the time of the experiment it was not technically feasible to show by independent means that cells from which recordings were made also released insulin. The presence of insulin-secreting cells in islets, however, was confirmed in each of the sections of pancreata by both insulin immunohistochemistry and by in situ hybridization, a result entirely consistent with previous studies (16). Individual endocrine cells and isolated cell clusters were used for studies of ion channel function by patch-clamp techniques (17) in the cell-attached and inside-out patch configurations. Standard NaCl- and KCl-rich solutions were added to the outside and the inside faces of the cell membrane, respectively. To correlate clinical data with in vitro studies of cell function, the external bathing solution also contained 2.5 mM glucose. Details of the recording apparatus, data collection, and analytical procedures have been recently described (15).

Results

Unlike normal human insulin-secreting cells (Fig. 2, *a* and *b*), ATP-sensitive K^+ channels were not operational in isolated pancreatic β cells from both groups of PHHI patients (14, 15) that were spontaneously electrically active and persistently generating Ca^{2+} dependent action potentials (14, 15, 17) (Fig. 2, *c* and *d*). In β cells isolated from the drug-responsive group, diazoxide, the diazoxide analogue BPDZ-44 (18), and somatostatin inhibited action potential generation (n = 26 separate intact cell recordings from this group of patients) (Fig. 2). As these effects occurred despite the absence of functional K_{ATP} channels (n = 87 separate recordings [inside-out and cell-attached patches]), the underlying mechanisms of action of these agents was further investigated using the cell-free recording configurations of the patch-clamp technique. Upon formation of isolated inside-out patches from the β cells of this group of patients, there was no spontaneous K^+ channel activity (15) unless the inner face of the plasma membrane was exposed to either ATP (500 μM) or ADP (500 μM) (Fig. 3), which then led to the appearance of a novel K^+ selective ion channel. As the properties of this channel distinguished it from any other K^+ channel reported from electrophysiological studies of β cells or insulin-secreting cell lines (6), we have designated this current I_{KPHHI}. This channel has a linear current-

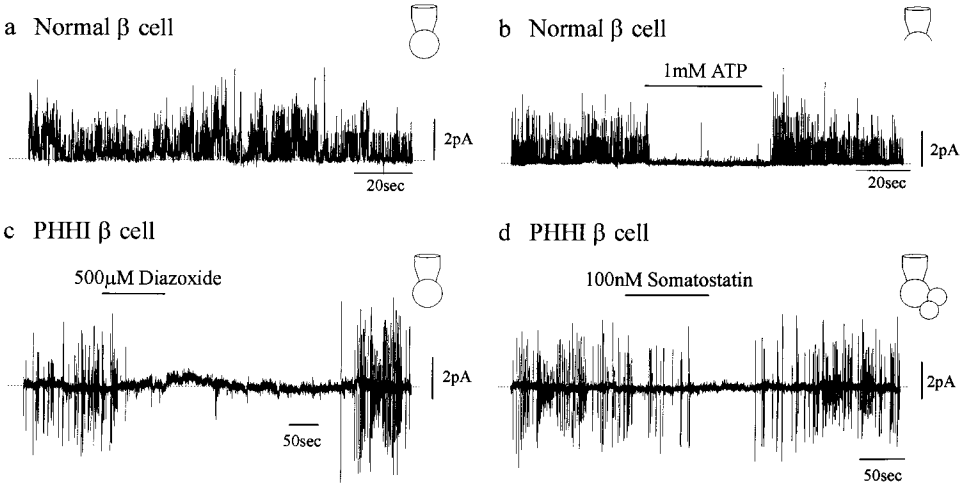

Figure 2. Termination of spontaneous electrical activity in isolated PHHI β cells by diazoxide and somatostatin. Controls (*a* and *b*) show ATP-sensitive K^+ channel recordings from normoglycemic infantile β cells obtained using the cell-attached patch (*a*) and the inside-out patch (*b*) configurations. Open K_{ATP} channels in β cells are responsible for establishing the cell membrane potential, and are inhibited by the intracellular addition of ATP (*b*). Note that upward deflections represent single K_{ATP} channel events. *c* and *d* are recordings made from PHHI β cells, and show how the spontaneous electrical events are terminated by diazoxide and somatostatin added to the intact cells, respectively. In both panels, note the absence of K_{ATP} channel openings, and that each deflection represents a single action potential current event resulting from Ca^{2+} influx. The patterns of electrical activity were recorded from different cells using the cell-attached patch-clamp configuration with symmetrical 140 mM NaCl-rich solutions added to the pipette and bath. For the cell-free recording (*b*), a KCl-rich solution was added to the inside of the cell membrane, and an NaCl-rich solution was added to the outside of the membrane. The data show that under these experimental conditions the action potential frequency decreased by on average 95±3% of the control value (100%). *b* is typical of eight, and *c*, seven other experiments.

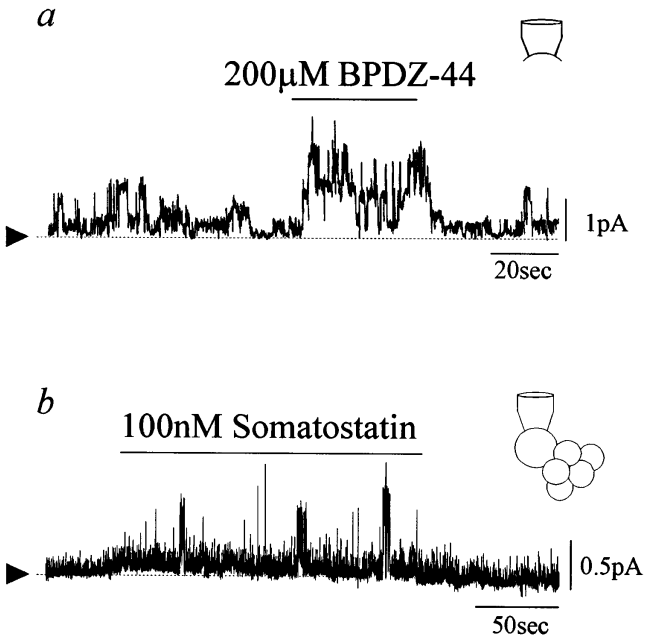

Figure 3. Modulation of novel K^+ channels by BPDZ-44 and somatostatin in isolated PHHI β cell membranes. (*a*) Potassium channel activation by BPDZ-44 in an excised inside-out patch, typical of ten other recordings. The data was obtained under physiological cation gradients comprising KCl- and NaCl-rich solutions (see Methods). The inner domain of the patch of membrane was silent before ATP addition, which was subsequently required to maintain channel openings. Under these conditions, BPDZ-44 caused a marked increase in channel activity. (*b*) Activation of a similar channel in the intact cell configuration by 100 nM somatostatin. This data was obtained using a KCl-rich solution in the pipette, and an NaCl-rich solution in the bath, and is typical of four other experiments. Similar effects were also found for diazoxide (n = 9) (see Fig. 4 *b*). Note in both *a* and *b* that the dotted line and triangle indicate the level of activity corresponding to all channels closed; upward deflections from this line represent outward current events.

voltage relationship plot, a single-channel conductance of ~ 20pS (Fig. 4 *a*), and is selectively activated by either diazoxide or BPDZ-44 (n = 24), and by the addition of somatostatin to intact cells (n = 8) (Figs. 3 and 4). In sharp contrast to the results of studies from this group of patients, K_{PHHI} channels were not recorded in β cells isolated from the drug-resistant patients with familial PHHI, (14) and there were no effects of diazoxide or somatostatin on isolated β cell function (n = 34).

Discussion

In normal insulin-secreting cells two subunits comprise the K_{ATP} channel. The α subunit is a K^+ channel core protein designated $K_{IR}6.2$, while the β subunit is a receptor with high affinity for sulphonylureas, SUR1 (10). As several gene mutations in familial PHHI patients have been identified at sites in the DNA encoding the K_{ATP} channel subunits (11, 12, 13, 19), we isolated DNA from each of the patients and confirmed that none of the drug-responsive group express mutations in nucleotide bases at any of these loci (Table II). By contrast, the genotypes of the drug-resistant patients were typical of familial PHHI since pancreatic β cell pathology was correlated with the previously described exon 35 mutation of the *SUR1* gene,

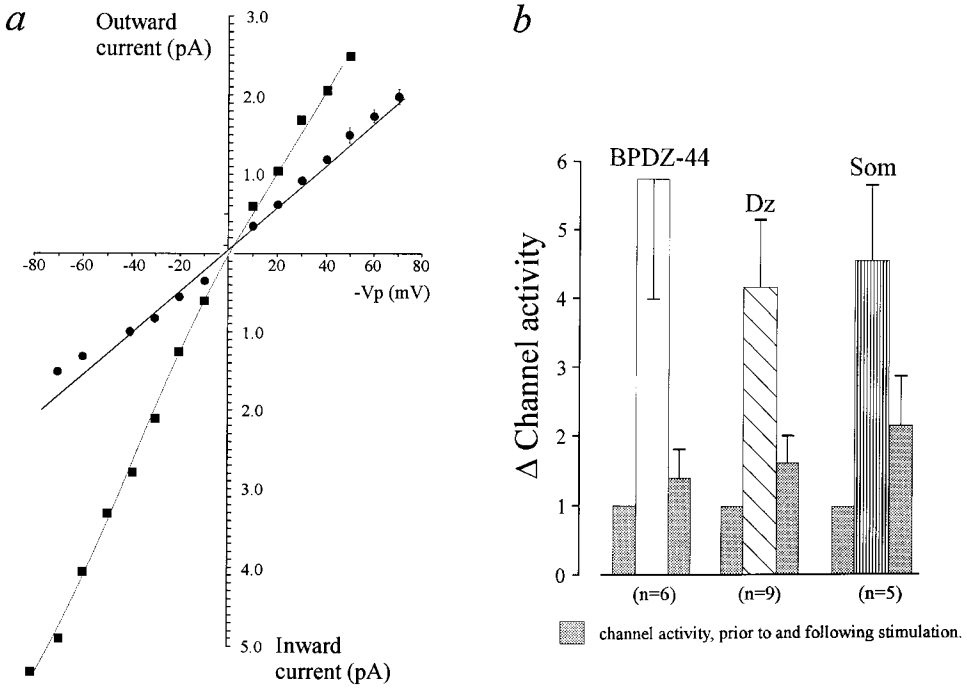

Figure 4. Properties of K_{PHHI} channels. *a* shows the current-voltage relationship plots for the K_{PHHI} channel recorded in PHHI β cells (*filled circles*) and the K_{ATP} channel recorded from normoglycaemic infantile human β cells. Both plots were obtained under the same experimental conditions, with both sides of the inside-out patch of membrane exposed to a symmetrical 140-mM K^+-rich solution. For the K_{PHHI} channel plot, each point on the graph represents an average value (mean±SEM) from four inside-out recordings. The gradients of the lines of best fit suggest that the single-channel current conductances are 20pS vs. 65pS for the K_{PHHI} and K_{ATP} channels, respectively. (*b*) Average data for the actions of diazoxide (500 μM), somatostatin (100 nM), and BPDZ-44 (200 μM) on K_{PHHI} channel activity in isolated PHHI β cells. Average data (mean±SEM values) has been expressed as a fraction of the immediate drug-free value (normalized to 1) under the same conditions as shown in Fig. 3, *a* (BPDZ-44) and *b* (diazoxide and somatostatin).

resulting in the premature truncation of SUR1 (14). Since the product of this gene mutation both removes that part of the protein incorporating the putative diazoxide and nucleotide-binding site (the second nucleotide binding fold) and leads to loss of K_{ATP} channels in recombinant studies (14), we therefore propose that the lack of drug responsiveness in familial PHHI is directly correlated to the absence of K_{ATP} channels. In addition, our data allude to the possibility that the *SUR1* gene defect is also associated with the loss of expression or functional activity of I_{KPHHI} in the familial phenotype of the disease.

Current medical therapy for PHHI is principally directed towards alleviation of hypoglycemia through a combination of a constant glucose infusion or elevated carbohydrate intake, as well as administration of agents that will inhibit insulin secretion, such as diazoxide and somatostatin analogues. These compounds act in normal pancreatic β cells to stimulate the activity of K_{ATP} channels and, in the case of somatostatin, by an additional decrease in cAMP accumulation (6). Pancreatic β cell function in patients with PHHI is compromised by the absence of K_{ATP} channels (14, 15), thus raising questions about the mechanism of action and efficiency of conventional PHHI therapy. In addition, PHHI is also complicated by the fact that the disease has a variable clinical phenotype with mild vs. severe, early-onset vs. late-onset, and familial vs. nonfamilial forms of the condition. In this study we have shown for the first time in a group of nonfamilial PHHI patients responding to medical therapy, that diazoxide and somatostatin activate a novel type of K^+ channel, (Figs. 3 and 4) and that this activation then leads to termination of Ca^{2+}-dependent action potentials in vitro (Fig. 2) and alleviation of hypoglycemia in vivo (Fig. 1). These findings are further supported by data from patients with a familial form of PHHI who were totally resistant to medical therapy in vivo (Fig. 1 *c*) (14), possessing β cells that lacked both K_{ATP} channels and K_{PHHI} channels. In these insulin-secreting cells there were no effects of K^+ channel modulators on cell electrical activity.

In summary, our data provide observations that explain the clinical significance of diazoxide and somatostatin as medical adjuncts to the treatment of PHHI. We have found that the presence of a novel K^+ channel sensitive to diazoxide and somatostatin in β cells isolated from PHHI patients with a drug responsive form of the disorder, leads to the inhibition of spontaneous electrical events in vitro. Since we have previously shown that the unregulated electrophysiological properties of PHHI β cells in vitro correlates to the hypersecretion of insulin in vivo (14, 15, 17), these findings provide a rationale for explaining the clinical profile of these patients. The universal responsiveness of nonfamilial PHHI to diazoxide in vitro questions whether our current dosage regimens are adequate, and the response of this group to somatostatin provides a basis for the suggested long-term use of this agent in treating PHHI (5). The synthesis of new K^+ channel modulators with selective efficacy for β cell channels may also prove medically useful, and may have major benefits in obviating the need for pancreatectomy not only in those patients who may respond to diazoxide and somatostatin, but also in those who are unresponsive to conventional drug therapy.

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