Original Article

Serum Glucagon Counterregulatory Hormonal Response to Hypoglycemia Is Blunted in Congenital Hyperinsulinism

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The mechanisms involved in the release of glucagon in response to hypoglycemia are unclear. Proposed mechanisms include the activation of the autonomic nervous system via glucose-sensing neurons in the central nervous system, via the regulation of glucagon secretion by intraislet insulin and zinc concentrations, or via direct ionic control, all mechanisms that involve high-affinity sulfonylurea receptor/inwardly rectifying potassium channel-type ATP-sensitive K^+ channels. Patients with congenital hyperinsulinism provide a unique physiological model to understand glucagon regulation. In this study, we compare serum glucagon responses to hyperinsulinemic hypoglycemia versus nonhyperinsulinemic hypoglycemia. In the patient group (n = 20), the mean serum glucagon value during hyperinsulinemic hypoglycemia was 17.6 ± 5.7 ng/l compared with 59.4 ± 7.8 ng/l in the control group (n = 15) with nonhyperinsulinemic hypoglycemia (P < 0.01). There was no difference between the serum glucagon responses in children with diffuse, focal, and diazoxide-responsive forms of hyperinsulinism. The mean serum epinephrine and norepinephrine concentrations in the hyperinsulinemic group were 2,779 ± 431 pmol/l and 2.9 ± 0.7 nmol/l and appropriately rose despite the blunted glucagon response. In conclusion, the loss of ATP-sensitive K^+ channels and or elevated intraislet insulin cannot explain the blunted glucagon release in all patients with congenital hyperinsulinism. Other possible mechanisms such as the suppressive effect of prolonged hyperinsulinemia on α-cell secretion should be considered. *Diabetes* 54:2946–2951, 2005

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ABCC8, ATP-binding cassette, subfamily C, member 8 (high-affinity sulfonylurea receptor); CHI, congenital hyperinsulinism; K_{ATP} channel, ATP-sensitive K^+ channel; KCNJ11, inwardly rectifying potassium channel, subfamily J, member 11; $K_{IR}6.2$, inwardly rectifying potassium channel; NEFA, nonesterified fatty acid; SUR1, high-affinity sulfonylurea receptor; VMH, ventromedial hypothalamus.

Congenital hyperinsulinism (CHI) is a cause of persistent hypoglycemia in children. Biochemically, there is unregulated insulin secretion from the pancreatic β-cells. So far, mutations in five different genes have been described that lead to unregulated insulin secretion (1–6). The most common causes of CHI are mutations in the ATP-binding cassette, subfamily C, member 8 (high-affinity sulfonylurea receptor) (*ABCC8*) and inwardly rectifying potassium channel, subfamily J, member 11 (*KCNJ11*) genes encoding the two subunits, high-affinity sulfonylurea receptor (SUR1) and inwardly rectifying potassium channel ($K_{IR}6.2$), respectively of the neuroendocrine-type ATP-sensitive K^+ channel (K_{ATP} channel) found in α- and β-cells and many neurons. Histologically, two types of CHI have been described, focal and diffuse (7). The focal form is found in ~40–50% of children and appears to be localized to one region of the pancreas. The focal form has been associated with uniparental disomy and genetic imprinting (8,9), whereas the diffuse form appears to be associated with autosomal recessive and dominant mutations in the *ABCC8* and *KCNJ11*genes.

The role played by the various counterregulatory hormones such as cortisol, growth hormone, epinephrine, norepinephrine, and glucagon in hyperinsulinemic hypoglycemia is not clear. Neonates with hyperinsulinemic hypoglycemia fail to generate an adequate serum cortisol counterregulatory response to symptomatic hypoglycemia (10). This appears to be related to the lack of drive from the hypothalamic-pituitary axis, with inappropriately low plasma adrenocorticotropic hormone concentrations at the time of hypoglycemia (10).

Glucagon, epinephrine, and norepinephrine form a primary defense against hypoglycemia. Glucagon is produced by the α-cells of the pancreatic islets and activates adenylate cyclase through a mechanism involving a guanine nucleotide-binding protein (11). The resulting accumulation of cAMP leads to activation of cAMP-dependent protein kinase, which, in turn, phosphorylates intracellular enzymes involved in the regulation of glycogen metabolism, gluconeogenesis, and glycolysis. Glucagon acutely stimulates glycogenolysis and has potential effects on hepatic gluconeogenesis and ketogenesis. The central role of glucagon in glucose physiology is exemplified by the fact that glucagon deficiency can cause hypoglycemia in humans and in mice (12,13). Despite the pivotal role of glucagon in maintenance of glucose homeostasis, there is

remarkably little literature on the glucagon counterregulatory response during hypoglycemia in childhood. The "normal" serum glucagon response at the time of hypoglycemia, for example, has yet to be reported.

The mechanism(s) that triggers the glucagon counterregulatory response to hypoglycemia is unclear. One may involve the activation of the autonomic nervous system (sympathetic, adrenomedullary, and parasympathetic) by glucose-sensing neurons located in the ventral medial hypothalamic (VMH) regions. These glucose-responsive neurons have K_{ATP} channels and in the VMH, increase their firing rate in response to elevation of extracellular glucose levels (14,15). VMH neurons in $K_{IR}6.2$-null mice, which lack functional neuroendocrine-type K_{ATP} channels, have a persistently elevated firing rate and exhibit a severely impaired glucagon counterregulatory response (16).

There is intense debate about the effects of systemic and intraislet insulin concentration upon plasma glucagon secretion during insulin-induced hypoglycemia. Some studies suggest that systemic and intra-islet insulin concentrations do not have any effect on the glucagon counterregulatory hormonal response (17), yet others demonstrate that intra-islet hyperinsulinemia prevents the glucagon response to hypoglycemia (18,19) (rev. in 20,21). Other studies show that glucagon secretion from α-cells is normally suppressed by the simultaneous activation of β-cells and that zinc released from β-cells may be implicated in this suppression (22–25). Specifically, a decrease in intra-islet insulin may be a signal for the glucagon secretory response to hypoglycemia in healthy humans (26).

Studies in mice have suggested that glucose can suppress glucagon secretion by inducing closure of K_{ATP} channels, leading to membrane depolarization and inactivation of the ion channels participating in action potential generation (27). Thus, K_{ATP} channels may be involved in the control of glucagon secretion by regulating the membrane potential in the α-cell in a way reminiscent to that documented in insulin-releasing β-cells. However, because α-cells possess a different complement of voltage-gated ion channels involved in action potential generation than the β-cell, moderate membrane depolarization in α-cells has been suggested to reduce rather than increase electrical activity and secretion (28).

Patients with CHI provide a unique in vivo physiological model to understand the effects of intra-islet hyper- and hypoinsulinemia on serum glucagon counterregulatory hormonal response to hypoglycemia. Patients with diffuse disease have unregulated insulin secretion from all of the pancreatic β-cells and therefore present disseminated intra-islet hyperinsulinemia. On the other hand, patients with focal CHI have a discrete lesion in which excess insulin secretion is confined to the focal domain. In the rest of the pancreas, the β-cells are functionally normal and are fully suppressed in the face of hypoglycemia (intra-islet hypoinsulinemia). In this study, we assessed the glucagon and catecholamine counterregulatory responses in a heterogeneous group of children with CHI and compared them with the responses of a group of children with nonhyperinsulinemic hypoglycemia. We retrospectively reviewed the serum glucagon and catecholamine counterregulatory responses of children with different forms of CHI (diffuse, focal, and diazoxide-responsive forms) and compared these with the responses of children with nonhyperinsulinemic hypoglycemia. Within the CHI group, we were able to compare the effect of physiological intra-islet hyperinsulinemia (in patients with diffuse disease) and intra-islet hypoinsulinemia (patients with focal disease) on glucagon release.

RESEARCH DESIGN AND METHODS

The glucagon and catecholamine responses of three groups of patients with CHI were reviewed retrospectively. The first group consisted of 10 consecutive children presenting with typical diffuse disease on histology. The second group consisted of five consecutive patients presenting with focal disease, which was confirmed histologically. The third group consisted of five patients with diazoxide-responsive CHI. The study was approved by the Ethics Committee of Great Ormond Street Children's Hospital and the Institute of Child Health; written informed consent was obtained from the parents or guardians.

Each of these patients was referred to a tertiary referral center (Great Ormond Street Children's Hospital NHS Trust, London, U.K.) for investigation of their hypoglycemia. None of the patients was hypoglycemic 48 h before the beginning of the study. Normoglycemia (4–8 mmol/l) was maintained in the CHI group by continuous intravenous infusion of dextrose using central venous catheters. The diagnostic work-up for the hypoglycemia involved carrying out a controlled fast that implicated stopping all enteral feeds and intravenous fluids. Blood glucose concentrations were measured hourly during the fast and more frequently if the patient was becoming hypoglycemic. Blood glucose was monitored at 15-min intervals in patients thought to have CHI and requiring high rates of intravenous dextrose infusion.

The fast was completed when the plasma glucose concentration fell to 2.6 mmol/l or if the child became symptomatic. At the time of hypoglycemia, each child had blood withdrawn for measurement of serum insulin, glucagon, epinephrine, norepinephrine, plasma nonesterified fatty acids (NEFAs), ketone bodies (aceto-acetate and 3-β-hydroxybutyrate), and serum ammonia through an indwelling intravenous catheter. The hypoglycemic event was then treated with either intravenous fluids (1 ml/kg 10% dextrose bolus) or enteral feeds if the child tolerated these. The measurements of serum insulin, glucagon, epinephrine, and norepinephrine are routine investigations performed in all children presenting with unexplained persistent hypoglycemia. The blood sample for measurement of serum glucagon was collected into a Trasylol tube and transported to the laboratory immediately. The diagnosis of CHI was made biochemically by the typical finding of hyperinsulinemic hypoglycemia with hypofattyacidemia and hypoketonemia and a raised glucose infusion rate required to maintain normoglycemia (29). Serum glucagon and catecholamine measurements were made before pancreatectomy in all CHI patients. Table 1 shows the characteristics of the patient group.

For the control group, the plasma glucagon and catecholamine responses were measured in a group of children presenting with nonhyperinsulinemic hypoglycemia. This included 15 children: 12 presenting with ketotic hypoglycemia, 2 with a disorder of fatty acid metabolism (medium-chain acyl-Co dehydrogenase), and 1 with glycogen storage disease type 1A. These children underwent the routine investigations described above. The diagnosis of "ketotic" hypoglycemia was made by excluding any of the other recognized causes of hypoglycemia and by the typical biochemical profile of raised NEFA concentration and appropriate ketone body formation. No other biochemical abnormality was noted in these patients. The diagnosis of medium-chain acyl-Co dehydrogenase deficiency was made by the finding of an abnormal NEFA–to–ketone body ratio, an elevated C8-acylcarnitine concentration, and a urinary organic acid profile showing abnormal excretion of the C6-C10 dicarboxylic acids (adipic-suberic-sebacic). Table 2 shows the characteristics of the control group.

Hormone assays. Serum insulin was measured using the Immulite immunometric assay with the Immulite analyzer. The IMMULITE Automated Immunoassay Analyzer is a continuous random access instrument that performs automated chemiluminescent immunoassays. Serum glucagon was measured by an established radioimmunoassay method at Hammersmith Hospital (London, U.K.) as previously described (30). This is a highly specific radioimmunoassay for serum glucagon with <0.01% reactivity for enteroglucagon.

Norepinephrine and epinephrine were measured after extraction from plasma using an alumina extraction procedure, then separated, and quantified by liquid chromatography with electrochemical detection (31). The interassay coefficients of variation within and above the normal range were 10.4–18.5% and 3.6–6.5% for epinephrine and 3.0–4.3% and 1.8–4.6% for norepinephrine, respectively.

Measurement of intermediary metabolites. The plasma concentration of NEFA and ketone bodies was determined using the Cobras Mira Plus Analyzer (Roche, Indianapolis, IN). The plasma glucose concentration was measured

TABLE 1
Clinical features, mutational analysis, and outcome of patients with CHI

Patient no.	Sex	Histological subtype	Age at investigation (months)	Genetics (ABCC8/KCNJ11)	Pancreatectomy
1	M	Diffuse-diazoxide unresponsive	1	No mutation	Near total
2	F	Diffuse-diazoxide unresponsive	2	No mutation	Near total
3	F	Diffuse-diazoxide unresponsive	1	No mutation	Near total
4	M	Diffuse-diazoxide unresponsive	1.5	Homozygous ABCC8 (D1193V)	Near total
5	F	Diffuse-diazoxide unresponsive	1	Homozygous ABCC8 (G111R)	Near total
6	M	Diffuse-diazoxide unresponsive	3	No mutation	Near total
7	F	Diffuse-diazoxide unresponsive	1	Homozygous ABCC8 (R1436Q)	Near total
8	M	Diffuse-diazoxide unresponsive	2	No mutation	Near total
9	M	Diffuse-diazoxide unresponsive	1	No mutation	Near total
10	F	Diffuse-diazoxide unresponsive	1	Homozygous ABCC8 (G1468T)	Near total
11	M	Diazoxide responsive	6	No mutation	Not applicable
12	M	Diazoxide responsive	8	No mutation	Not applicable
13	F	Diazoxide responsive	3	No mutation	Not applicable
14	M	Diazoxide responsive	5	No mutation	Not applicable
15	F	Diazoxide responsive	4	No mutation	Not applicable
16	M	Focal-diazoxide unresponsive	2	Paternal ABCC8 (exon 5, G228D)	Partial
17	F	Focal-diazoxide unresponsive	2	Paternal ABCC8 (exon 7, A355T)	Partial
18	F	Focal-diazoxide unresponsive	3	No mutation	Partial
19	M	Focal-diazoxide unresponsive	1	Paternal ABCC8 (exon 3, A113V)	Partial
20	F	Focal-diazoxide unresponsive	2	No mutation	Partial

M, male; F, female.

using the standard glucose oxidase method on a Kodak Vitrous 750 instrument.

Genetic. All exons and flanking introns of the *ABCC8*/SUR1 gene and the entire *KCNJ11*/K_{IR}6.2 open reading frame were subjected to PCR amplification and tested for small deletions, insertions, or point mutations using denaturing high-performance liquid chromatography (Wave 3500; Transgenomic). Sequencing and analysis were performed using kits provided by Amersham Biosciences.

Statistical methods. The results are expressed as mean serum hormone concentrations (±1 SD), and significant tests were performed using the Student's *t* test.

RESULTS

The counterregulatory hormones (insulin, glucagon, epinephrine, and norepinephrine) and intermediary metabolite results in the patient group with CHI are shown in Table 3, whereas Table 4 shows the counterregulatory hormonal responses in children with nonhyperinsulinemic hypoglycemia. Consistent with the clinical diagnoses, the patient group shows excess insulin release for the degree of hypoglycemia when compared with the control group. The serum insulin concentration was undetectable in all children with nonhyperinsulinemic hypoglycemia, whereas an increase in serum insulin was present in all patients with CHI, with an average value of 107.9 ± 104.8 pmol/l.

TABLE 2
Clinical features and causes of hypoglycaemia in patients with nonhyperinsulinemic hypoglycemia

Patient no.	Sex	Age at investigation (months)	Diagnosis
1	F	24	Ketotic hypoglycemia
2	F	19	Ketotic hypoglycemia
3	M	18	Ketotic hypoglycemia
4	M	15	Ketotic hypoglycemia
5	M	21	Ketotic hypoglycemia
6	F	16	Ketotic hypoglycemia
7	M	19	Ketotic hypoglycemia
8	M	3	GSD type 1A
9	F	19	Ketotic hypoglycemia
10	M	4	MCAD
11	F	4	MCAD
12	F	18	Ketotic hypoglycemia
13	M	17	Ketotic hypoglycemia
14	F	16	Ketotic hypoglycemia
15	M	17	Ketotic hypoglycemia

M, male; F, female; MCAD, medium-chain acyl-Co dehydrogenase; GSD, glycogen storage disease.

In the patient group, the mean serum glucagon value at the time of hyperinsulinemic hypoglycemia was 17.6 ± 5.7 ng/l. In comparison, the mean serum glucagon value in the control group with nonhyperinsulinemic hypoglycemia was 59.4 ± 7.8 ng/l. There was a significant difference ($P < 0.01$) between the mean serum glucagon response in children with nonhyperinsulinemic hypoglycemia and those with CHI. Serum insulin concentrations were undetectable in all children with nonhyperinsulinemic hypoglycemia versus an increase in all patients with CHI. There was no correlation ($r = 0.02$) between the serum insulin and serum glucagon concentrations in the CHI group.

The degree of hypoglycemia achieved was comparable in the two groups, with plasma glucose values between 1.7 and 2.8 mmol/l in the nonhyperinsulinemic group and between 1.6 and 2.6 mmol/l in the CHI group. The two groups were different with respect to the time taken to develop hypoglycemia during the controlled fast. In the CHI group, the mean time taken to become hypoglycemic was 60 min (range 15–240), whereas in the nonhyperinsulinemic group, the mean time was 360 min (120–600).

As shown in Table 4, the mean serum epinephrine and norepinephrine concentrations in the CHI group were 2,779 ± 431 pmol/l and 2.9 ± 0.7 nmol/l, respectively. In comparison, the mean serum epinephrine and norepinephrine concentrations in the nonhyperinsulinemic group were 2,800 ± 446 and 2.7 ± 0.6 nmol/l, respectively. There were no significant differences between the mean serum

TABLE 3
Serum glucagon and catecholamine counterregulatory hormonal responses in patients with CHI

Patient no.	Glucose (mmol/l)	Insulin (pmol/l)	Glucagon (ng/l)	Epinephrine (pmol/l)	Norepinephrine (nmol/l)	NEFAs (mmol/l)	Ketone bodies (mmol/l)
1	1.6	96	19.6	2,980	2.6	<0.05	<0.05
2	1.7	61.2	17	2,160	2.5	<0.05	<0.05
3	1.6	140	19	2,480	2.8	0.09	<0.05
4	2.0	54	23	3,650	2.9	<0.05	<0.05
5	2.5	112.2	13	2,800	2.8	0.08	<0.05
6	2.2	103.2	16	2,500	3.2	<0.05	<0.05
7	2.1	156	11	2,360	2.5	<0.05	<0.05
8	1.2	532.2	19	2,300	2.5	<0.05	<0.05
9	2.0	67.8	12.9	3,700	5.7	0.12	<0.05
10	1.6	102	12	2,450	3.6	<0.05	<0.05
11	2.1	97.8	34	2,630	2.6	<0.05	<0.05
12	2.6	72.6	16	3,250	2.9	0.12	0.09
13	1.9	87.6	26	2,580	2.4	0.14	0.08
14	2.0	35.4	13	2,650	2.5	0.21	0.10
15	1.6	41.4	20	3,120	2.1	0.18	0.10
16	2.3	75.6	13	3,260	2.9	0.23	0.10
17	1.5	57.6	13	2,680	2.6	0.15	0.09
18	2.1	53.4	22	2,580	2.5	0.90	<0.05
19	1.8	91.8	13	2,980	3.2	<0.05	<0.05
20	1.6	121.8	14	2,480	2.5	0.26	0.15
Mean ± SD		107.9 ± 104.8	17.6 ± 5.7	2,779 ± 431	2.9 ± 0.7		

epinephrine and norepinephrine concentrations between the two groups.

The serum glucagon responses in the CHI were analyzed further according to the histological subtype of CHI. The mean serum glucagon concentrations in children with diffuse, focal, and diazoxide-responsive forms of hyperinsulinism were 16.3 ± 3.9, 15 ± 3.9, and 21.8 ± 8.4 ng/l, respectively. There was no significant difference between these groups, although the number of children within the focal and diazoxide-responsive groups was small.

Homozygous mutations in the *ABCC8* were found in 4 of 12 patients with diffuse disease, and paternal *ABCC8* gene mutations were found in 3 of 5 patients with histologically proven focal disease. No mutations were found in *ABCC8* and *KCNJ11* genes in the diazoxide-responsive forms of CHI.

DISCUSSION

This study shows that serum glucagon counterregulatory hormonal responses are blunted in diffuse, focal, and diazoxide-responsive forms of CHI when compared with children who have the same degree of hypoglycemia due to nonhyperinsulinemic causes. Interestingly, there were

TABLE 4
Serum glucagon and catecholamine responses in patients with nonhyperinsulinemic hypoglycemia

Patient no.	Glucose (mmol/l)	Insulin (pmol/l)	Glucagon (ng/l)	Epinephrine (pmol/l)	Norepinephrine (nmol/l)	NEFAs (mmol/l)	Ketone bodies (mmol/l)
1	2.4	<1	54	3,000	2.9	2.5	1.7
2	2.6	<1	60	2,600	2.3	2.4	3.2
3	2.7	<1	55	2,700	3.1	2.3	3.5
4	1.9	<1	47	3,600	3.2	2.0	2.1
5	2.8	<1	63	2,900	2.6	2.2	3.3
6	1.9	<1	64	2,700	3.6	2.3	3.1
7	2	<1	54	2,500	2.9	3.1	2.9
8	2.5	<1	59	2,500	2.8	2.6	2.0
9	2.3	<1	65	3,600	3.9	2.4	3.1
10	2.1	<1	63	2,200	2.6	3.5	0.5
11	1.9	<1	79	2,800	2.9	3.2	0.7
12	2.1	<1	48	2,900	2.6	2.6	1.9
13	1.8	<1	59	2,500	2.8	2.9	2.1
14	1.9	<1	56	2,800	2.4	3.7	2.9
15	1.7	<1	65	2,700	2.6	2.6	1.9
Mean ± SD			59.4 ± 7.8	2,800 ± 446	2.7 ± 0.6		

Normal values: plasma glucose, 3.5–5.5 mmol/l; serum insulin, <6 pmol/l at the time of hypoglycemia; serum glucagon, up to 50 ng/l [during normoglycemia in adults (30,43)]; serum epinephrine, 100–800 pmol/l [during normoglycemia (44,45)]; serum norepinephrine, 0.5–3.5 nmol/l [during normoglycemia (44,45)]; NEFAs, value depends on the degree of fasting, plasma glucose, and serum insulin levels (<0.05 means undetectable). Should increase in response to hypoglycemia. Ketone bodies, value depends on the degree of fasting, plasma glucose, and serum insulin levels (<0.05 means undetectable). Should increase in response to hypoglycemia.

no differences in the serum glucagon counterregulatory response between children with diffuse, focal, or diazoxide-responsive forms of CHI. The epinephrine and norepinephrine counterregulatory responses to hypoglycemia were appropriate in both the CHI and nonhyperinsulinemic groups.

Diffuse CHI is characterized by the inappropriate and excessive insulin secretion from all of the pancreatic β-cells reflecting elevated intra-islet insulin levels/Zn^{2+} levels. In focal CHI, excess insulin secretion is confined to a discrete focal lesion. In the rest of the pancreas, however, the β-cells are functionally normal and are fully suppressed in the face of hypoglycemia. Thus, most of the islets of patients with focal CHI would be expected to have low intra-islet insulin levels/Zn^{2+} levels and hence should have raised serum glucagon levels in response to hypoglycemia. The fact that children with focal CHI also have blunted serum glucagon responses suggests that intra-islet hyperinsulinemia cannot be the only mechanism leading to a reduced glucagon response to hyperinsulinemic hypoglycemia, as suggested in rodent models (22–25) and in healthy humans (26). Intra-islet insulin levels have not been measured directly in children with CHI, but transhepatic pancreatic portal venous sampling has been used in an attempt to differentiate focal from diffuse disease (32,33). In diffuse disease, portal venous sampling shows elevated insulin levels from all of the venules draining the pancreas, whereas in focal disease, insulin levels are only elevated in the venules draining the focal lesion (32,33).

Recurrent recent antecedent hypoglycemia is unlikely to lead to the blunting of the glucagon response because the CHI patients were not hypoglycemic 48 h before the induction of the controlled hypoglycemia. The normal serum epinephrine/norepinephrine levels argue strongly against recurrent recent antecedent hypoglycemia as the cause of the low serum glucagon levels. The level of hypoglycemia achieved was comparable in the hyperinsulinemic and nonhyperinsulinemic groups, hence that is unlikely to account for the different glucagon responses in the two groups. The children in the nonhyperinsulinemic group were older than children in the hyperinsulinemic group, this reflecting the fact that ketotic hypoglycemia is observed in children after the age of ~15 months (34). Again, the appropriate serum epinephrine/norepinephrine levels observed in the two groups strongly argues against age as a determinant of the serum glucagon responses to hypoglycemia.

Patients with CHI have continuously unregulated insulin secretion and hence have persistently elevated serum insulin levels in relation to the blood glucose concentration (29). Arterial and portal vein hyperinsulinemia have been shown to decrease plasma glucagon levels, and high circulating insulin levels are known to have a direct inhibitory effect on the α-cell (35,36). Prolonged hyperinsulinemia results in a selective blunting of the plasma glucagon response to hypoglycemia, perhaps because of a direct suppressive effect of insulin on α-cell secretion (37). Hence, it is possible that the serum glucagon response is blunted in all CHI patients because of the continued exposure of the α-cell to unregulated insulin secretion.

Appropriate epinephrine and norepinephrine counterregulatory responses to hypoglycemia were observed in both the CHI and nonhyperinsulinemic groups. This result was surprising because chemical injury of areas of the brain show that the VMH area has a role in the release of catecholamines in response to hypoglycemia (38), K_{ATP}-containing glucose-sensing neurons have been implicated in the catecholamine response (15), and intracerebral ventricular injections of sulfonylureas (glibenclamide or tolbutamide) have been reported to suppress the epinephrine counterregulatory responses to brain glucopenia and/or systemic hypoglycemia (39). On the other hand, other centers have been implicated in K_{ATP}-independent glucose sensing and control of catecholamine release in response to hypoglycemia (40,41). None of our patients had a mutation in the *KCNJ11*/$K_{IR}6.2$ gene; thus, we are unable to assess whether a mutation of the $K_{IR}6.2$ subunit will impair glucose sensing independently of SUR1 as observed in $K_{IR}6.2$-null mice (15).

Patients with CHI can have extremely severe hypoglycemia with glucose infusion rates of 20 $mg \cdot kg^{-1} \cdot min^{-1}$, (with an average of 4–6 $mg \cdot kg^{-1} \cdot min^{-1}$), but there is no correlation between serum insulin levels and the severity of hypoglycemia (29). Our findings indicate that CHI patients are relatively glucagon deficient. Glucagon has been used in the diagnosis and management of severe forms of CHI (42). Subcutaneous infusion of glucagon produces higher serum levels than those achieved by endogenous secretion (K.H., unpublished data) and generates an increase in blood glucose concentration in CHI patients presumably by increasing glycogenolysis.

In summary, this study has shown for the first time that children with CHI display blunted serum glucagon counterregulation with normal epinephrine and norepinephrine responses. The precise mechanism of this blunted glucagon response is not clear but may be related to prolonged hyperinsulinemia, causing a selective blunting of the plasma glucagon response to hypoglycemia, intra-islet hyperinsulinemia, or defects in α-/β-cell communication secondary to loss of SUR1/$K_{IR}6.2$ -type K_{ATP} channels at least in those patients with diffuse disease. CHI is therefore associated not only with unregulated insulin secretion but also with impaired glucagon counterregulation. Thus, relative deficiency of serum glucagon counterregulation is another factor that predisposes these children to severe hypoglycemia.

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