**Dysregulation of Insulin Secretion in Children With Congenital Hyperinsulinism due to Sulfonlurea Receptor Mutations**


Mutations in the high-affinity sulfonlurea receptor (SUR)-1 cause one of the severe recessively inherited diffuse forms of congenital hyperinsulinism or, when associated with loss of heterozygosity, focal adenomatosi. We hypothesized that SUR1 mutations would render the β-cell insensitive to sulfonlureas and to glucose. Stimulated insulin responses were compared among eight patients with diffuse hyperinsulinism (two mutations), six carrier parents, and ten normal adults. In the patients with diffuse hyperinsulinism, the acute insulin response to intravenous tolbutamide was absent and did not overlap with the responses seen in either adult group. There was positive, albeit significantly blunted, acute insulin response to intravenous dextrose in the patients with diffuse hyperinsulinism. Graded infusions of glucose, to raise and then lower plasma glucose concentrations over 4 h, caused similar rises in blood glucose but lower peak insulin levels in the hyperinsulinemic patients. Loss of acute insulin response to tolbutamide can identify children with diffuse SUR1 defects. The greater response to glucose than to tolbutamide indicates that ATP-sensitive potassium (KATP) channel–independent pathways are involved in glucose-mediated insulin release in patients with diffuse SUR1 defects. The diminished glucose responsiveness suggests that SUR1 mutations and lack of KATP channel activity may contribute to the late development of diabetes in patients with hyperinsulinism independently of subtotal pancreatectomy. *Diabetes* 50:322–328, 2001

Several distinct forms of congenital hyperinsulinism have been identified in recent years (1). Sporadic nongenetic cases of transient hyperinsulinism can be associated with maternal diabetes and with perinatal stresses such as birth asphyxia or small-for-dates birth weight (2). Dominant genetic forms include activating glucokinase mutations that lower the glucose threshold for insulin release (3), gain-of-function mutations in glutamate dehydrogenase that cause both hyperinsulinism and hyperammonemia (4), and other types whose genetic bases have not yet been identified (5,6). However, mutations in the ATP-sensitive potassium (KATP) channel complex of the pancreatic β-cell plasma membrane cause some of the most severe clinical disease (7–11). Encoded by two adjacent genes on chromosome 11p, the sulfonlurea receptor (SUR)-1 regulates the channel activity, whereas the inwardly rectifying potassium channel (Kir6.2) constitutes the ion pore (12,13). Patients with KATP channel mutations who present the severe form of the disease are clinically diazoxide unresponsive, and many require 95% subtotal pancreatectomy to prevent recurrent hypoglycemia. They also exhibit a high risk of later developing diabetes, which is often attributed to their surgical treatment (14–16). SUR1 and Kir6.2 mutations can be expressed in two ways: autosomal recessive inheritance of two abnormal SUR1 or Kir6.2 alleles results in diffuse hyperinsulinism (formerly called nesidioblastosis), whereas inheritance of an abnormal paternal SUR1 allele with somatic loss of the maternal chromosome 11p15 leads to focal adenomatosi (17–19).

The KATP channel complex transduces the metabolic status of the β-cell into cell membrane electrical activity and thereby links insulin release with metabolic demands. SUR1, a member of the ATP-binding cassette superfamily, forms a hetero-octamer with Kir6.2 (20,21). Glucose entry and metabolism increase the ratio of ATP to ADP within the β-cell. At very high concentrations, ATP binding to Kir6.2 inhibits the channel activity, whereas magnesium nucleotides can antagonize this inhibition through interactions with the nucleotide binding folds of the SUR1 (22). The increased ATP-to-ADP ratio leads to closure of the KATP channel and hence depolarization of the β-cell membrane. The depolarization opens voltage-gated calcium channels, and the resultant elevation of the intracellular calcium concentration triggers exocytosis of insulin granules (23,24). Sulfonlureas modulate insulin secretion by binding to...
SURI; some, like tolbutamide, stimulate insulin secretion, whereas others, like diazoxide, inhibit it.

The present study was undertaken to test the hypothesis that mutations in SURI, the metabolic transducer, would render the β-cell unresponsive to sulfonylureas and insensitive to high or rising glucose levels as well as to falling glucose levels. Insulin responses to tolbutamide, a sulfonylurea that stimulates insulin release, and to both acute and prolonged graded glucose stimulation were measured in children with diffuse SURI−/− hyperinsulinism. The effects of heterozygous SURI mutations were investigated by evaluating insulin secretion in the heterozygous parents of these children.

**RESEARCH DESIGN AND METHODS**

**Subject characteristics.** The clinical characteristics of the eight children with diffuse SURI−/− hyperinsulinism who were studied are shown in Table 1. They were aged 2–20 years. Patients 1 and 2 were sisters, and patients 5, 6, and 8 were brothers. All eight children were unresponsive to treatment with diazoxide, a drug that inhibits insulin secretion by opening the potassium channel through its effect on SURI. Five of the children had undergone subtotal pancreatectomy for <12 h. The other three had similarly severe hypoglycemia and were found SURI mutations in the Ashkenazi Jewish population (25,26); four of the children studied were homozygous for DelF 1388 and 3992→g→a, and four were compound heterozygous DelF 1388/3992→g→a.

<table>
<thead>
<tr>
<th>SURI genotype</th>
<th>Patient number</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Age at presentation</th>
<th>Age at pancreatectomy</th>
<th>Current treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>DelF 1388/3992→g→a</td>
<td>1</td>
<td>F</td>
<td>20</td>
<td>4 h</td>
<td>2 months</td>
<td>Frequent feedings</td>
</tr>
<tr>
<td>DelF 1388/3992→g→a</td>
<td>2</td>
<td>F</td>
<td>14</td>
<td>Newborn</td>
<td>8 days</td>
<td>None</td>
</tr>
<tr>
<td>DelF 1388/3992→g→a</td>
<td>3</td>
<td>F</td>
<td>13</td>
<td>11 months</td>
<td>ND*</td>
<td>None since age 8 years</td>
</tr>
<tr>
<td>DelF 1388/3992→g→a</td>
<td>4</td>
<td>M</td>
<td>2</td>
<td>3 days</td>
<td>1, 2, 30 months*</td>
<td>Glucagon-octreotide infusion</td>
</tr>
<tr>
<td>3992→g→a/3992→g→a</td>
<td>5</td>
<td>M</td>
<td>13</td>
<td>10 days</td>
<td>ND</td>
<td>Frequent feedings</td>
</tr>
<tr>
<td>3992→g→a/3992→g→a</td>
<td>6</td>
<td>M</td>
<td>11</td>
<td>2 days</td>
<td>ND</td>
<td>Frequent gastrostomy feedings</td>
</tr>
<tr>
<td>3992→g→a/3992→g→a</td>
<td>7</td>
<td>F</td>
<td>6</td>
<td>1–2 days</td>
<td>1 month</td>
<td>None</td>
</tr>
<tr>
<td>3992→g→a/3992→g→a</td>
<td>8</td>
<td>M</td>
<td>4</td>
<td>At birth</td>
<td>1 month</td>
<td>None</td>
</tr>
</tbody>
</table>

*Surgery done after the time of the study; ND, no surgery.

**RESULTS**

**AIRs.** The AIRs to glucose and tolbutamide in a normal adult and those in child number 6 with SURI−/− hyperinsulinism are shown in Fig. 1A and B, respectively. The normal adult responded briskly to both stimuli, with AIRs of similar magnitude (45 µU/ml to glucose and 34 µU/ml to tolbutamide). In contrast, the SURI−/− child had a smaller AIR to glucose (31 µU/ml), despite an even greater elevation in blood glucose concentration (345 vs. 290 mg/dl in the normal control), and no AIR to tolbutamide (4 µU/ml).

Table 2 compares the results of the AIR tests in the group of eight children with diffuse SURI−/− hyperinsulinism, their carrier parents, and normal adults. Baseline insulin levels in the SURI−/− group were comparable to those in the normal adults. AIR to tolbutamide was absent in the children with diffuse hyperinsulinism (P < 0.005). The 95% CI of the AIR to tolbutamide in the diffuse hyperinsulinemic patients (−2.3 to 3.7 µU/ml) did not overlap with the normal control subjects (26.4–79.7 µU/ml). Glucose stimulation provoked a positive but smaller AIR in the diffuse hyperinsulinemic patients (P < 0.05). The glucose disposal rates among the SURI−/− children and the normal adult groups were similar. Mean insulin responses to the two AIR tests did not differ among the four children with homozygous 3992→g→a mutations and the four children with compound heterozygous DelF 1388/3992→g→a mutations (AIR to glucose 333 vs. 335 µU/ml and AIR to tolbutamide 149 vs. 157 µU/ml).
Insulin dysregulation in congenital hyperinsulinism

As shown in Table 2, AIRs to both glucose and tolbutamide were similar in the SUR1+/– carriers and the normal adults. The 95% CI of the AIR to tolbutamide in the diffuse hyperinsulinemic patients did not overlap with that of the carriers (9.7–52.3 µU/ml). The glucose disposal rates of the carriers were also similar to those of the normal adults.

**Insulin response to graded glucose infusion.** Graded glucose infusion studies were performed in three children with SUR1–/– diffuse hyperinsulinism who had not undergone subtotal pancreatectomy. Surgery-naive patients were chosen for this study to eliminate subtotal pancreatectomy as a potential confounding variable that could cause impaired glucose responsiveness. Figure 2 shows the blood glucose and plasma insulin concentrations in the graded glucose infusion studies of the same SUR1–/– child (number 6) and normal adult control as in Fig. 1. In the normal adult (Fig. 2A), the rise and subsequent fall in blood glucose was associated with a parallel, though slightly delayed, rise and fall in insulin concentration (peak 87 µU/ml). As shown in Fig. 2B, child number 6 with SUR1–/– hyperinsulinism had a similar rise and fall in blood glucose but achieved a peak insulin concentration of only 29 µU/ml despite a higher peak blood glucose concentration. Figure 3 shows the insulin concentrations plotted against blood glucose concentrations from the studies of Fig. 2. The slope of the up curve was steeper in the normal adult (37 µU · mg⁻¹ · 10⁻²) than in the child with SUR1–/– hyperinsulinism (4 µU · mg⁻¹ · 10⁻³), indicating diminished sensitivity to glucose for insulin secretion in the patient.

Table 3 summarizes the results of the graded glucose infusion studies in the three children with SUR1–/– diffuse hyperinsulinism, five SUR1+/– carriers, and four normal adults. Because there was no difference between the carriers and normal control subjects on either AIR or on any of the graded glucose infusion parameters assessed, the two adult groups were combined for data analysis. Peak blood glucose concentration in the SUR1–/– children (298 ± 50 mg/dl) was equivalent to that in the adults. The peak plasma insulin concentrations in the SUR1–/– children overlapped the lower end of values seen in the adults, but the mean insulin concentration was significantly less in the former group (P < 0.05). Glucose sensitivity

**FIG. 1.** AIRs to glucose and tolbutamide in children with diffuse SUR1–/– hyperinsulinism. A: Normal adult control. B: Patient 6 with diffuse SUR1–/– hyperinsulinism.

**TABLE 2**

<table>
<thead>
<tr>
<th>Subject group</th>
<th>n</th>
<th>Baseline plasma insulin concentration (µU/ml)</th>
<th>Peak blood glucose concentration (mg/dl)</th>
<th>AIR to glucose (µU/ml)</th>
<th>Glucose disposal rate (%/min)</th>
<th>AIR to tolbutamide (µU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffuse SUR1–/–</td>
<td>8</td>
<td>11 ± 2 (NS*)</td>
<td>293 ± 17 (NS)</td>
<td>14 ± 4 (&lt;0.05)</td>
<td>2 ± 0.3 (NS)</td>
<td>0.7 ± 1 (&lt;0.005)</td>
</tr>
<tr>
<td>hyperinsulinism</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SUR1+/– carriers</td>
<td>6</td>
<td>10 ± 2 (NS)</td>
<td>198 ± 12 (NS)</td>
<td>37 ± 13 (NS)</td>
<td>2 ± 0.2 (NS)</td>
<td>31 ± 8 (NS)</td>
</tr>
<tr>
<td>Normal adults</td>
<td>10</td>
<td>8 ± 1</td>
<td>262 ± 16</td>
<td>42 ± 9</td>
<td>2 ± 0.5</td>
<td>53 ± 12</td>
</tr>
</tbody>
</table>

Data are means ± SE (P) or means ± SE. *Significance values are presented for the difference between each group and the normal adults.
in the SUR1−/− children was also less than that in the adults (P < 0.01). There was no insulin displacement at 150 mg/dl glucose in the children with SUR1−/− hyperinsulinism. When insulin levels were plotted against blood glucose concentrations, the control subjects showed a hysteresis loop that resulted in a greater slope for the down curve compared with the up curve (124 ± 43 vs. 57 ± 13 µU · mg−1 · 10−2, P < 0.05 by a paired non-parametric test). The SUR1−/− children had loss of the hysteresis loop, with down slopes that were similar to the up slopes (11 ± 3 vs. 4 ± 2 µU · mg−1 · 10−2; NS).

DISCUSSION

The results of these studies show that children with SUR1−/− hyperinsulinism do not respond to the insulin secretagogue, tolbutamide, and have a diminished, although positive, AIR to intravenous glucose compared with control subjects. AIR testing did not differentiate children with diffuse SUR1−/− hyperinsulinism homozygous for the 3992–9g→a mutation from those compound heterozygous for DelF 1388/3992–9g→a mutations. During graded glucose infusion studies, children with diffuse SUR1−/− hyperinsulinism who had never undergone subtotal pancreatectomy exhibited reduced β-cell glucose sensitivity. Thus, SUR1−/− hyperinsulinism causes blunted but not absent glucose responsiveness. The abnormalities found on AIRs and graded glucose infusion studies of these patients were not age related because the SUR1−/− children were as old as 20 years and the adults as young as 19 years.

Patients with SUR1−/− hyperinsulinism had no AIR to tolbutamide. A previous report by Ehrlich and Martin (27) found excessive insulin release in response to intravenous tolbutamide in 15 children with idiopathic hypoglycemia. The earlier study, however, measured glucose and insulin responses from 15 to 120 min after tolbutamide and did not assess the immediate response. Furthermore, the cause(s) of hypoglycemia in these children was not known and likely represented a heterogeneous group of disorders (not necessarily diffuse SUR1−/− hyperinsulinism). Intravenous tolbutamide tests have also been used in the assessment of patients with insulinomas, again looking only at delayed (120–180 min) responses (28).
The loss of AIR to tolbutamide in children with SUR1<sup>−/−</sup> hyperinsulinism is not surprising given the fact that tolbutamide binds to SUR1 and these children have a lack of K<sub>ATP</sub> channel activity due to defective SUR1 proteins (29). Sharma et al. (30) have reported that the truncated and misfolded mutant SUR1 proteins do not transit to the plasma membrane. Similarly, these children are clinically unresponsive to treatment with diazoxide, which inhibits insulin secretion by binding to SUR1. Loss of AIR to tolbutamide can therefore serve as a diagnostic marker for children who will not benefit from diazoxide therapy, although it is remotely possible that an SUR1 mutation different from those studied here may allow the molecule to respond to one or the other ligand. Patients with mutations in Kir6.2 would be expected to have the same responses as those with SUR1 mutations, since both components of the K<sub>ATP</sub> channel are required for normal channel activity.

The complete loss of AIR to tolbutamide but only partial blunting of AIR to glucose provides evidence that K<sub>ATP</sub> channel–independent pathways are involved in glucose-mediated insulin secretion in children with SUR1<sup>−/−</sup> hyperinsulinism. The existence of K<sub>ATP</sub> channel–independent pathways has been suggested by studies of islets from rats and mice (31–33) and was recently demonstrated in human islets in vitro (34). The precise K<sub>ATP</sub> channel–independent pathways are still unclear (35). Proposed mechanisms include glucose-mediated elevations in intracellular calcium concentrations via mobilization of calcium sequestered in the endoplasmic reticulum (36–38). Intracellular calcium release may involve inositol-1,4,5-triphosphate (39) or the calcium release–activated nonselective cation channel (i<sub>i,CRAN</sub>) (40,41). Furthermore, a non–calcium-dependent mechanism involving protein kinases A and C, ATP, and GTP has been suggested (42).

The reduced AIR to glucose and the diminished glucose sensitivity on graded glucose infusion studies suggest that SUR1<sup>−/−</sup> β-cells are less glucose responsive. Additionally, the insulin displacement at 150 mg/dl glucose is lost in children with SUR1<sup>−/−</sup> hyperinsulinism. Both factors may lead to postprandial hyperglycemia in children with SUR1<sup>−/−</sup> hyperinsulinism. These findings corroborate the clinical observation that hyperinsulinemic patients can exhibit episodes of both hypoglycemia and hyperglycemia, sometimes even in the same day. Recent studies in SUR1<sup>−/−</sup> knockout (and Kir6.2 knockout) mice did not have the severe hypoglycemia seen in the human disease but demonstrated a loss of first-phase and attenuated second-phase glucose-stimulated insulin secretion, consistent with the impairments in the AIR to glucose and glucose sensitivity observed in the present studies (43). Insulin secretory defects on intravenous glucose tolerance testing and graded glucose infusions have been reported in different types of maturity-onset diabetes of the young (MODY) and autosomal-dominantly inherited forms of diabetes caused by non-SUR1 single-gene mutations (44–46). In one MODY family studied, reductions in insulin secretory oscillations during prolonged glucose infusion were also detected in genetic marker–positive family members who were not yet diabetic (47). This closely resembles our children with SUR1<sup>−/−</sup> hyperinsulinism who, because of their single gene defect, exhibited abnormal insulin release on graded glucose infusion, but who were not diabetic. The finding of normal glucose disposal rates in the children with diffuse SUR1<sup>−/−</sup> hyperinsulinism suggests that they may have adapted to impaired insulin release by increasing peripheral sensitivity to insulin. The AIR to glucose did not differ between the children with diffuse SUR1<sup>−/−</sup> hyperinsulinism who had and those who had not undergone subtotal pancreatectomy, and the abnormal response to graded glucose infusion was found in our SUR1<sup>−/−</sup> hyperinsulinemic patients without surgical intervention. This finding suggests that SUR1<sup>−/−</sup> β-cells may contribute, independently of subtotal pancreatectomy, to the increased risk of diabetes seen in diffuse SUR1<sup>−/−</sup> hyperinsulinemic patients.

The children with SUR1<sup>−/−</sup> hyperinsulinism had no insulin displacement at 150 mg/dl glucose. It is unclear whether this signifies loss of the normal glucose potentiation of insulin release or loss of the normal lag in the suppression of the insulin secretory response to a falling glucose. It is also possible that the flat glucose sensitivity curve of the patients with diffuse SUR1<sup>−/−</sup> hyperinsulinism made it difficult to detect any glucose potentiation. In any case, the hysteresis loop formed by the difference in insulin levels along the up and down curves in the control subjects was not apparent in the children with SUR1<sup>−/−</sup> hyperinsulinism. Presumably, this observation is due to the loss of K<sub>ATP</sub> channel activity and provides further evidence of the glucose blindness of SUR1<sup>−/−</sup> β-cells. How this effect relates to the glucose potentiation of insulin secretion (48–51) is unknown. Further studies of glucose responsiveness in children with hyperinsulinism are needed to better understand how this phenomenon is altered by SUR1 mutations.

The heterozygous SUR1<sup>+/−</sup> carrier parents demonstrated normal insulin release on both AIRs and graded glucose infusion.

### Table 3

<table>
<thead>
<tr>
<th>Peak blood glucose (mg/dl)</th>
<th>Peak plasma insulin (µU/ml)</th>
<th>Glucose sensitivity (µU · mg⁻¹ · 10⁻²)</th>
<th>Insulin displacement at 150 mg/dl glucose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUR1&lt;sup&gt;−/−&lt;/sup&gt; children</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 3</td>
<td>350</td>
<td>56</td>
<td>0</td>
</tr>
<tr>
<td>Patient 5</td>
<td>198</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>Patient 6</td>
<td>345</td>
<td>51</td>
<td>4</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>298 ± 50 (NS*)</td>
<td>42 ± 12 (P &lt; 0.05)</td>
<td>4 ± 2 (P &lt; 0.01)</td>
</tr>
</tbody>
</table>

Adults (n = 9)

| Mean ± SE | 257 ± 15 | 120 ± 31 | 57 ± 13 | 47 ± 7 |
| 95% CI    | 223–292  | 48–191   | 27–86   | 32–63  |

*Significance of the difference between the mean values of the children and adults by the alternate Welch t test.
sion studies. Thus, performance of AIR testing and graded glucose infusion studies on parents of children with hyperinsulinism cannot serve as a simple clinical way of identifying heterozygous carriers and thereby predicting which families potentially transmit paternal-only or autosomal recessive SUR1 mutations. The normal responses seen in the heterozygous SUR1<sup>+/−</sup> parents also suggest that in nonobese individuals without a history of diabetes, carrying one mutated SUR1 allele does not increase the risk of hypoglycemia or diabetes.

Because patients with the different types of hyperinsulinism follow markedly different clinical courses, including responsiveness to medical therapies such as diazoxide, identifying the type of hyperinsulinism in any given patient allows individualized tailoring of the therapeutic plan for that patient. Furthermore, the diagnosis of diffuse versus focal hyperinsulinism is currently based on histopathologic examination of subtotal pancreatectomy specimens; genetic analysis of the SUR1 mutations is still available only through research laboratories. This distinction is important because local excision in focal hyperinsulinism is far preferable to blind 95% subtotal pancreatectomy; local excision carries a lower risk of complications and is potentially curative. Complete loss of AIR to tolbutamide constitutes a potentially valuable clinical method for preoperatively identifying children with diffuse hyperinsulinism. Because children with focal hyperinsulinism also have a subpopulation of normal β-cells outside their focal lesion, they would be expected to retain an AIR to tolbutamide and have an AIR to glucose intermediate between that seen in the patients with diffuse hyperinsulinism and that seen in normal individuals. Further studies of patients with congenital hyperinsulinism at the time of diagnosis (both diffuse and focal) are needed to confirm the predictive value of the AIR to tolbutamide as a clinical marker for diffuse disease.

In summary, the loss of AIR to tolbutamide may be a useful clinical identifier of children with congenital hyperinsulinism due to diffuse SUR1<sup>−/−</sup> mutations. The reduced glucose responsiveness of SUR1<sup>−/−</sup> also has ramifications for the care of children with congenital hyperinsulinism. The primary glucose-sensing defect in diffuse SUR1<sup>−/−</sup> hyperinsulinism may directly contribute, independently from surgical treatment, to the increased risk of diabetes or impaired glucose tolerance in these patients. Furthermore, investigations of the insulin secretory dynamics in patients with diffuse SUR1<sup>−/−</sup> mutations may shed light on normal β-cell signaling. Our study provides the first in vivo evidence of the involvement of K<sub>ATP</sub> channel–independent pathways in glucose-mediated insulin secretion in humans.

Acknowledgments

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REFERENCES

INSULIN DYSREGULATION IN CONGENITAL HYPERINSULINISM


