Arginine-Induced Insulin Release in Glucokinase-Deficient Subjects

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OBJECTIVE — In eight glucokinase (GCK)-deficient subjects, we have investigated insulin secretion rates (ISRs) in response to intravenous arginine. Impairment in the enzymatic activity of mutant GCK leads to a reduced glycolytic flux in β-cells. This defect translates in vivo as a right shift in the glucose/ISR dose-response curve. Insulin secretion in response to other secretagogues has not been reported.

RESEARCH DESIGN AND METHODS — The arginine test was performed immediately after a 2-h hyperglycemic (10 mM) clamp. ISR was computed by deconvolution of peripheral C-peptide levels. Linear regression analyses were performed to assess correlations between the β-cell secretory responses to the arginine test, an intravenous glucose tolerance test (IVGTT), and a hyperglycemic clamp (areas under the C-peptide curves), and between these parameters and the glucose tolerance status (area under the glucose curve during an oral glucose tolerance test).

RESULTS — Two minutes after the injection of arginine, the increment in ISR was 30.17 ± 10.01 pmol insulin · kg^{-1} · min^{-1} in patients and 36.25 ± 15.46 pmol insulin · kg^{-1} · min^{-1} in control subjects (P = 0.38). Throughout the experiment, increments in ISR were comparable in both groups. The amount of insulin secreted in response to arginine (0–5 min) was similar in patients and control subjects: 81 ± 28 vs. 119 ± 55 pmol/kg (P = 0.16), respectively. The arginine test C-peptide response was not correlated with the IVGTT or hyperglycemic clamp responses. The arginine test and hyperglycemic clamp responses were not correlated to the glucose tolerance status. The best predictor of the glucose tolerance was the C-peptide response to the IVGTT (r^2 = 0.78; P = 0.002).

CONCLUSIONS — β-cell secretory increment in response to arginine was found to be in the normal range in GCK-deficient subjects. The arginine test does not seem to reflect either the β-cell secretory defect or the glucose tolerance status of these subjects. IVGTT seems to be the best predictor of the latter parameter in this population.

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GCK, glucokinase; ISR, insulin secretion rate; IGT, impaired glucose tolerance; IFG, impaired fasting glucose; NIDDM, non-insulin-dependent diabetes mellitus; ANOVA, analysis of variance; IVGTT, intravenous glucose tolerance test; OGTT, oral glucose tolerance test.

Mutations in the coding regions of the glucokinase (GCK) gene on chromosome seven result in a familial form of mild, non-insulin-dependent chronic hyperglycemia with autosomal dominant inheritance and high penetrance (1). GCK is expressed in the liver and pancreatic β-cells where it phosphorylates glucose to glucose-6-phosphate and thus regulates glucose flux (2). In vitro enzymatic activity of mutant GCK varies according to the nature and position of the mutation ranging from no activity at all to a slight decrease in the V_{max} of the enzyme and its affinity for glucose (3,4). Affected individuals show a right shift in vivo in the dose-response curve of glucose-induced insulin secretion (5,6), which correlates with the degree of the enzymatic activity defect observed in vitro (6). Taken together, these findings support the concept that GCK acts as the glucose sensor of pancreatic β-cells and suggest that the primary insulin secretory defect in GCK-deficient diabetes is related to a reduced glucose metabolism in β-cells (7). In view of these results, it would be interesting to evaluate in GCK-deficient subjects, a population presenting a well-defined insulin secretion defect β-cell function in response to secretagogues other than glucose. Here we report plasma insulin and C-peptide profiles and insulin secretion rates (ISRs) in response to intravenous arginine, which is a powerful amino acid stimulus of β-cell secretion. We compared acute intravenous arginine and glucose responses and investigated the correlation of these parameters with the glucose tolerance status and with estimations of the β-cell secretory defect obtained during a hyperglycemic clamp (5).

RESEARCH DESIGN AND METHODS

Metabolic studies were performed in eight patients (six men and two women) from four different kindreds. GCK mutations in these individuals are (1,8) a glutamic acid-300→glutamine (kindred

Table 1—*Clinical profile of GCK-deficient patients*

	Sex	Age (years)	BMI (kg/m^2)	Glucose tolerance status	Age at diagnosis (years)	Treatment
Patient (kindred)						
A (F51)	F	26	22.1	IFG	10	Diet
B (F51)	F	42	24.3	Diabetes	24	Diet
C (F51)	M	38	24.5	IGT	19	Diet
D (F51)	M	47	25.1	IGT	28	Diet
E (F423)	M	42	21.0	Diabetes	22	Sulfonylurea
G (F386)	M	41	21	IGT	3	Sulfonylurea
H (F386)	M	13	17.3	IGT	12	Diet
I (F85)	M	21	21.1	IGT	20	Diet
Patients	—	34 ± 12	22.0 ± 2.6	—	17 ± 8	—
Control group	—	27 ± 3	21.8 ± 1.7	—	—	—

Data are means ± SD. Individuals in the kindred F51 are siblings, except D, who is a distant cousin; in the kindred F386 they are father and son.

F51), a glycine-175→arginine (F386), a 15-base pair deletion at splice donor site of intron 4/exon 4 (F423), and a point mutation of splice acceptor site of intron 6/exon 7 (F85). All kindreds responded to the criteria of maturity-onset diabetes of the young: presence of non-insulin-dependent chronic hyperglycemia in three consecutive generations and at least two patients diagnosed before 25 years of age (9).

Individuals selected for this evaluation presented either diabetes (n = 2) or impaired glucose tolerance (IGT) (n = 5) according to the World Health Organization definition (10) or a fasting plasma glucose between 6.1 and 7.8 mM in two separate measurements with a 2-h post oral glucose load plasma glucose <7.8 mM (n = 1). This criterion of impaired fasting glucose (IFG) represents values of fasting plasma glucose >2 SD above the mean of the normal population (11). Apart from chronic hyperglycemia, patients were in good general health and did not present with late complications of diabetes. Individual profiles are shown in Tables 1 and 2.

Seven lean, healthy individuals (five men and two women) with no diabetic relatives were used as a control group (Table 1). Patients and individuals in the control group gave fully informed consent before taking part in the study.

Evaluations were started between 7:00 and 8:00 A.M. after an overnight fast. A catheter was placed in the left cubital vein for glucose or arginine infusion, and a right arm vein was cannulated for intermittent blood sampling. Subjects remained supine throughout the test.

Arginine-induced insulin secretion is proportionate to the steady-state plasma glucose concentration preceding the stimulation; the magnitude of the acute insulin response is a linear function of plasma glucose between 3.3 and 13.9 mM glucose (12). Thus, to standardize patient and control group responses, plasma glucose was clamped at 10 mM for 2 h (time −120 to time 0 min). Clamping procedures and results were previously reported (5). At time 0 min, the clamping system was disconnected, and 5 g of 10% arginine chlorhydrate was injected intravenously in 30 s. Throughout the experiment, blood was regularly sampled for glucose, insulin, and C-peptide measurements. Plasma insulin and C-peptide were measured by radioimmunoassays. Plasma glucose was measured by the glucose-oxidase method. ISRs were obtained by deconvolution of C-peptide values using ISEC software (13). Individual kinetics of C-peptide disappearance are computed by ISEC from the subject's weight, height, age, sex, and classification (normal, non-insulin-dependent diabetes mellitus [NIDDM], and obese) based on parameters validated in a population study (14).

Data are expressed as means ± SD. Statistics were performed on data normalized by logarithmic transformation. Unpaired Student's t tests were used for comparisons between groups. Repeated measures analysis of variance (ANOVA) were used to compare ISRs across a period of time. Simple linear regression analyses were performed to test for possible relations between the acute β-cell secretory response to arginine and responses to glucose administration—a 0.5-g/kg intravenous glucose tolerance test (IVGTT) and a 10 mM glucose hyperglycemic clamp—and between these parameters and glucose tolerance. For these regression analyses, IVGTT, the arginine test, and the hyperglycemic clamp were expressed as the area under the C-peptide curve (0–10 min for the former two tests and 60–120 min for the hyperglycemic clamp), and the glucose tolerance was expressed as the area under the glucose curve during an oral glucose tolerance test (OGTT). Plasma C-peptide values were used preferentially to plasma insulin values because they seem to better reflect portal insulin levels. IVGTT and hyperglycemic clamp data were computed from results partially reported previously (5).

Table 2—*Metabolic profile of GCK-deficient patients*

		OGTT			IVGTT			Arginine test		
	HbA_{1c} (%)	Glucose 0 min (mM)	Glucose 120 min (mM)	Glucose area (mM/min)	Insulin area (pM/min)	C-peptide area (nM/min)	Glucose area (mM/min)	Insulin area (pM/min)	C-peptide area (nM/min)	Amount of secreted insulin (pmol/kg)
Subject (kindred)										
A (F51)	6.0	7.0	7.2	1,005	1,632	9.5	185	1,064	6.8	82
B (F51)	6.0	7.4	11.7	1,402	344	4.4	175	918	7.8	72
C (F51)	6.0	6.6	8.4	1,107	1,670	12.4	168	1,584	8.4	105
D (F51)	6.4	7.2	9.2	1,004	1,757	10.6	210	2,142	9.3	83
E (F423)	6.1	6.3	11.2	1,443	214	3.7	165	539	3.3	41
G (F386)	6.0	6.1	5.8	905	2,418	16.2	188	1,362	6.0	49
H (F386)	6.3	6.3	10.6	1,293	1,055	5.4	170	1,248	6.9	129
I (F85)	6.6	7.3	9.5	1,396	1,171	7.9	167	1,560	7.3	89
Patients	6.2 ± 0.2	6.8 ± 0.5	9.2 ± 2.0	1,194 ± 193	1,283 ± 744	8.8 ± 4.3	179 ± 15	1,302 ± 485	7.0 ± 1.8	81 ± 28
Control group		4.9 ± 0.5	4.6 ± 1.0	691 ± 113	2,216 ± 787	11.1 ± 3.3	150 ± 23	3,658 ± 1,434	9.6 ± 5.0	119 ± 55
Statistical significance		$P = 0.0001$	$P = 0.0001$	$P = 0.0001$	$P = 0.048$	$P = 0.18$	$P = 0.013$	$P = 0.0004$	$P = 0.29$	$P = 0.17$

Data are means ± SD. Standard HbA_{1c} values in our laboratory for nondiabetic subjects: 4.9 ± 0.7%. Arginine test is expressed as incremental data over time 0 min.

RESULTS — At the end of the clamping procedure (time 0 min), plasma glucose was not significantly different in patients (9.9 ± 1.0 mM) and in the control group (10.1 ± 0.2 mM; $P = 0.5$). However, because of the GCK deficiency, plasma insulin and C-peptide values and ISRs were significantly lower in patients than in the control group: 56 ± 26 vs. 320 ± 116 pM insulin ($P = 0.0001$), 1.1 ± 0.2 vs. 3.1 ± 0.6 nM C-peptide ($P = 0.0001$), and 4.51 ± 1.07 vs. 11.49 ± 2.64 pmol insulin · kg^{-1} · min^{-1} ($P = 0.0001$). To account for these differences, the responses to the arginine challenge were expressed as the increment over levels at time 0 min. Incremental data are shown in Fig. 1, Table 2, and the text below; actual data are shown in Table 3. Two minutes after the intravenous injection of arginine (Fig. 1), plasma insulin increment peaked at 305 ± 97 pM in patients and 852 ± 470 pM in control subjects ($P = 0.0025$). Concomitant plasma C-peptide increment was 1.5 ± 0.5 nM in patients and 1.7 ± 0.8 nM in control subjects ($P = 0.4$). ISR increment at 2 min was 30.17 ± 10.01 pmol insulin · kg^{-1} · min^{-1} in patients and 36.25 ± 15.46 pmol insulin · kg^{-1} · min^{-1} in control subjects ($P = 0.38$). Throughout the experiment, ISR increments were comparable in both groups (repeated measures ANOVA, $P = 0.17$). The area under the insulin and C-peptide increment curves (time 0–10 min) expresses the overall responses to arginine: insulin area was 1,302 ± 484 vs. 3,658 ± 1,434 pM/min ($P = 0.0004$), and C-peptide area was 7.0 ± 1.8 vs. 9.6 ± 5.0 nM/min ($P = 0.29$), respectively, in patients and in the control group (Table 2). The amount of insulin secreted in response to arginine and computed from increments in ISR (0–5 min) was similar in patients and control subjects: 81 ± 28 vs. 119 ± 55 pmol/kg ($P = 0.16$), respectively (Table 2). Plasma glucose levels remained similar in patients and control subjects during the insulin secretion peak (9.2 ± 0.6 vs. 8.4 ± 1.1 mM, $P = 0.1$ at 2 min and 9.0 ± 0.9 vs. 8.1 ± 1.1 mM, $P = 0.1$

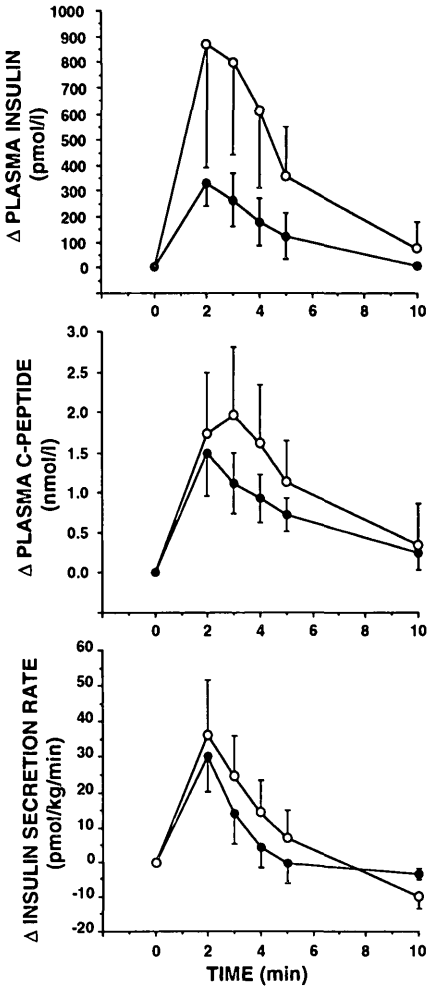

Figure 1—*Plasma insulin and C-peptide concentrations, and ISRs following intravenous arginine administration. Results are expressed as the increment over levels at time 0 min.* ●, *GCK-deficient subjects* (n = 8); ○, *control group* (n = 7). *Data expressed as means* ± *SD.*

at 3 min). At the end of the experiment, glucose levels were higher in patients than in control subjects: 8.6 ± 0.9 vs. 7.5 ± 0.9 mM, $P = 0.03$ at 5 min, and 8.3 ± 0.9 vs. 5.9 ± 1.3 mM, $P = 0.001$ at 10 min.

The C-peptide response during the arginine test was not significantly correlated to responses obtained during the IVGTT ($P = 0.23$) or the hyperglycemic clamp ($P = 0.62$; data not shown). C-peptide responses to the arginine test and hyperglycemic clamp were not correlated to glucose tolerance either ($P = 0.44$ and $P = 0.92$, respectively). The best predictor of glucose tolerance in GCK-deficient subjects was the C-peptide response to the IVGTT (coefficient of determination $r^2 = 0.78$; $P = 0.002$). To test the robustness of this correlation, analyses were performed including six additional GCK-deficient subjects from these and other families for whom IVGTT and OGTT data were available (6). A stronger statistical correlation was observed ($r^2 = 0.77$; $P = 0.0001$). A weaker but still significant correlation was observed when insulin instead of C-peptide values were used in the computations: $r^2 = 0.61$, $P = 0.013$ for $n = 8$ patients; $r^2 = 0.41$, $P = 0.014$ for $n = 14$ patients.

CONCLUSIONS— We report normal increment of ISRs in response to arginine in a panel of patients with chronic hyperglycemia caused by mutations in the GCK gene. Plasma C-peptide increments throughout the test, assessed as the area under the curve, as well as the calculated amount of insulin secreted in response to arginine, were not significantly different in patients and control subjects. Values of both parameters were >1 SD below the mean of the control group in all patients but subject E, who seems to have a more pronounced secretory defect to both arginine and glucose. Contrastingly, plasma insulin increment during the arginine test was constantly lower in patients than it was in control subjects.

The difference between plasma insulin and C-peptide responses may be ascribed to the first-pass hepatic extraction of insulin as well as to its peripheral clearance, both of which are known to vary largely in different physiological situations (15). Conversely, C-peptide is not extracted by the liver and has constant peripheral clearance, the kinetics of which were shown to be in the normal range in GCK-deficient subjects (6). In this way, peripheral C-peptide concentrations reflect better than insulin concentrations the β-cell secretory rates. Incidentally, peripheral levels of insulin, but not of C-peptide, are significantly reduced in patients compared with control subjects in response to both glucose and arginine (Table 2). This suggests that liver extraction and/or peripheral clearance of insulin might be enhanced in GCK-deficient patients. No data on these processes are currently available.

The mechanisms of the insulinotropic action of arginine, as well as of the glucose/arginine interaction, are largely unknown. It has been suggested that the accumulation of arginine, a positively charged molecule, inside β-cells leads to the depolarization of the plasma membrane and eventually to insulin secretion as a purely biophysical effect (16). However, it is known that in pancreatic islets, arginine is metabolized in several pathways with resulting conversion in L-ornithine and urea, or L-glutamate, and generation of polyamines or nitric oxide (17,18). The role that these reactions, or other changes in the oxidative catabolism of nutrients, might have in the insulinotropic action of arginine is not fully understood (19). Whatever might be its mechanism of action, the insulinotropic effect of arginine does not seem to be affected by the impairment in the glycolytic flux in β-cells resulting from GCK mutations.

We have observed, by means of glucose clamps and multiple dose glucose infusions, that the prominent metabolic feature of GCK deficiency is a right shift in the glucose/ISR dose-response curve (5,6). This shift results in a 60% reduction in ISRs in GCK-deficient subjects compared with control subjects relative to concomitant glucose levels. As a consequence, in the present set of experiments, control subjects may have depleted more insulin during the hyperglycemic clamp (time −120 to 0 min) than GCK-deficient subjects. One might argue that this higher depletion of insulin might have led to a relatively smaller response to arginine than would otherwise have been seen. However, this seems unlikely, as we have observed sustained ISR, both in GCK-deficient subjects and control subjects,

Table 3—*Arginine test (actual data)*

	0 min		2 min (peak)		Area	
	Insulin (pM)	C-peptide (nM)	Insulin (pM)	C-peptide (nM)	Insulin (pM/min)	C-peptide (nM/min)
Patient (kindred)						
A (F51)	36	1.1	372	2.8	1,424	17.8
B (F51)	42	1.0	348	3.2	1,338	17.8
C (F51)	72	1.2	528	3.2	2,304	20.4
D (F51)	66	1.3	384	2.6	2,802	22.3
E (F423)	30	1.1	180	1.7	839	14.3
G (F386)	30	0.7	216	1.7	1,662	13.0
H (F386)	102	0.9	468	2.2	2,268	15.9
I (F85)	72	1.4	396	2.8	2,280	21.3
Patients	56 ± 26	1.1 ± 0.2	362 ± 117	2.5 ± 0.6	1,865 ± 652	17.8 ± 3.4
Control group	320 ± 116	3.1 ± 0.6	1,171 ± 550	4.8 ± 1.1	6,938 ± 2,058	41.2 ± 8.5
Statistical significance	$P = 0.0001$	$P = 0.0001$	$P = 0.0002$	$P = 0.0002$	$P = 0.0001$	$P = 0.0001$

Data are means ± SD.

with up to 42 h of hyperglycemic stimulation (6).

In this panel of GCK-deficient subjects, the acute β-cell secretory responses to arginine and glucose were in the normal range in all but one subject. However, they were not correlated with each other and did not correlate with the β-cell secretory response to the hyperglycemic clamp. These results suggest that neither the arginine test nor the IVGTT reflect the β-cell defect present in GCK-deficient subjects. Indeed, during an IVGTT, the secretory defect associated with GCK deficiency is masked by the higher than normal concomitant glucose levels observed in the patients (Table 2), which leads to ISRs that are in the same range as those of control subjects (6). However, when expressed as a C-peptide: glucose ratio, IVGTT responses are significantly lower in patients than in control subjects (data not shown). Interestingly, the glucose tolerance assessed by the OGTT in GCK-deficient subjects did not correlate with the β-cell secretory response to the arginine test. More paradoxically, it did not correlate with the response to the hyperglycemic clamp either, but was strongly correlated with the response to the IVGTT. These results suggest that the first-phase insulin secretion with its priming effect on the liver (20) is one of the main determinants of glucose tolerance in this population.

The normal secretory increment in response to arginine observed in these GCK-deficient patients contrasts with results of studies on islet cell antibody positive subjects at the early prediabetic state (21–23). Responses to several secretagogues, including intravenous arginine, glucose, glucagon, and tolbutamide, were shown to be severely impaired in these subjects. In this regard, results of animal studies, performed after partial pancreatectomy (24) or streptozocin administration (25), as well as studies on hemipancreatectomized healthy human donors (26), suggest that a decrease in arginine-stimulated insulin secretion is well correlated with a progressive reduction in the β-cell mass. It is tempting to speculate that this might be an explanation for the more severe β-cell secretory impairment in response to both arginine and glucose observed in subject E. Our data also contrast with those observed in the most common form of NIDDM with the late age of onset. Although normal or even higher than normal insulin responses to an arginine challenge have been reported in NIDDM (27), these studies suffer from the bias resulting from different glucose levels in patients and control subjects. In NIDDM subjects with glucose levels matched to control subjects, insulin response to arginine was found to be significantly reduced (28). It should be pointed out that these NIDDM patients were truly diabetic, while most GCK-deficient subjects present a mild form of chronic hyperglycemia (1).

In conclusion, β-cell secretory response to arginine was found to be preserved in GCK-deficient subjects. These results suggest that the mechanism of action of arginine-induced insulin secretion is not affected by the impairment in the glycolytic flux resulting from GCK mutations. No correlation was observed between the response to arginine and responses to IVGTT and hyperglycemic clamp. The arginine test does not seem to reflect either the β-cell secretory defect or the glucose tolerance of these subjects. IVGTT seems to be the best predictor of the latter parameter in this population.

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