Reduced Pancreatic Polypeptide Response to Hypoglycemia and Amylin Response to Arginine in Subjects With a Mutation in the HNF-4α/MODY1 Gene

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Subjects with the Q268X mutation in the hepatocyte nuclear factor (HNF)-4α gene (RW pedigree/maturity-onset diabetes of the young [MODY]-1) have diminished insulin and glucagon secretory responses to arginine. To determine if pancreatic polypeptide (PP) secretion is likewise involved, we studied PP responses to insulin-induced hypoglycemia in 17 RW pedigree members: 6 nondiabetic mutation-negative [ND(–)], 4 nondiabetic mutation-positive [ND(+)], and 7 diabetic mutation-positive [D(+)]. Subjects received 0.08 U/kg body wt human regular insulin as an intravenous bolus to produce moderate self-limited hypoglycemia. PP areas under the curve (PP-AUCs) were compared among groups. With hypoglycemia, the PP-AUC was lower in the D(+) group (14,907 ± 6,444 pg/ml, *P* = 0.03) and the ND(+) group (14,622 ± 6,015 pg/ml, *P* = 0.04) compared with the ND(–) group (21,120 ± 4,158 pg/ml). In addition, to determine if the β-cell secretory defect in response to arginine involves amylin in addition to insulin secretion, we analyzed samples from 17 previously studied RW pedigree subjects. We compared the AUCs during arginine infusions for the 3 groups both at euglycemia and hyperglycemia as well as their C-peptide–to–amylin ratios. The D(+) and ND(+) groups had decreased amylin AUCs during both arginine infusions compared with the ND(–) group, but had similar C-peptide–to–amylin ratios. These results suggest that the HNF-4α mutation in the RW/MODY1 pedigree confers a generalized defect in islet cell function involving PP cells in addition to β- and α-cells, and β-cell impairment involving proportional deficits in insulin and amylin secretion. *Diabetes* 49:961–968, 2000

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Δ, change from baseline; AAR, acute amylin response; ACR, acute C-peptide response; ALP, amylin-like peptide; AUC, area under the curve; CV, coefficient of variation; D(+), diabetic mutation-positive; HNF, hepatocyte nuclear factor; IPF, insulin promoter factor; MODY, maturity-onset diabetes of the young; ND(–), nondiabetic mutation-negative; ND(+), nondiabetic mutation-positive; PP, pancreatic polypeptide; RIA, radioimmunoassay.

Maturity-onset diabetes of the young (MODY) is a heterogeneous monogenic form of diabetes characterized by autosomal dominant inheritance, onset at a young age, and a primary defect in insulin secretion (1–3). Currently, 5 MODY subtypes and genes have been identified: MODY1 associated with a mutation in the hepatocyte nuclear factor (HNF)-4α gene on chromosome 20 (4), MODY2 associated with a mutation in the glucokinase (GCK) gene on chromosome 7p (5), MODY3 associated with a mutation in the HNF-1α gene on chromosome 12q (6), MODY4 associated with a mutation in the insulin promoter factor (IPF)-1 gene on chromosome 13q (7), and MODY5 associated with a mutation in the HNF-1β gene on chromosome 17 (8).

The RW pedigree is a large MODY1 pedigree with a nonsense mutation (Q268X) in the HNF-4α gene (1–4). In this pedigree, both nondiabetic and diabetic MODY subjects with the HNF-4α gene mutation have a defect in both insulin and glucagon secretion in response to infused arginine, suggesting involvement of the pancreatic α-cell as well as the β-cell (9). Thus, we wished to ascertain whether the HNF-4α gene mutation affects a pancreatic islet cell line other than β- and α-cells, i.e., the pancreatic polypeptide (PP) cell. This cell of the endocrine pancreas is distinct from β-, α-, and δ-cells and produces PP, a 36–amino acid peptide (10). Insulin-induced hypoglycemia and ingestion of a protein meal (beef) are good stimuli to the release of PP and exercise is a moderate stimulus, whereas intravenous administration of amino acids and oral or intravenous administration of fat and glucose are modest stimuli. The intact vagus nerve is requisite for the full expression of a protein-induced increase in plasma PP (10,11). Basal plasma levels of PP have been reported to be increased in diabetic patients proportional to the degree of hyperglycemia and with aging (10), but this has not been confirmed. The PP response to insulin-induced hypoglycemia was the same in type 2 diabetic patients without autonomic neuropathy as in control subjects, but was impaired in diabetic subjects with autonomic neuropathy (12).

Although the primary objective of this work was to study the PP response to hypoglycemia in subjects of the RW/MODY1 pedigree with and without the Q268X mutation in the HNF-4α gene, we were also able to ascertain whether the mutation confers a defect in the α-cell response to hypoglycemia similar to that seen in response to arginine (9).

To further characterize the β-cell dysfunction in HNF-4α/MODY1 and to determine if amylin secretion is affected similarly to insulin secretion, we re-analyzed samples from a previous study (9) to examine the effects of arginine on amylin and amylin-like peptide (ALP) concentrations under euglycemic and hyperglycemic conditions. Amylin (13), or islet amyloid polypeptide (14), is a normal secretory product of pancreatic β-cells (15) produced from a single gene located on the short arm of chromosome 12 (16,17). There are at least 2 forms of amylin: nonglycosylated amylin and glycosylated plus nonglycosylated ALP (18). Amylin itself is a 37–amino acid peptide with a single NH_2-terminal intramolecular disulfide bond and amidated COOH-terminus, whereas ALP represents a mixture of proamylin, amylin aggregates, and posttranslationally modified amylin (18). Amylin is colocalized with insulin in β-cell secretory granules, and amylin and insulin are cosecreted under different experimental conditions (18–26). Both type 1 and type 2 diabetic subjects display insulin and amylin deficiency (16,22). There have been no studies to date describing the plasma concentration or secretory responses of amylin in MODY in general and in MODY1 in particular.

The experimental design of the PP study also afforded the opportunity to determine the degree of C-peptide suppression in response to induced hypoglycemia to determine if the HNF-4α gene mutation confers a defect in the β-cell's ability to suppress insulin secretion in response to hypoglycemia.

In summary, we wished to test the hypothesis that insulin-induced hypoglycemia could uncover possible defects in PP, in addition to β- and α-cell function, and that arginine infusion would uncover defects in amylin secretion in subjects with the HNF-4α/MODY1 gene mutation.

RESEARCH DESIGN AND METHODS

Evaluation of PP- and glucagon secretion, and C-peptide suppression during insulin-induced hypoglycemia

Subjects. We studied 17 members of the RW pedigree from generations III, IV, and V (1,2). Most of the subjects were described in detail in a previous study (9). Of the subjects from the previous study, 2 (III-35 and V-20) were not available for the present study. Subject IV-166 was not recruited because of a history of seizures. There were 2 nondiabetic mutation-positive [ND(+)] subjects (V-23 and V-25) from the previous study who developed diabetes; they have now been included in the diabetic mutation-positive [D(+)] group. In addition, 2 D(+) subjects not previously studied participated (IV-137 and IV-167). (Subjects in generation V have revised numbers as given by Fajans and Bell [27]). The study was reviewed and approved by the Institutional Review Board of the University of Michigan Medical Center, and all subjects provided written informed consent.

Protocol. Subjects were studied in the University of Michigan General Clinical Research Center, where they were admitted the evening before the study. Subjects were studied in the recumbent position after an overnight fast of 10–12 h. An intravenous sampling catheter was inserted in a retrograde direction in a dorsal vein of the hand, and the hand was kept in a box thermostatically heated to 60°C to achieve arterialization of venous blood. A second catheter for insulin administration was inserted into the contralateral antecubital vein. In nondiabetic subjects and diabetic subjects with fasting plasma glucose <115 mg/dl, blood samples for glucose, PP, glucagon, and C-peptide were drawn at –75, –60, –45, –30, –15, and 0 min. In these subjects, the means of the glucose and hormone levels at –75 to 0 min are referred to as the basal levels. At 0 min, human regular insulin (0.08 U/kg) was administered as an intravenous bolus injection. After the intravenous bolus injection of human regular insulin, samples were drawn at 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 min. Further samples were drawn at 75, 90, 105, and 120 min. Glycosylated hemoglobin was measured at –75 min.

There were 3 diabetic subjects with fasting plasma glucose of >115 mg/dl (128–141 mg/dl) who received a small intravenous bolus of human regular insulin (0.007 U/kg or ~0.5 U) at –95 min to lower the plasma glucose without causing hypoglycemia. In these subjects, a single sample was obtained before the administration of ~0.5 U insulin and used for calculation of basal levels given in Table 1. After a 20-min equilibration period, blood samples were obtained as above. Glycosylated hemoglobin was measured at –95 min.

TABLE 1
Characteristics of subjects from the RW/MODY1 pedigree by glucose tolerance and mutation status

	Genotype*		
	Nondiabetic		Diabetic
	(–)	(+)	(+)
n (M/F)	4/2	3/1	2/5
Age (years)	28 ± 11	27 ± 11	29 ± 11
BMI (kg/m^2)	25 ± 3	24 ± 2	22 ± 3
GHb (%)	5.8 ± 0.0	5.9 ± 0.0†	7.4 ± 0.0‡
Fasting glucose (mg/dl)	90 ± 10	88 ± 13	106 ± 29
Fasting PP (pg/ml)	87 ± 13	114 ± 29‡	116 ± 36‡
Fasting glucagon (pg/ml)	128 ± 24	150 ± 31	139 ± 21
Fasting C-peptide (ng/ml)	1.7 ± 0.3	1.4 ± 0.7	1.5 ± 0.4

Data are means ± SE. *(–), Normal/normal; (+), normal/Q268X mutation. †$P < 0.05$, ND(+) vs. D(+); ‡$P < 0.05$, ND(+) or (D+) vs. ND(–).

At bedside, patients were monitored for symptoms of hypoglycemia, and glucose levels were measured by a Beckman Glucose Analyzer (Beckman Instruments, Brea, CA) before insulin administration and each time blood was drawn after insulin administration until recovery from hypoglycemia. Spontaneous recovery of glucose levels occurred in all subjects. None required treatment with intravenous or oral glucose.

Assay procedures. All blood samples were collected on ice and stored at –70°C until assayed. Total glycosylated hemoglobin was measured by the Glyc-Affin affinity-based chromatography assay (Isolab, Akron, OH). Glucagon and PP were measured by double-antibody radioimmunoassays (RIAs) (intra-assay coefficient of variation [CV] 4 and 15%, respectively). C-peptide was measured by a specific RIA (intra-assay CV 11%). Plasma glucose was measured on a Cobas Mira Plus analyzer using a hexokinase method (intra-assay CV 4%). All assays were performed in the Michigan Diabetes Research and Training Center Chemistry Core Laboratory.

Data analysis. Glucose, PP, glucagon, and C-peptide areas under the curve (AUCs) were calculated with the trapezoidal rule for the time interval of 5–120 min when the insulin bolus was given at 0 min. The mean glucose and hormone levels measured at –75, –60, –45, –30, –15, and 0 min preceding the insulin bolus (baseline values) were subtracted from the AUCs. Slopes of decrease or increase were calculated as the absolute change in the glucose or hormone level from baseline to the peak or nadir divided by the time from 0 min to when the peak or nadir was achieved. Results are expressed as means ± SE. Statistical significance of differences among groups was assessed with an unpaired Student's *t* test using Microsoft Excel software (Seattle, WA) . $P < 0.05$ was defined as the limit of statistical significance.

Evaluation of amylin and ALP secretion in response to arginine during euglycemia and hyperglycemia

Subjects and protocol. A detailed description of the subjects and the protocol was published previously (9) and is shown in Fig. 3. One ND(+) subject (V-24) was excluded from this analysis because of technical problems with the amylin assay.

Assay procedures. Collection of blood samples and methods for glucose and C-peptide were described previously (9). Amylin and ALP were measured using 2-site monoclonal antibodies with F024 and F002 fluorescent-linked enzyme detection (intra-assay CVs <15% in both assays) (28,29). The F024 assay measures only human amylin, whereas the F002 assay measures human amylin as well as at least 2 additional species of amylin-like peptides, which have been shown by Western blotting to be larger than human amylin, possibly representing incompletely processed proamylin (29). The minimum detectable concentrations for the amylin and ALP assays were between 0.8 and 0.6 pmol/l, respectively. To incorporate values less than minimum detectable concentration into our analyses, we assumed that values reported as less than minimum detectable concentration were the midpoint between 0 and the lowest reported value in the assay.

All samples were measured in duplicate, and their means were used. Amylin and ALP assays were performed by Amylin Pharmaceuticals in the Linco Research Laboratory (San Diego, CA).

Data analysis. Acute C-peptide responses (ACRs), acute amylin responses (AAR), and acute ALP responses were calculated as the mean of the hormone

concentrations obtained 2, 3, 4, and 5 min after the arginine boluses minus the mean of the hormone concentrations obtained 20, 10, and 0 min before the arginine boluses. C-peptide, amylin, and ALP AUCs were calculated with the trapezoidal rule for the time interval of 10–60 min when the arginine bolus was administered at time 0 and the arginine infusion begun at 5 min. Hormone concentrations obtained 20, 10, and 0 min before the arginine bolus were subtracted from the AUCs. C-peptide–to–amylin ratios were calculated in picomoles per liter at 20, 10, and 0 min before the arginine boluses and during and after the arginine infusions (10–60 min). All indexes of C-peptide, amylin, and ALP secretion were assessed during euglycemia, during arginine administration at euglycemia, during hyperglycemia, and during arginine administration at hyperglycemia. Results are expressed as means ± SE. Statistical significance of differences among groups was assessed with the Wilcoxon's rank-sum test, and the significance of differences within groups was assessed with the Wilcoxon's signed-rank test and paired difference *t* test. $P < 0.05$ was defined as the limit of statistical significance.

RESULTS

Hypoglycemia protocol. To evaluate PP and glucagon secretion and C-peptide suppression with insulin-induced hypoglycemia, 17 members of the RW pedigree were studied: 6 nondiabetic mutation-negative [ND(–)], 4 ND(+), and 7 D(+) members (Table 1). No significant differences were observed among groups with regard to sex or age, and all subjects were nonobese. Mean total glycosylated hemoglobin concentrations did not differ between ND(–) and ND(+) subjects but were higher in the D(+) group compared with either nondiabetic group. Basal glucose, glucagon, and C-peptide did not differ significantly among groups. Basal PP was higher in the ND(+) and D(+) groups compared with the ND(–) group. After induction of hypoglycemia, all subjects experienced self-limited hypoglycemic symptoms (e.g., perspiration and warmth) to a similar degree.

Figure 1 demonstrates the protocol and plasma levels of glucose, PP, glucagon, and C-peptide before and after induction of hypoglycemia. Table 2 shows glucose and plasma hormone slopes, peaks and nadirs, and change from baseline (Δ), which quantify the trends illustrated in Fig. 1. Figure 2 shows the plasma hormone areas under the curve for the time period of 5–120 min for glucose, PP, glucagon, and C-peptide.

Effect of insulin on plasma glucose. The baseline glucose level before induction of hypoglycemia was slightly higher in the D(+) group compared with the 2 nondiabetic groups, but there were no statistically significant differences among groups (Fig. 1*A*). Rates of glucose decline after the insulin bolus were similar among the 3 groups, and mean glucose nadirs were reached at an average of 30 min in all groups. There was no difference in the glucose slope, glucose nadir, and Δglucose among groups (Table 2). Glucose-negative AUC was significantly larger in the D(+) group compared with the ND(–) group ($P = 0.03$), whereas the value for the ND(+) group was intermediate between these groups (Fig. 2*A*).

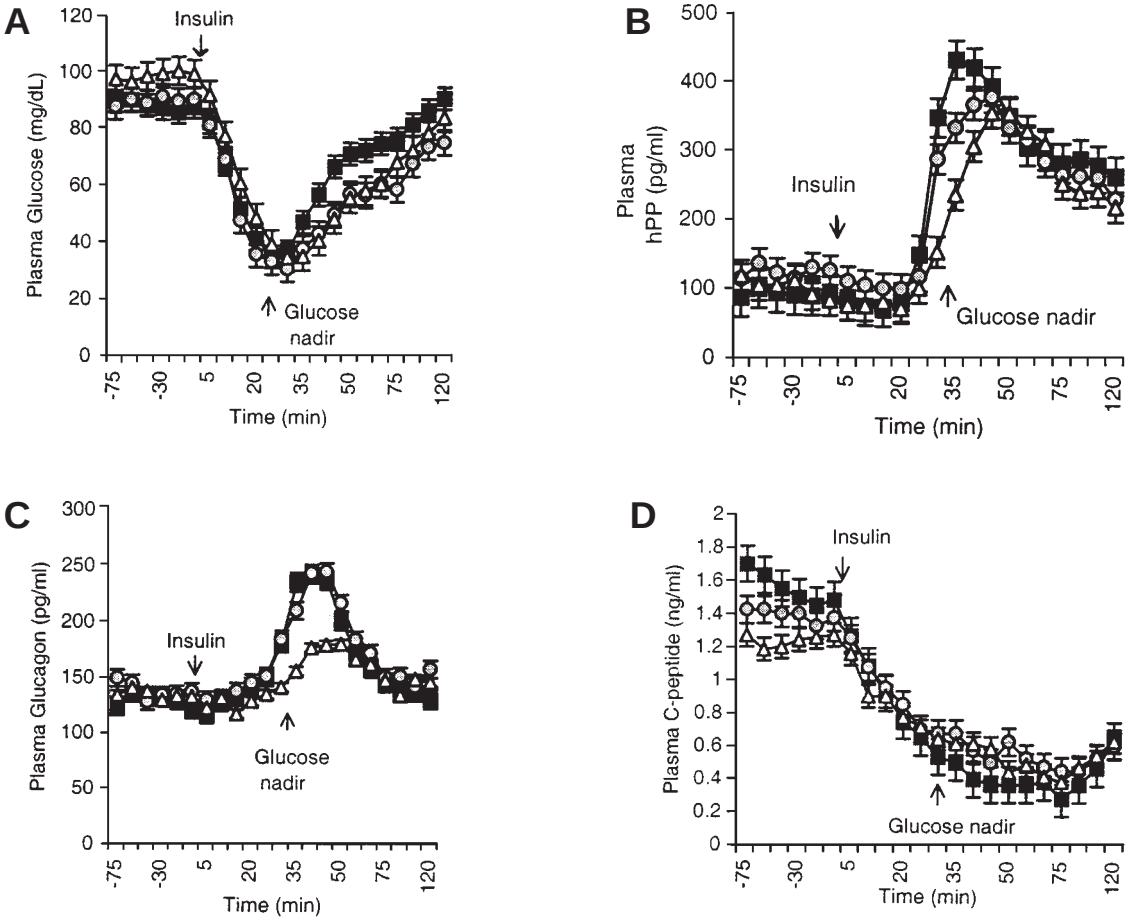

FIG. 1. Effect of insulin-induced hypoglycemia on plasma concentrations of glucose (*A*), PP (*B*), glucagon (*C*), and C-peptide (*D*) in 3 groups of subjects of the RW/MODY1 pedigree. ■, ND(–); ◉, ND(+); △, D(+).

TABLE 2
Plasma glucose, PP, glucagon, and C-peptide responses after induction of hypoglycemia in 3 groups of subjects from the RW/MODY1 pedigree

	Genotype		
	Nondiabetic		Diabetic
	(–)	(+)	(+)
Plasma glucose			
Slope (mg · dl^{-1} · min^{-1})	–2.0 ± 0.5	–2.1 ± 0.2	–2.0 ± 0.4
Nadir (mg/dl)	32 ± 7	28 ± 7	32 ± 7
ΔGlucose (mg/dl)	56 ± 12	61 ± 14	66 ± 17
Plasma PP			
Slope (pg · ml^{-1} · min^{-1})	3.4 ± 1.1	2.7 ± 1.6	2.5 ± 2.9
Peak (pg/ml)	457 ± 60	426 ± 80	401 ± 128
ΔPP (pg/ml)	366 ± 46	299 ± 72*	299 ± 140
Plasma glucagon			
Slope (pg · ml^{-1} · min^{-1})	2.0 ± 0.5	2.1 ± 0.2	1.5 ± 1.4*
Peak (pg/ml)	253 ± 75	251 ± 70	202 ± 47
ΔGlucagon (pg/ml)	125 ± 62	113 ± 66	67 ± 48*
Plasma C-peptide			
Slope (ng · ml^{-1} · min^{-1})	–0.028 ± 0.008	–0.020 ± 0.006	–0.015 ± 0.009*
C-peptide nadir (ng/ml)	0.27 ± 0.20	0.38 ± 0.32	0.30 ± 0.20
ΔC-peptide (ng/ml)	1.33 ± 0.28	1.01 ± 0.37	0.91 ± 0.37*

Data are means ± SE. *P < 0.05, ND(+) or D(+) vs. ND(–).

Effect of hypoglycemia on plasma PP. After the insulin bolus, there was a more robust increase and higher peak in plasma PP in the ND(–) group compared with the D(+) group. The ND(+) group showed intermediate values. These trends are reflected in the PP slopes and peaks of the 3 groups in Table 2, although differences were not statistically significant. ΔPP was similar in the ND(+) and D(+) groups and lower than in the ND(–) group, and the difference between the ND(+) and ND(–) groups was statistically significant. Plasma PP AUCs after hypoglycemia for the D(+) and ND(+) groups were significantly smaller than the PP AUCs for the ND(–) group (P = 0.03 and 0.04, respectively) (Fig. 2*B*). This result occurred despite the larger negative glucose AUCs in the D(+) and ND(+) groups compared with the ND(–) groups (Fig. 2*A*). PP AUC was similar for the D(+) and ND(+) groups (Fig. 2*B*).

Effect of hypoglycemia on plasma glucagon. With induction of hypoglycemia, there was a slower rise and lower peak in plasma glucagon in the D(+) group compared with the

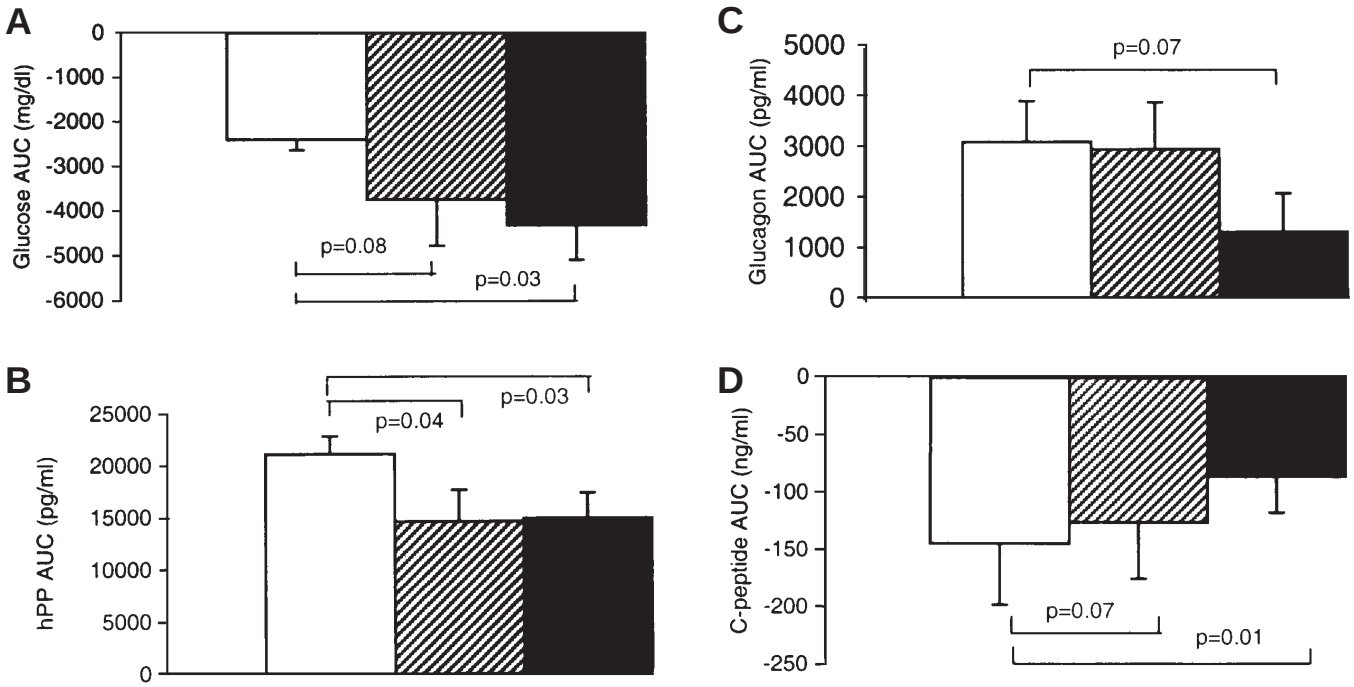

FIG. 2. Plasma AUCs of glucose (*A*), PP (*B*), glucagon (*C*), and C-peptide (*D*) responses after induction of hypoglycemia in 3 groups of subjects from the RW/MODY1 pedigree. □, ND(–); ▨, ND(+); ■, D(+).

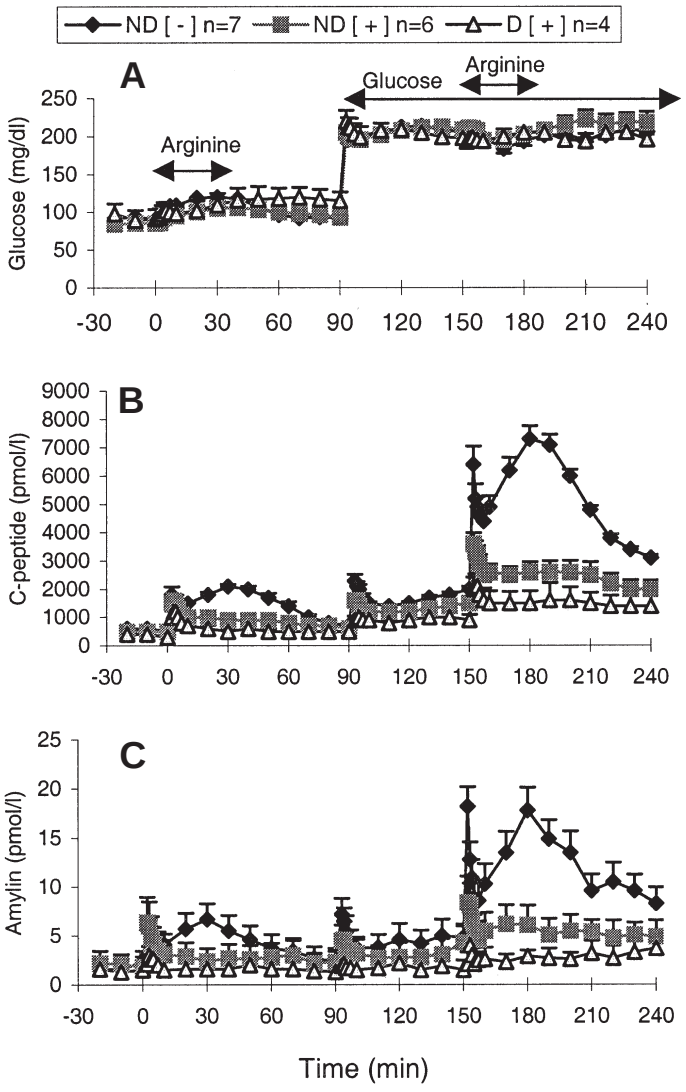

FIG. 3. Effect of bolus and infusion of arginine, glucose, and glucose plus arginine on plasma concentrations of glucose (*A*), C-peptide (*B*), and amylin (*C*) in 3 groups of subjects.

ND(–) group (Fig. 1*C*). This is reflected in the glucagon slope, glucagon peak, Δglucagon, and glucagon AUC. The differences in the glucagon slope and Δglucagon were significant (Table 2). There were no differences between the ND(+) and ND(–) groups.

Effect of hypoglycemia on plasma C-peptide. After the insulin bolus, the slowest rate of decrease and lowest ΔC-peptide was in the D(+) group and was significantly lower than that in the ND(–) group (Table 2). The ND(+) group had the highest C-peptide nadir, with intermediate C-peptide slope and ΔC-peptide compared with the 2 other groups. C-peptide–negative AUC was smallest in the D(+) group and was significantly smaller than the ND(–) group (P = 0.01) (Fig. 2*D*). C-peptide–negative AUC for the ND(+) group was intermediate between ND(–) and D(+) but did not reach statistical significance.

Arginine infusion protocol. For the amylin studies, the demographic characteristics of the 3 groups were similar, as previously reported (9). Figure 3 illustrates the protocol for arginine stimulation at euglycemia and hyperglycemia and demonstrates the glucose, C-peptide, and amylin concentrations in the ND(–), ND(+), and D(+) groups.

Amylin studies during euglycemia. At baseline, the average amylin concentrations were not significantly different among the groups (Table 3).

After the 5-g arginine bolus, ACR did not differ significantly among groups but tended to be lower in the D(+) group (Fig. 3, data reported previously [9]). AAR was significantly lower in the D(+) group compared with the ND(–) group (Table 3).

During the arginine infusion, C-peptide and amylin concentrations peaked at 30 min in the ND(–) group but were lower in the ND(+) and D(+) groups (Fig. 3). The C-peptide AUC was significantly lower in the D(+) group compared with the ND(+) and ND(–) groups (Fig. 3, data reported previously [9]). The amylin AUC was also significantly lower in the D(+) and ND(+) groups compared with the ND(–) group (Table 3). Amylin AUC did not differ significantly between the D(+) and the ND(+) groups (Table 3).

Amylin studies during the hyperglycemic clamp. During the hyperglycemic clamp, but before arginine administration, C-peptide and amylin concentrations were signifi-

TABLE 3
Plasma concentrations of amylin, acute amylin responses, glucose concentrations, and AUCs (10–60 min) for amylin during the study phase

	ND(–)	ND(+)	D(+)
n	7	6	4
Before arginine at euglycemia			
Amylin (pmol/l)	1.6 ± 0.4	1.8 ± 0.6	1.1 ± 0.5
Arginine administration at euglycemia			
AAR (pmol/l)	3.3 ± 0.6	3.5 ± 1.7	1.4 ± 0.2‡
Average glucose (mg/dl)*	107 ± 3	103 ± 3	115 ± 15
Amylin AUC (pmol/l)	262.6 ± 38.6	126.6 ± 40.2†	64.2 ± 20.2‡
Before arginine during hyperglycemic clamp			
Amylin (pmol/l)	4.8 ± 0.8	3.2 ± 1.0	1.5 ± 0.5‡
Arginine administration during hyperglycemic clamp			
AAR (pmol/l)	7.9 ± 0.6	4.5 ± 1.3	1.5 ± 0.2‡
Average glucose (mg/dl)*	198 ± 2	212 ± 7	201 ± 6
Amylin AUC (pmol/l)	690.9 ± 69.1	277.2 ± 65.6†	133.1 ± 17.7‡

Data are means ± SE. *Mean for period of 10–60 min. $P < 0.05$: †ND(+) vs. ND(–); ‡D(+) vs. ND(–).

cantly lower in the D(+) group compared with the ND(–) group (Fig. 3, data reported previously for C-peptide [9]; Table 3). Although amylin concentrations were lower in the ND(+) group compared with the ND(–) group, the difference was not significant (Table 3).

After the second arginine bolus during the hyperglycemic clamp, ACR was significantly lower in the D(+) and ND(+) groups compared with the ND(–) group (Fig. 3, data reported previously [9]). AAR was significantly lower in the D(+) than in the ND(–) group; the difference between the D(+) and ND(+) groups was not significant (Table 3).

During arginine infusion at hyperglycemia, the differences in C-peptide and amylin concentrations between the 3 groups were greatly magnified compared with corresponding concentrations during euglycemia (Fig. 3). The average glucose concentrations did not differ among the groups during the entire period of the hyperglycemic clamp (Fig. 3, Table 3). C-peptide AUC and amylin AUC in the D(+) and ND(+) groups were significantly lower than in the ND(–) group (Fig. 3), but the 2 mutation-positive groups were not significantly different from each other (Table 3).

Changes in amylin secretion with hyperglycemia. The change in amylin AUC from euglycemic to hyperglycemic glucose concentrations for the ND(–) group was 428 ± 47 (P < 0.02), for the ND(+) group was 151 ± 34 (P < 0.03), and for the D(+) group was 69 ± 25 (P < 0.03).

C-peptide–to–amylin molar ratios. Table 4 shows the C-peptide–to–amylin molar ratios before and after the arginine infusions. The C-peptide–to–amylin ratios before and during arginine infusion (10- to 60-min interval) did not differ significantly between the groups at both euglycemic and hyperglycemic plasma glucose concentrations (Table 4). Moreover, there were no significant changes in the C-peptide–to–amylin ratios within groups (Table 4).

ALP results. For all studies with arginine infusion, similar changes were obtained for ALP as for amylin; thus, data are not reported.

DISCUSSION

Pancreatic β-cell dysfunction and not insulin resistance has been established as the genetic or primary defect of MODY1 (3,9,30,31). β-Cell dysfunction, manifested by decreased insulin secretion in response to glucose (3,30) and arginine (9), occurs in nondiabetic and diabetic subjects with a mutation in the HNF-4α/MODY1 gene. β-Cell dysfunction has also been demonstrated in studies involving subjects with glucokinase/MODY2 (32,33) and HNF-1α/MODY3 (34,35). α-Cell dysfunction, manifested by an impaired glucagon secretory response to arginine, has been reported by us in subjects of the HNF-4α/MODY1 RW pedigree (9) and by others in a subject with HNF-1α/MODY3 (36). Thus, it is important to ascertain whether islet cells other than the β- and α-cells are affected by the mutation of the HNF-4α gene. PP secretion was tested during insulin-induced hypoglycemia. This testing gave us further opportunity to study glucagon secretion as well as C-peptide suppression. Somatostatin secretion from the pancreatic δ-cells cannot be evaluated in humans because somatostatin is released from multiple endocrine-like cells of the body, and there is no specific assay for pancreatic δ-cell somatostatin (37).

After induction of hypoglycemia, there was a delayed and decreased PP response in the ND(+) and D(+) groups compared with the ND(–) group (Figs. 1*B* and 2*B*). This response occurred despite a greater stimulus for PP secretion, as assessed by the relatively lower plasma glucose concentrations in the ND(+) and D(+) groups than in the ND(–) group (Figs. 1*A* and 2*A*). These findings suggest that there is a defect in the hypoglycemia-induced secretion of PP conferred by the HNF-4α/MODY1 gene. Because the same defect in the PP response was seen in both D(+) and ND(+) groups, it cannot be related to any preceding hyperglycemia in the D(+) group.

Hypoglycemia is also a stimulus for glucagon secretion. Thus, the glucagon responses in the 3 groups were examined. There was a diminished glucagon response in the D(+) group compared with the ND(–) group (Fig. 1*C*), but the differences in glucagon AUC did not reach conventional limits of statistical significance. Because the stimulus for glucagon secretion, that is, the negative glucose AUC, was larger in the D(+) group than in the ND(–) group, it is possible that this caused relatively more glucagon secretion in the D(+) group, masking the difference in the glucagon response in the 2 groups. Other parameters measuring the magnitude of the hypoglycemic stimulus, such as the slope of glucose decrease, the glucose nadir, and Δglucose, were similar among groups, indicating that the differences in negative glucose AUCs were due to a delayed recovery from hypoglycemia. It is possible that the suggestive defect in hypoglycemia-induced glucagon secretion shown in the D(+) group may be conferred by the mildly diabetic state and not by the HNF-4α mutation per se. This is, however, not consistent with the impairment in argi-

TABLE 4
C-peptide–to–amylin molar ratios before (–20–0 min) and during (10–60 min) arginine infusion

	ND(–)	ND(+)	D(+)
n	7	6	4
Euglycemia			
Before arginine	677.7 ± 166.2	503.3 ± 159.4	566.5 ± 180.8
During arginine	418.5 ± 70.5	695.4 ± 242.5	688.5 ± 244.2
Change	–259.0 ± 138.6	192.1 ± 190.0	122 ± 153.7
Hyperglycemia			
Before arginine	460.2 ± 68.6	1,178.1 ± 484.0	1,073.6 ± 337.5
During arginine	526.0 ± 82.0	610.6 ± 155.4	637.7 ± 202.7
Change	65.8 ± 33.2	–567.5 ± 401.4	–435.9 ± 348.1

Data are means ± SE. None of the differences among groups or between treatments are statistically significant.

nine-stimulated glucagon secretion demonstrated in the HNF-4α/MODY1 subjects (9). It is also possible that the HNF-4α gene mutation in MODY1 affects the signaling pathway for arginine-induced glucagon secretion but not the signaling pathway for hypoglycemia-induced glucagon secretion. A resolution of these questions will be determined in experiments in which hypoglycemia of greater magnitude and/or duration is induced in these MODY1 subjects. Of note, an impaired glucagon secretory response to arginine was also reported in a subject with HNF-1α/MODY3 (36). The HNF-4α gene regulates HNF-1α gene expression (38).

We have previously speculated that reductions in β- and α-cell function in HNF-4α/MODY1 subjects could be due either to a common defect in signal transduction in both islet cell types or to a decrease in pancreatic islet cell mass (9), since all pancreatic islet cells are thought to be derived from a common precursor stem cell (39). Data are not available in humans to distinguish between these 2 possibilities. There is no animal model for HNF-4α diabetes (40). HNF-4α is a transcription factor and like other transcription factors associated with MODY, such as IPF-1 (also known as IDX-1), may be involved in pancreatic islet cell development and growth (38,41). It is likely that HNF-4α plays a role not only in β-cell but also in α-cell and PP cell development or function, and a mutation in the HNF-4α gene, as occurs in MODY1, may explain the generalized involvement of β-, α-, and PP cell function.

To further evaluate the β-cell dysfunction in HNF-4α/MODY1, we determined the amylin response to infused arginine during euglycemia and hyperglycemia from samples obtained in our previous study (9). In this study, the mean plasma concentrations of amylin in the ND(–) subjects during both the euglycemic and hyperglycemic phases of the study were within the range of 1.0–2.0 pmol/l, similar to concentrations reported for healthy nondiabetic subjects (23). Concentrations of amylin increased significantly in the ND(–) group after the arginine bolus and infusion at both euglycemia and hyperglycemia, as reported in previous studies of healthy subjects (22,25). Amylin secretory defects occurred in response to arginine in the ND(+) and D(+) groups compared with the ND(–) group at both euglycemic and hyperglycemic plasma glucose concentrations. This finding indicates that subjects with the HNF-4α/MODY1 mutation are not only insulin deficient (as reported in previous studies [3,9,30,31]) but are also amylin deficient. The fact that secretion of amylin is severely affected in prediabetic MODY1 subjects suggests that the amylin deficiency is due to a β-cell abnormality secondary to the mutation in the HNF-4α gene and not due to the diabetic state or hyperglycemia per se. Because the ratios of C-peptide to amylin before and during the arginine infusion and at both euglycemic and hyperglycemic plasma glucose concentrations are similar in all groups, deficient amylin secretion is in proportion to the deficiency in insulin secretion. Thus, similar quantitative defects in both insulin and amylin secretion by the β-cell exist, consistent with other studies that used C-peptide as a measure of insulin secretion (22,42). The data do not indicate whether the observed amylin deficiency is secondary to insulin deficiency caused by the HNF-4α mutation or due to a direct effect of the mutation on amylin secretion.

We also had the opportunity to ascertain whether the HNF-4α mutation-induced β-cell dysfunction is manifested by a possible abnormality in β-cell suppressibility by hypoglycemia. With hypoglycemia, there was diminished C-peptide suppression in the D(+) group compared with the ND(–) group (Fig. 1*D*) despite a greater hypoglycemic stimulus in the D(+) group, as assessed by the larger negative glucose AUC (Fig. 1*A*). In addition to the lesser negative C-peptide AUC, slope of C-peptide suppression and ΔC-peptide were significantly less for the D(+) group than the ND(–) group (Table 2). All values for the ND(+) group were intermediate between the D(+) and ND(–) groups (Fig. 2*D*, Table 2). This finding suggests that there may be a defect in C-peptide suppression with hypoglycemia, perhaps reflecting dysregulation of insulin secretion conferred by the mutation in the HNF-4α gene.

In summary, the reduced PP secretion in response to hypoglycemia in the D(+) and ND(+) groups compared with the ND(–) group suggests that the HNF-4α/MODY1 gene affects pancreatic islet PP cells in addition to the β- and α-cells. The HNF-4α mutation in MODY1 may therefore confer a generalized defect in pancreatic islet cell function involving β-, α-, and PP cell types. This generalized defect could either be due to defects in β-, α-, or PP cell signaling pathways or due to a reduction in the mass of pancreatic islet cells. These conclusions need to be confirmed by similar studies in other HNF-4α/MODY1 pedigrees. Deficient amylin secretion in proportion to deficient insulin secretion and decreased suppressibility of β-cell secretion in response to insulin-induced hypoglycemia provide additional evidence of β-cell dysfunction in HNF-4α/MODY1 prediabetic and diabetic subjects.

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