GLP-1 Receptor Antagonist Exendin-(9-39) Elevates Fasting Blood Glucose Levels in Congenital Hyperinsulinism Owing to Inactivating Mutations in the ATP-Sensitive K$^+$ Channel

Andrew C. Calabria,[1] Changhong Li,[1,2] Paul R. Gallagher,[3] Charles A. Stanley,[1,2] and Diva D. De León[1,2]

Infants with congenital hyperinsulinism owing to inactivating mutations in the K_{ATP} channel (K_{ATP}HI) who are unresponsive to medical therapy will require pancreatectomy to control the hypoglycemia. In preclinical studies, we showed that the GLP-1 receptor antagonist exendin-(9-39) suppresses insulin secretion and corrects fasting hypoglycemia in $SUR-1^{-/-}$ mice. The aim of this study was to examine the effects of exendin-(9-39) on fasting blood glucose in subjects with K_{ATP}HI. This was a randomized, open-label, two-period crossover pilot clinical study. Nine subjects with K_{ATP}HI received either exendin-(9-39) or vehicle on two different days. The primary outcome was blood glucose; secondary outcomes were insulin, glucagon, and GLP-1. In all subjects, mean nadir blood glucose and glucose area under the curve were significantly increased by exendin-(9-39). Insulin-to-glucose ratios were significantly lower during exendin-(9-39) infusion compared with vehicle. Fasting glucagon and intact GLP-1 were not affected by treatment. In addition, exendin-(9-39) significantly inhibited amino acid–stimulated insulin secretion in pancreatic islets isolated from neonates with K_{ATP}HI. Our findings have two important implications: 1) GLP-1 and its receptor play a role in the regulation of fasting glycemia in K_{ATP}HI; and 2) the GLP-1 receptor may be a therapeutic target for the treatment of children with K_{ATP}HI. *Diabetes* 61:2585–2591, 2012

Inactivating mutations in the genes encoding the ATP-sensitive K$^+$ channel (K_{ATP} channel) are responsible for the most common and most severe form of congenital hyperinsulinism (K_{ATP}HI). In most cases, medical therapy is not effective and a pancreatectomy is required to control the hypoglycemia. For infants with a focal form of K_{ATP}HI, total excision of the lesion can be curative. However, those with diffuse disease may require a near-total pancreatectomy. Unfortunately, this surgical approach carries a high risk of either persistent hypoglycemia or insulin-requiring diabetes (1,2).

Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted from intestinal L cells in response to ingested nutrients. In addition to its insulinotropic actions, GLP-1 glucose-lowering effects include inhibition of glucagon secretion, hepatic glucose production, gastric emptying, and appetite (rev. in 3). Genetic and pharmacologic disruption of GLP-1 receptor signaling leads to fasting hyperglycemia (4–10), suggesting that in addition to contributing to postprandial glucose regulation, GLP-1 plays a role in the control of fasting glucose. Exendin-(9-39), a truncated form of the GLP-1 agonist exendin-4, is a specific GLP-1 receptor antagonist in mice and humans (5,11,12). In preclinical studies, we showed that exendin-(9-39) decreases cAMP levels and insulin secretion in $SUR-1^{-/-}$ mouse islets (13). In vivo, continuous subcutaneous infusion of exendin-(9-39) significantly raised fasting blood glucose levels in $SUR-1^{-/-}$ mice without affecting glucose tolerance or insulin sensitivity (13). These findings suggest that GLP-1 and its receptor play a key role in the control of insulin secretion in this mouse model.

We hypothesized that exendin-(9-39) can elevate fasting blood glucose levels in children and adults with K_{ATP}HI and, thus, may have a potential therapeutic application for this disorder. To evaluate this hypothesis, we examined the effect of exendin-(9-39) on glucose homeostasis in subjects with congenital hyperinsulinism. Given the dearth of available effective medical therapies for individuals with K_{ATP}HI, these studies are important in understanding the pathophysiology of this disorder and evaluating the potential therapeutic applications of antagonists of the GLP-1 receptor in the treatment of this serious condition.

RESEARCH DESIGN AND METHODS

Nine subjects with confirmed genetic and clinical diagnosis of K_{ATP} hyperinsulinism were recruited from the Hyperinsulinism Center at the Children's Hospital of Philadelphia (CHOP). Exclusion criteria included acute medical illnesses; a history of systemic chronic diseases such as cardiac failure, renal insufficiency, hepatic insufficiency, chronic obstructive pulmonary disease, anemia, or uncontrolled hypertension; pregnancy; diabetes; and use of medications that affect glucose metabolism, such as glucocorticoids, β-agonists, octreotide, and diazoxide.

This was a randomized, open-label, two-period complete crossover pilot study to evaluate the effect of the GLP-1 receptor antagonist exendin-(9-39), on glucose metabolism in subjects with K_{ATP}HI. The study was approved by the human subjects committee of CHOP and the U.S. Food and Drug Administration. Written informed consent was obtained from all subjects or their parent/guardian. Assent was obtained from the children when appropriate.

Subjects were admitted to the CHOP Clinical and Translational Research Center (CTRC) inpatient unit. All subjects were administered 5 ng exendin-(9-39) (0.05 μg/mL) intradermally as a test of immediate hypersensitivity. Baseline chemistry profiles were obtained to evaluate liver and kidney function in all subjects, and a pregnancy test was performed in all postmenarchal females.

An antecubital vein was cannulated in each forearm for infusions and blood sampling. Each subject underwent two experiments in random order and on consecutive days. On one day, after a 12-h overnight fast, subjects received an intravenous infusion of vehicle (0.9% NaCl) for 1 h followed by an intravenous infusion of exendin-(9-39) at 100 pmol/kg/min (0.02 mg/kg/h) for 2 h and then 300 pmol/kg/min (0.06 mg/kg/h) for 2 h, followed by 500 pmol/kg/min (0.1 mg/kg/h) for the last 2 h. The doses of exendin-(9-39) were selected based on previously published data demonstrating that at a dose of 300 pmol/kg/min, exendin-(9-39) abolishes the effects of physiologic postprandial plasma concentrations of GLP-1 and that a higher dose of 500 pmol/kg/min increases fasting plasma glucose concentration in normal subjects (5,12). On the other day, after a 12-h overnight fast, subjects received an intravenous infusion of vehicle for 7 h. The infusion rates of vehicle were identical to the volume infused during the exendin-(9-39) study day. The primary outcome for this study was fasting blood glucose concentration. Secondary outcomes include fasting plasma insulin, C-peptide, glucagon, intact GLP-1, and insulin/glucose. Blood samples for blood glucose, insulin, glucagon, and intact GLP-1 were obtained at multiple time points during the infusions (−60, 0, 40, 60, 80, 120, 160, 180, 200, 220, 240, 280, 300, 320, 340, and 360 min). During the infusion, blood glucose was monitored by a bedside glucose meter (Surestep) as needed to avoid hypoglycemia (defined as <3.9 mmol/L [70 mg/dL]). An intravenous infusion of dextrose was started if blood glucose levels fell to <3.3 mmol/L (60 mg/dL) during the study period.

Peptide. Exendin-(9-39) was synthesized by the American Peptide Company (Sunnyvale, CA) under cGMP guidelines. The peptide was purified to ≥97% by high-performance liquid chromatography, and the sequence and mass were verified. The peptide was stored in a lyophilized form at −20°C. For administration, the peptide was diluted in 0.9% NaCl and added to 0.25% human serum albumin (final concentration of 0.1 mg/mL). Aliquots were tested for sterility and pyrogenicity through the Investigational Drug Service at the University of Pennsylvania. The use of synthetic exendin-(9-39) is approved under the U.S. Food and Drug Administration Investigational New Drug no. 76612.

Islet studies. Fresh pancreata from surgical specimens from three neonates (age 4–6 weeks) with K_{ATP}HI who were homozygous for mutations in either *KCNJ11* (R136L) or *ABCC8* (R248X and E824X) were procured through an institutional review board–approved protocol. The pancreas was injected with collagenase (Sigma-Aldrich; St. Louis, MO). Islets were handpicked under microscopy and cultured in RPMI-1640 medium containing 10 mmol/L glucose for 3 days prior to the studies. Batches of 50 islets were preincubated in glucose-free Krebs-Ringer bicarbonate buffer for 60 min. Exendin-(9-39) was added 30 min into the preincubation period. Then, islets were exposed to stimulation with 10 mmol/L glucose or a mixture of amino acids at a concentration of 4 mmol/L as previously described (14). Media were collected for determination of insulin concentration.

Assays. Whole blood glucose was measured using a Siemens Rapid Point 400 Blood Gas analyzer (Siemens Healthcare Diagnostics, Deerfield, IL). The analyzer has a resolution of 1 mg/dL and a within-run SD of ±4 mg/dL. Plasma insulin was measured using an ELISA kit from ALPCO (cat. no. 08-10-1113-99; ALPCO Diagnostics, Salem, NH). The assay has a sensitivity of 0.798 uIU/mL and an intra-assay coefficient of variation (CV) of <5%. C-peptide was measured using an RIA kit (cat. no. HCP-20K, Millipore; Linco Research, St. Charles, MO). The assay has a sensitivity of 0.1 ng/mL and an intra-assay CV of <10%. Glucagon was measured using an RIA kit (cat. no. GL-32K, Millipore; Linco Research). The assay has a sensitivity of 20 pg/mL and an intra-assay CV of <10%. Intact GLP-1 was measured using a GLP-1 ELISA kit (cat. no. EGLP-35K, Millipore; Linco Research) in samples collected with dipeptidyl peptidase IV inhibitor (cat. no. DPP4, Millipore; Linco Research) (10 μL/mL blood) to prevent proteolytic cleavage. The kit has a sensitivity of 2 pmol/L and an intra-assay CV of <10%. Insulin concentrations from the islet studies were measured by RIA (Millipore; Linco Research).

Statistical analysis. All results are presented as means ± SD. Area under the plasma concentration–time curve (AUC) was calculated for each outcome, under each treatment condition, using the linear trapezoid method. Histograms and one-sample Kolmogorov-Smirnov tests were used to examine outcome variables for normality of distribution. Effects of carryover, period, and treatment were examined using mixed-effects models (SAS proc mixed). Results from the islet studies were analyzed by one-way ANOVA.

RESULTS

Subject characteristics. Nine subjects (six female and three male) ages 15–47 years with K_{ATP}HI were included in the study (Table 1). Six subjects were heterozygous for dominant *ABCC8* mutations, two were homozygous for the common recessive Ashkenazi mutation (*ABCC8*, 3992–9G>

TABLE 1
Subject characteristics

Subject	Age (years)	Sex	Mutation (ABCC8)	Pancreatectomy
1	29	F	delF1388/3992–9 G>A	85%
2	44	M	delS1387*	None
3	35	M	S408P*	None
4	17	F	3992–9 G>A/3992–9 G>A	95%
5	15	F	3992–9 G>A/3992–9 G>A	95%
6	18	M	delS1387*	None
7	16	F	delS1387*	None
8	47	F	R1353H*	None
9	37	F	R521Q*	None

F, female; M, male. *Dominant mutation.

A), and one was a compound heterozygous for recessive *ABCC8* mutations. The latter three subjects with recessive *ABCC8* mutations had undergone a pancreatectomy during infancy. Three of the six dominant K_{ATP}HI subjects had been treated with diazoxide until their teenager years. None of the subjects were receiving medical therapy for hyperinsulinism at the time of the study, although many of them reported having episodes of hypoglycemia that they manage by avoiding prolonged fasting.

Effect of exendin-(9-39) on fasting blood glucose. After a 12-h overnight fast, mean baseline blood glucose concentration at time 0 was not different on vehicle and exendin-(9-39) days (5.8 ± 1.3 mmol/L [105.3 ± 23 mg/dL] vs. 5.9 ± 1.2 mmol/L [105.6 ± 22.3 mg/dL], respectively; $P = 0.819$) (Fig. 1). Of note, three of the subjects had a baseline blood glucose concentration >7 mmol/L (127 mg/dL). During vehicle infusion, blood glucose decreased in all subjects, and eight of the subjects developed hypoglycemia with blood glucose concentrations <3.9 mmol/L (70 mg/dL); three of the eight required an intravenous infusion of dextrose for symptomatic hypoglycemia and blood glucose concentrations <3.3 mmol/L (60 mg/dL). The time to hypoglycemia during vehicle infusion varied
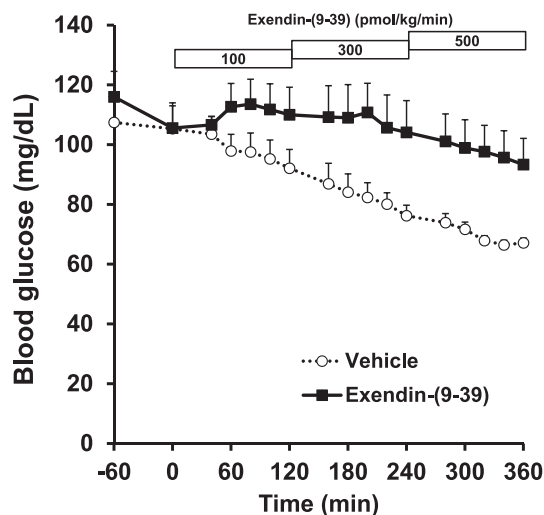

FIG. 1. Effect of exendin-(9-39) on fasting blood glucose. Mean blood glucose ± SEM during vehicle and exendin-(9-39). Subjects received vehicle from time −60 to 0 min, followed by vehicle or exendin-(9-39) for 6 h. Exendin-(9-39) was infused at a dose of 100 pmol/kg/min from 0 to 120 min, 300 pmol/kg/min from 120 to 240 min, and 500 pmol/kg/min from 240 to 360 min.

among the subjects, with three subjects developing hypoglycemia within the first 3 h of the study (or an ~15-h fast), while the other five developed hypoglycemia after 3 h. In contrast, during exendin-(9-39) infusion, hypoglycemia did not occur in any of the subjects. Mean nadir blood glucose concentration was significantly greater during exendin-(9-39) infusion compared with vehicle infusion (4.9 ± 1.1 mmol/L [88.4 ± 19.5 mg/dL] vs. 3.5 ± 0.3 mmol/L [63.4 ± 5.3 mg/dL]; $P = 0.009$) (Fig. 1). Mean blood glucose AUC from 0 min to the end of the infusion at 360 min was significantly greater during exendin-(9-39) infusion compared with vehicle (2,096 ± 454 mmol · min/L [37,734 ± 8176 mg · min/dL] vs. 1,678 ± 281 mmol · min/L [30,201 ± 5,050 mg · min/dL]; $P = 0.013$). Blood glucose AUC was significantly greater during exendin-(9-39) infusion compared with vehicle at all dose levels (100 pmol/kg/min: 489 ± 88 mmol · min/L [8,808 ± 1,580 mg · min/dL] vs. 425 ± 87 mmol · min/L [7,654 ± 1,573 mg · min/dL], $P = 0.012$; 300 pmol/kg/min: 480 ± 124 mmol · min/L [8,642 ± 2,227 mg · min/dL] vs. 365 ± 64 mmol · min/L [6,562 ± 1,158 mg · min/dL], $P = 0.023$; and 500 pmol/kg/min: 433 ± 106 mmol · min/L [7,789 ± 1,903 mg · min/dL] vs. 303 ± 31 mmol · min/L [5,456 ± 563 mg · min/dL], $P = 0.009$).

There was a carryover effect on blood glucose concentration at baseline ($P = 0.011$), implying that the value of day 2 may have been affected by the interventions on day 1. There were no carryover or period effects on nadir blood glucose or blood glucose AUC.
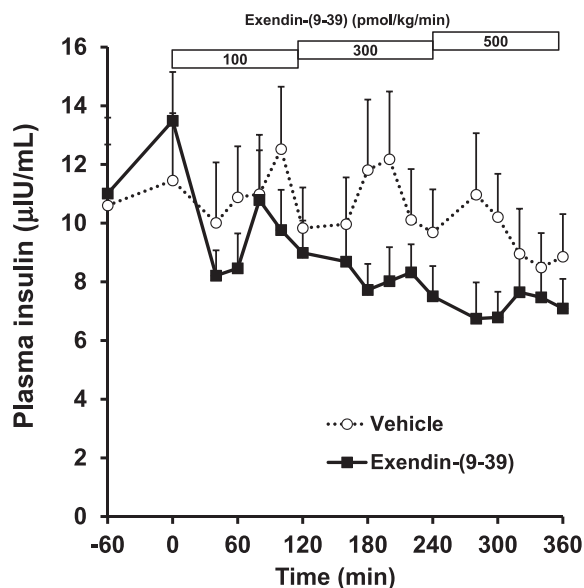
Effects of exendin-(9-39) on plasma insulin and C-peptide. After the 12-h overnight fast, mean baseline plasma insulin concentration at time 0 was not significantly different on vehicle and exendin-(9-39) days (79.9 ± 47.9 pmol/L [11.5 ± 6.9 μIU/mL] vs. 93.8 ± 34.7 pmol/L [13.5 ± 5 μIU/mL], respectively; $P = 0.13$) (Fig. 2). There were no significant differences in mean maximal plasma insulin concentration during the study period [100.7 ± 45.8 pmol/L (14.5 ± 6.6 μIU/mL) for vehicle vs. 99.3 ± 38.2

pmol/L (14.3 ± 5.5μIU/mL) for exendin-(9-39); $P = 0.60$] (Fig. 2). During the entire infusion period, the mean insulin AUC was greater for vehicle (25,842 ± 12,397 pmol · min/L [3,721 ± 1,785 μIU · min/mL] than for exendin-(9-39) (21,016 ± 7,410 pmol · min/L [3,026 ± 1,067 μIU · min/dL]) overall and at each dose level, though this difference was not statistically significant (all P values >0.29) (Fig. 2). While no carryover effects were identified in overall AUC, a statistically significant period effect was identified at the 500 pmol/kg/min dose ($P = 0.034$).

The overall insulin/glucose AUC (Fig. 3) was greater for vehicle compared with exendin-(9-39) (43.6 ± 15.1 vs. 29.2 ± 11.8) but was only borderline significant ($P = 0.053$). However, the mean insulin-to-glucose AUC ratio was significantly lower during infusion of exendin-(9-39) at the higher doses compared with vehicle (100 pmol/kg/min: 6.7 ± 3.2 vs. 8.9 ± 3.2, $P = 0.11$; 300 pmol/kg/min: 6.2 ± 2.5 vs. 10.3 ± 3.6, $P = 0.016$; and 500 pmol/kg/min: 6.0 ± 2.2 vs. 10.7 ± 4.6, $P = 0.045$). No carryover or period effects were identified for AUC overall or at any dose level (all P values >0.094).

Mean baseline C-peptide concentration was not significantly different on the vehicle and exendin-(9-39) days. Similar to the insulin AUC, mean C-peptide AUC was greater overall [268.4 ± 83.9 nmol · min/L (806.1 ± 252 ng · min/mL) for vehicle vs. 233.9.1 ± 67.3 ng · min/mL (702.5 ± 202.1 ng · min/mL) for exendin-(9-39)] and in all dose-level time frames in the vehicle group compared with the exendin-(9-39) group but failed to reach statistical significance. There were no carryover or period effects for any of these parameters. Insulin–to–C-peptide ratio did not change across time or between experimental conditions (data not shown).
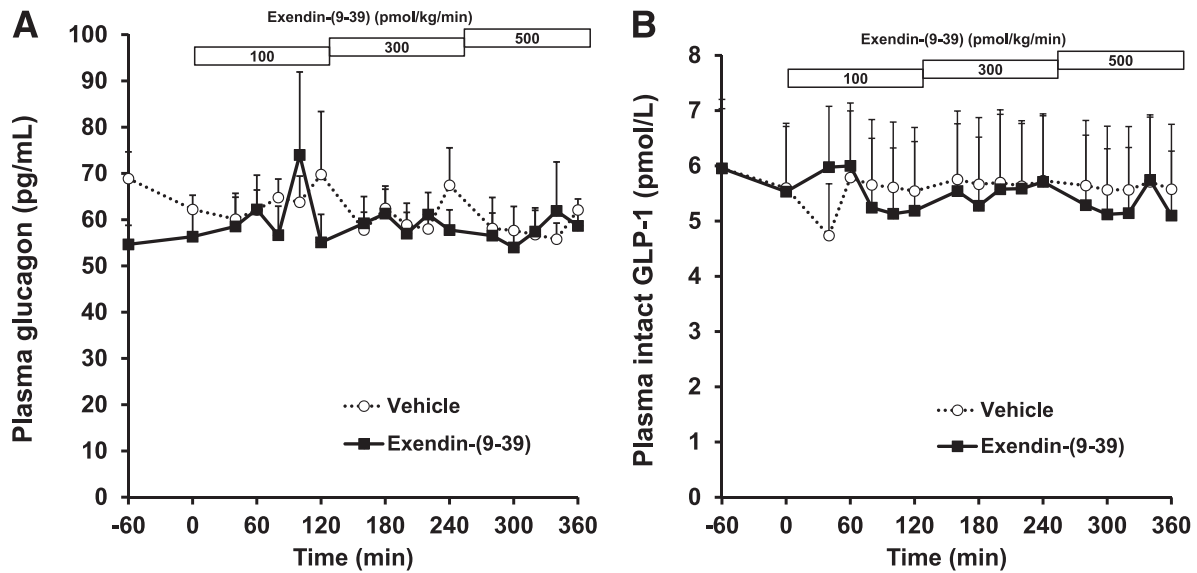
Effects of exendin-(9-39) on plasma glucagon and intact GLP-1. Mean glucagon AUC was not significantly different between the vehicle and exendin-(9-39) days (21,867 ± 4,432 pg · min/mL vs. 21,243 ± 5,836 pg · min/dL;

FIG. 2. Effect of exendin-(9-39) on fasting plasma insulin. Mean plasma insulin ± SEM during vehicle and exendin-(9-39). Subjects received vehicle from time −60 to 0 min, followed by vehicle or exendin-(9-39) for 6 h. Exendin-(9-39) was infused at a dose of 100 pmol/kg/min from 0 to 120 min, 300 pmol/kg/min from 120 to 240 min, and 500 pmol/kg/min from 240 to 360 min.
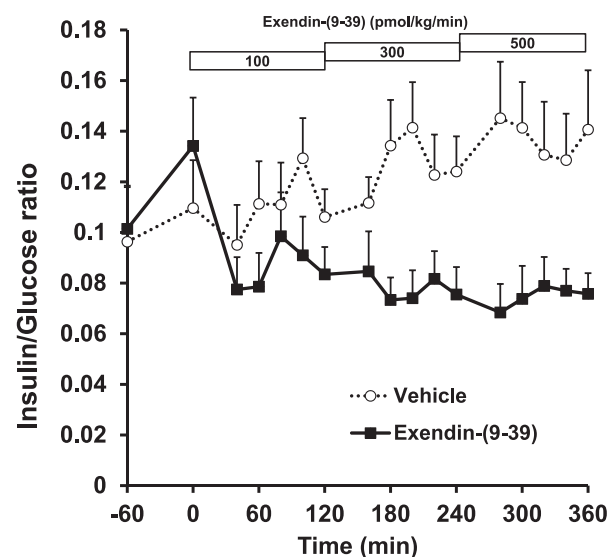
FIG. 3. Effect of exendin-(9-39) on insulin-to-glucose ratio. Mean insulin-to-glucose ratio ± SEM during vehicle and exendin-(9-39). Subjects received vehicle from time −60 to 0 min, followed by vehicle or exendin-(9-39) for 6 h. Exendin-(9-39) was infused at a dose of 100 pmol/kg/min from 0 to 120 min, 300 pmol/kg/min from 120 to 240 min, and 500 pmol/kg/min from 240 to 360 min.

FIG. 4. Effect of exendin-(9-39) on glucagon and intact GLP-1. *A*: Mean plasma glucagon ± SEM during vehicle and exendin-(9-39). *B*: Mean plasma intact GLP-1 ± SEM during vehicle and exendin-(9-39). Subjects received vehicle from time −60 to 0 min, followed by vehicle or exendin-(9-39) for 6 h. Exendin-(9-39) was infused at a dose of 100 pmol/kg/min from 0 to 120 min, 300 pmol/kg/min from 120 to 240 min, and 500 pmol/kg/min from 240 to 360 min.

$P = 0.9$) (Fig. 4*A*). Similarly, there was no effect of treatment on mean intact GLP-1 AUC [1,992 ± 1,203 pmol · min/L for vehicle vs. 1,970 ± 1,284 pmol · min/L for exendin-(9-39); $P = 0.8$] (Fig. 4*B*).

Effect of exendin-(9-39) on stimulated insulin secretion in K$_{ATP}$HI islets. To further examine the effect of exendin-(9-39) on insulin secretion, we examined its effects on stimulated insulin secretion in islets isolated from neonates with K$_{ATP}$HI. Pancreatic islets from subjects with inactivating mutations in the K$_{ATP}$ channel exhibited responses to fuel stimulation similar to those in *SUR-1*$^{-/-}$ mouse islets (14). Insulin secretion increased by fourfold in

response to stimulation with a physiologic mixture of amino acids, while the response to stimulation with 10 mmol/L glucose was minimal (Fig. 5). Pretreatment with exendin-(9-39) inhibited amino acid–stimulated insulin secretion in human K$_{ATP}$HI islets [$P < 0.001$, ANOVA; post hoc Bonferroni: 0 mmol/L glucose vs. 10 mmol/L glucose $P = 1$, 0 mmol/L glucose vs. amino acids mixture (AAM) $P = 0.001$, AAM vs. AAM plus exendin-(9-39) $P = 0.003$)]. These results indicate that GLP-1 receptor antagonism can inhibit amino acid–stimulated insulin secretion in islets from patients with K$_{ATP}$HI, similar to observations we have previously reported in the mouse model (13).
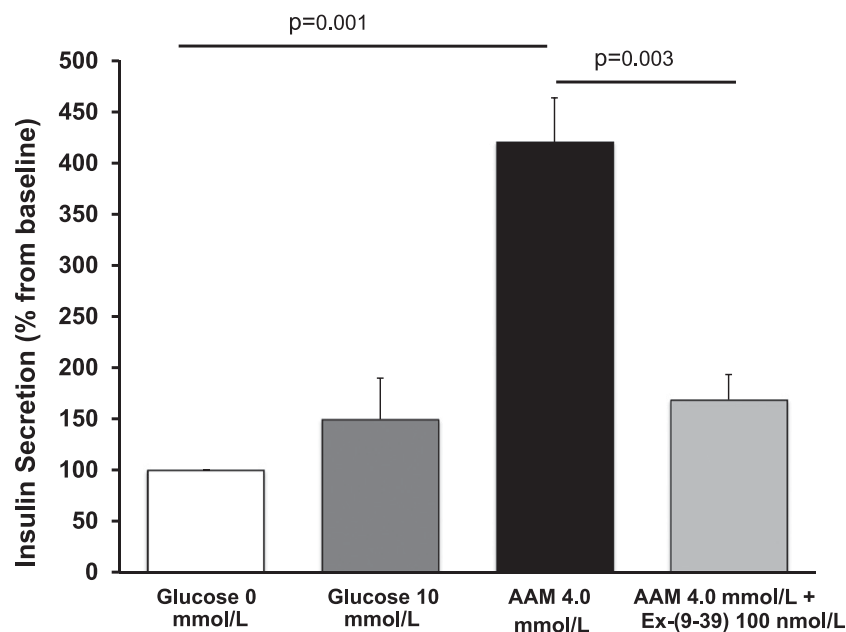
FIG. 5. Effect of exendin-(9-39) on amino acid–stimulated insulin secretion in human K$_{ATP}$HI islets. Insulin secretion ± SEM at baseline, 0 mmol/L glucose after stimulation with 10 mmol/L glucose, 4 mmol/L amino acids, and 4 mmol/L amino acids in the presence of 100 nmol/L exendin-(9-39). $N = 3$; ANOVA, $P = 0.001$; post hoc Bonferroni, 10 mmol/L glucose vs. AAM $P = 0.001$, AAM vs. AAM plus exendin-(9-39) $P = 0.003$.

DISCUSSION

The results from this pilot study clearly demonstrate that exendin-(9-39) prevents hypoglycemia and maintains stability of blood glucose during a prolonged fast in individuals with $K_{ATP}HI$. These findings have two important implications: *1*) GLP-1 and its receptor play a role in the regulation of fasting glycemia not only in normal individuals but also in individuals with $K_{ATP}HI$ and *2*) the GLP-1 receptor may be a therapeutic target for the treatment of children with $K_{ATP}HI$.

The importance of endogenous GLP-1 in the regulation of glucose metabolism is well established. Thus, GLP-1 receptor agonists have been introduced as a new therapeutic approach to treat diabetes. Less is known about the potential role of GLP-1 and its receptor in disorders of insulin regulation resulting in hypoglycemia. Evidence from studies involving pharmacologic doses of GLP-1 (15,16) and excessive endogenous GLP-1 secretion (17) raises the possibility that despite the "glucose dependency" of its insulinotropic actions, GLP-1 actions can result in overt hypoglycemia. The clinical use of the GLP-1 receptor agonist exenatide (Byetta) has not been associated with significant hypoglycemia in patients with diabetes, except when given concomitantly with sulfonylureas (18). This latter interesting observation suggests that K_{ATP} channel integrity is important to maintain the glucose dependency of GLP-1 actions in the β-cell. This clinical observation is in agreement with the results from our preclinical studies in the mouse model of K_{ATP} channel inactivation (13) and the clinical studies presented here. Whether the mechanism of GLP-1 involvement in $K_{ATP}HI$ is through increased GLP-1 secretion or due to a constitutively active receptor remains to be determined. Clinically, we have observed that in neonates with severe $K_{ATP}HI$, withdrawal of enteral feedings results in a decrease in glucose requirements to maintain euglycemia. These unpublished observations would suggest that GLP-1 secretion in response to ingested nutrients is increased in these children, perhaps resulting from dysfunctional K_{ATP} channels in L cells (19). However, in our present clinical study, the effects of exendin-(9-39) on glucose were seen during fasting. We have not yet examined its effect during feeding. Interestingly, Salehi, Prigeon, and D'Alessio (20) have reported that exendin-(9-39) inhibits insulin secretion in the absence of elevated circulating concentrations of GLP-1. This finding is in agreement with in vitro studies (21) and our mouse islets studies (13), suggesting that exendin-(9-39) is an inverse agonist of the GLP-1 receptor. This implies that there is a tonic regulation via ligand-independent activity of the GLP-1 receptor that is inhibited by exendin-(9-39). The data supporting constitutive activity of the GLP-1 receptor include not only models in which exendin-(9-39) was used but also data from GLP-1 receptor–null islets (22,23). It is therefore plausible that in depolarized pancreatic islets (due to inactivating mutations in K_{ATP} channels or due to hyperglycemia or sulfonylureas) the constitutive activity of the GLP-1 receptor has an amplifying effect on insulin secretion that can be reversed by exendin-(9-39).

Our study was not designed to determine comprehensively the mechanism by which exendin-(9-39) exerts its effects on blood glucose in $K_{ATP}HI$. In healthy individuals, previous studies have shown that the effects of exendin-(9-39) on fasting plasma glucose are associated with upregulated glucagon secretion (6,9,10), but others had failed to demonstrate an effect on glucagon during fasting (5,6). In all of these studies, there was no significant effect on fasting insulin. It has been suggested that the effect on fasting glucose is mediated by exendin-(9-39) blockade of the purported enhanced peripheral glucose disposal effects of GLP-1; however, there is no evidence supporting this hypothesis. Studies in healthy humans fasted overnight demonstrated that small transient increases in plasma insulin and decrease in glucagon in response to GLP-1 result in a decline in glucose appearance most likely owing to suppression of hepatic glucose release but without any significant change in glucose disappearance (24). Similar findings have been reported in rats (25). We would speculate that the lack of fall in glucose observed in our study is most likely due to an increase in endogenous glucose production (R_a) rather than a decrease in glucose utilization. However, the mechanism by which exendin-(9-39) increases fasting plasma glucose in normal individuals remains unclear. In isolated $SUR-1^{-/-}$ mouse pancreatic islets, exendin-(9-39) very clearly inhibits insulin secretion at baseline and in response to amino acid stimulation. In the present clinical study, insulin concentrations in the subjects with $K_{ATP}HI$ were lower during exendin-(9-39), although not statistically significant, suggesting that inhibition of insulin secretion is, at least in part, responsible for the maintenance of glucose concentration. There was no evidence of an effect of exendin-(9-39) on hepatic extraction of insulin, as insulin–to–C-peptide ratio was not different between the experimental conditions or across time. In this population, however, the lack of significant change in insulin concentrations has to be interpreted taking into consideration the following: *1*) the study was not powered to detect a significant effect on insulin; *2*) in $K_{ATP}HI$, very small changes in insulin concentration have a very large effect on fasting blood glucose, and therefore a very large number of subjects may be needed to detect small effects on insulin with statistical significance; and *3*) when interpreting plasma insulin concentration, one has to consider the glucose concentration at the same time, and thus while blood glucose concentration was decreasing during vehicle infusion, insulin concentration was either increasing or unchanged. Therefore, insulin-to-glucose ratios during vehicle and exendin-(9-39) infusion were shown to be significantly different at the highest doses of exendin-(9-39), indicating that, at least in part, the effects of exendin-(9-39) in $K_{ATP}HI$ are mediated by its effects on the β-cells. Further supporting this, amino acid–stimulated insulin secretion in islets isolated from children with $K_{ATP} HI$ was inhibited by exendin-(9-39).

While it is plausible that the hyperglycemic effects of exendin-(9-39) are in part mediated by its effects on glucagon, we did not detect any treatment effects on glucagon concentration. Although the sample size is too small to make any definite conclusions, it is possible that GLP-1 receptor blockade does not overcome the glucagon secretory defect characteristic of the K_{ATP} channel defects (26–28). Similar to findings of previous reports (20), fasting intact GLP-1 plasma concentration was not affected by treatment with exendin-(9-39), contrary to the increased GLP-1 response to a meal observed by other investigators during exendin-(9-39) infusion (9,10,20). This suggests that GLP-1 actions directly or indirectly regulate GLP-1 secretion after meals but not in the fasting state. We did not evaluate the effects of exendin-(9-39) on extraislet GLP-1 actions, but given that these studies were in the fasting state and of very short duration, it is unlikely that they played any significant role.

Independent of the mechanism of action, the effect of exendin-(9-39) on fasting blood glucose is clinically significant for this population. As observed during this study, even older subjects and those subjects with a milder phenotype are at risk for symptomatic hypoglycemia during prolonged periods of fasting. Despite the impressive recent advances in the understanding of the molecular biology of congenital hyperinsulinism, there have not been any advances in medical therapies for >20 years. Diazoxide, the mainstay of medical therapy for hyperinsulinism, suppresses insulin by activating the opening of the β-cell K$_{ATP}$ channel and is therefore ineffective in the majority of K$_{ATP}$HI cases. The second line of therapy for hyperinsulinism is octreotide, a somatostatin analog. The initial response to octreotide is good in most infants with hyperinsulinism, but tachyphylaxis develops after a few doses, rendering therapy inadequate for long-term use in most cases (29). Moreover, while generally well tolerated, octreotide therapy has recently been associated with fatal necrotizing enterocolitis in neonates with hyperinsulinism. This may be due to reduced splanchnic blood flow with increasing doses: a finding that may limit the use of octreotide in the treatment of hyperinsulinism (30). Thus, the finding that exendin-(9-39) raises fasting blood glucose levels in human subjects with K$_{ATP}$HI shows great promise for the development of a potential therapeutic mechanism by antagonism of the GLP-1 receptor.

Our study has some limitations that should be discussed. Because the clinical research experience with exendin-(9-39) has been limited, we chose to study older individuals first. As expected, given the natural history of the disease and the fact that some of the subjects had undergone a pancreatectomy, blood glucose concentrations after an overnight fast were not low and three of the subjects had fasting hyperglycemia; however, during the vehicle infusion these levels declined, resulting in symptomatic hypoglycemia in most of the subjects. Exendin-(9-39) prevented this decline without causing overt hyperglycemia during the fasting period. Because of safety concerns, subjects with symptomatic hypoglycemia were rescued with dextrose to maintain blood glucose levels 3.3 mmol/L (>60 mg/dL), therefore limiting the treatment effect that we could detect. All the subjects included in this study have mutations in *ABCC8*, but some mutations were recessive and biallelic, while others were dominant and monoallelic. Nevertheless, the hyperglycemic effects of exendin-(9-39) were seen in all subjects, irrespective of their mutation.

Overall, similar to the findings of our preclinical studies, our data show that exendin-(9-39) elevates fasting glucose in human subjects with K$_{ATP}$HI. These data do not necessarily indicate that there is a specific involvement of GLP-1 signaling in the pathogenesis of K$_{ATP}$HI. Nevertheless, our findings offer some insight into the pathophysiology of K$_{ATP}$HI and also point to the GLP-1 receptor as a therapeutic target for this condition. Our promising results suggest a significant role for GLP-1 receptor action as an integral mechanism in the control of fasting glycemia in K$_{ATP}$HI. Given the lack of available therapies, antagonism of the GLP-1 receptor represents a novel therapeutic target to control hypoglycemia in congenital hyperinsulinism and could have a beneficial impact on morbidity and improve long-term outcomes for this devastating disorder.

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