Amplified Hormonal Counterregulatory Responses to Hypoglycemia in Rats After Systemic Delivery of a SUR-1–Selective K^+ Channel Opener?

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OBJECTIVE—In glucose-sensing neurons, ATP-sensitive K^+ channels (K_{ATP} channels) are thought to translate metabolic signals into an alteration in neuronal firing rates. Because these neurons express the Kir6.2/SUR-1 isoform of the K_{ATP} channel, we sought to examine the therapeutic potential of the SUR-1–selective potassium channel opener (KCO), NN414, to amplify counterregulatory response to hypoglycemia.

RESEARCH DESIGN AND METHODS—In vivo dose-response studies with NN414 delivered intravenously to normal Sprague-Dawley rats before the induction of controlled hypoglycemia were performed. Based on these studies, the potential for NN414 to restore counterregulatory responses in chronically cannulated nondiabetic and diabetic BB rats was explored using the in vivo hyperinsulinemic-hypoglycemic clamp technique.

RESULTS—NN414 delivered systemically amplified epinephrine responses during acute hypoglycemia and showed a persisting effect to amplify the epinephrine response when given 24 h before the hypoglycemic study. Local delivery of a potassium-channel blocker to the ventromedial hypothalamus reversed the effects of systemic NN414. In addition, NN414 amplified the epinephrine response to hypoglycemia in both nondiabetic and diabetic BB rats with defective hormonal counterregulation.

CONCLUSIONS—These studies demonstrate in a variety of rodent models that systemic delivery of Kir6.2/SUR-1–selective KCOs enhance the glucose counterregulatory response to insulin-induced hypoglycemia. Future studies in human subjects are now required to determine their potential as a therapy for hypoglycemia-associated autonomic failure in type 1 diabetes. ***Diabetes* 57:3327–3335, 2008**

The benefits of lowering average blood glucose levels in type 1 diabetes to reduce the risk of long-term microvascular complications are well established. In clinical practice, the degree to which this can be achieved is often limited by the increased risk of severe hypoglycemia that accompanies intensified glucose-lowering regimens (1,2). Individuals with type 1 diabetes are, moreover, particularly prone to develop hypoglycemia because of defects in the normal compensatory homeostatic defense response. Almost all individuals with type 1 diabetes fail to release glucagon in response to hypoglycemia, a defect that appears to relate to the progressive loss of β-cell function (3) and is thought to arise predominantly through the loss of intraislet insulin signaling (4). This leaves epinephrine as the major hormonal counterregulatory defense against low blood glucose. However, a majority of patients will also develop additional deficiencies in the epinephrine counterregulatory response (5). It has now been established in both rodent (6) and human (7) studies that antecedent exposure to hypoglycemia is a major factor involved in the genesis of this defect. Several small trials in human subjects have shown that strict avoidance of hypoglycemia can improve epinephrine (8,9) and symptomatic (10) responses during a subsequent hypoglycemic clamp study. The difficulty in achieving strict hypoglycemia avoidance in both of these trials means that this intervention has not become part of routine clinical practice. Thus, therapeutic options designed to limit the impact of hypoglycemia during intensive insulin therapy remain limited.

The stimulus to epinephrine release during hypoglycemia is thought to result from activation of specialized glucose-sensing neurons within the brain (11–14) and periphery (15). Glucose-sensing neurons use glucose as a signaling molecule to alter their firing rate and are of two predominant subtypes, namely, glucose-excited neurons, whose firing rate increases, and glucose-inhibited neurons, whose firing rate decreases, as ambient glucose levels rise (16–18). Based largely on data in rodents, it is currently believed that glucose-sensing neurons react to alterations in extracellular glucose using mechanisms similar to those used by the pancreatic β-cell, with glucokinase and the ATP-sensitive K^+ channel (K_{ATP}) as key steps in this process (19,20).

K_{ATP} channels provide a link between neuronal metabolism and membrane potential in many tissues (21,22). Classical K_{ATP} channels comprise two subunits: a receptor (SUR-1, SUR-2A, or SUR-2B) of sulfonylureas and an inward-rectifier K^+ channel member, Kir6.2 (22,23). Skeletal muscle and cardiac K_{ATP} channels comprise SUR-2A and Kir6.2, whereas the pancreatic β-cell K_{ATP} channel, the prototype glucose-sensing cell, comprises SUR-1 and Kir6.2 (21–24). In the pancreas, the K_{ATP} channel has been shown to play a key role in the mechanism by which β-cells regulate insulin release in response to changes in the glucose to which they are exposed (25,26). K_{ATP} channels have been demonstrated throughout the brain, including hypothalamic regions thought to be involved in glucose sensing (27–29). Examination of gene expression in glucose-sensing neurons using single-cell RT-PCR identified mRNA for SUR-1 and Kir6.2 (30). Electrophysiological studies of rat (20,31,32) and mouse brain slice

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TABLE 1
Effect of different intravenous doses of the SUR-1–selective KCO, NN414, on glucose, insulin, and C-peptide under basal and hyperinsulinemic-hypoglycemic conditions

Variable	Time point	Control	0.06 mg/kg NN414	0.6 mg/kg NN414	6 mg/kg NN414
n		8	6	10	8
Glucose (mg/dl)	Basal	119 ± 4	120 ± 6	126 ± 4	122 ± 4
	After injection	111 ± 5	115 ± 4	125 ± 4	115 ± 6
	Hypoglycemia	46 ± 1	51 ± 1*	52 ± 2*	41 ± 2
Insulin (pmol/l)	Basal	491 ± 107	322 ± 68	589 ± 213	519 ± 130
	After injection	480 ± 95	285 ± 62	346 ± 105	451 ± 108
	Hypoglycemia	3,860 ± 237	4,009 ± 583	3,409 ± 611	4,520 ± 550
C-peptide (pmol/l)	Basal	360 ± 51	477 ± 65	611 ± 47	308 ± 53
	After injection	271 ± 38	400 ± 40	426 ± 57	75 ± 10†
	Hypoglycemia	49 ± 2	45 ± 2	50 ± 2	41 ± 4

*$P < 0.05$ vs. control; †$P < 0.05$ after injection vs. baseline.

preparations (33) have demonstrated that sulfonylureas can stimulate the firing of glucose-excited neurons and can alter the response of glucose-excited neurons to changes in ambient glucose levels. In animal models, transgenic Kir6.2 knockout mice show impaired glucose counterregulation (33), and we have recently shown in vivo that pharmacological closure of the K_{ATP} channel in the ventromedial hypothalamus (VMH; a key glucose-sensing region) via direct microinjection of glibenclamide suppressed (34), whereas K_{ATP} channel openers (KCOs) amplified, the counterregulatory response to hypoglycemia in both normal and recurrently hypoglycemic rats (35). In this later study, the SUR-1–selective KCO NN414, when microinjected into the VMH, a key brain glucose-sensing region (36), amplified hypoglycemic counterregulation at significantly lower concentrations than the nonselective KCO, diazoxide (35).

Taken together, these data suggest that K_{ATP} channels allow glucose-sensing neurons to translate the metabolic signal into an alteration in neuronal firing rates and, moreover, that KCOs may offer a potential therapeutic option for individuals with type 1 diabetes. In the current study, this hypothesis is explored in a series of rodent models. Our data show that systemic administration of a SUR-1–selective KCO has a consistent effect to amplify the epinephrine response to controlled hypoglycemia in nondiabetic and diabetic rats and in those with defective counterregulation.

RESEARCH DESIGN AND METHODS

Male Sprague-Dawley ($n = 102$; weight 305 ± 4 g [means ± SE]) and diabetic BB rats ($n = 12$; disease duration 2–4 weeks) were housed in the Yale Animal Resource Center, fed a standard pellet diet (Agway Prolab 3000), and maintained on a 12-h/12-h day/night cycle. Diabetic BB rats were maintained on once-daily protamine zinc insulin, with doses based on body weight, 9:00 A.M. glucose, and protocol. The animal care and experimental protocols were reviewed and approved by the Yale Institutional Animal Care and Use Committee.

Surgery. One week before each study, all animals were anesthetized with an intraperitoneal injection (1 ml/kg) of a mixture of xylazine (20 mg/ml AnaSed; Lloyd Laboratories, Shenandoah, IA) and ketamine (100 mg/ml Ketaset; Aveco, Fort Dodge, IA) in a ratio of 1:2 (vol:vol), before undergoing vascular surgery for the implantation of vascular catheters in a carotid artery and jugular vein. In some rodents, after this procedure, microinjection guide cannulas were bilaterally inserted into the VMH (coordinates from bregma: antero-posterior −2.6 mm, medio-lateral ±3.8 mm, and dorso-ventral −8.3 mm at an angle of 20°), as described previously (34).

Recurrent hypoglycemia protocol. In these studies, rats received an intraperitoneal injection of human regular insulin (Eli Lilly, Indianapolis, IN) at a dose of 10 units/kg given once daily for the 3 consecutive days before the glucose clamp study. Each episode of hypoglycemia (tail vein blood glucose 1.7–2.2 mmol/l [30–40 mg/dl]) lasted for ~3 h, after which the rats were given free access to food and intraperitoneal dextrose, if required. Any rats experiencing a seizure were excluded and did not undergo a subsequent clamp study. The model of recurrent hypoglycemia used in our laboratory has been previously reported in detail and has been shown to induce suppression of epinephrine responses to subsequent hypoglycemia (37).

KCOs. This study used intravenous delivery of the SUR-1–selective KCO NN414 (Novo Nordisk A/S, Malvo, Denmark) (38). NN414 was prepared in 1.5 $mg\ m^{-1}$ artificial extracellular fluid (aECF) for systemic delivery. In all studies, NN414 was delivered systemically 30 min before the induction of hypoglycemia.

Microinjection. On the morning of the study, 26-gauge microinjection needles, designed to extend 1 mm beyond the tip of the guide cannula (Plastics One, Roanoke, VA), were inserted bilaterally to the VMH. Rats were then microinjected over 2.5 min (0.1 μl/min) with either glibenclamide (12.4 ng dissolved in aECF and 0.5% DMSO or control [aECF with 0.5% DMSO]) using a CMA-102 infusion pump (CMA Microdialysis, North Chelmsford, MA) (34). Microinjection occurred immediately before the hyperinsulinemic-hypoglycemic clamp study as detailed below. At the end of the study, the rats were killed, and probe position was confirmed in all rats.

Infusion protocol. In all experiments, the same hyperinsulinemic-hypoglycemic clamp infusion protocol was used. Overnight-fasted rats had their vascular catheters opened and were then allowed to settle over 90 min. Thereafter, a hyperinsulinemic-hypoglycemic clamp technique as adapted for the rat (6) was initiated. Briefly, at time = 0, a 90-min 20 $mU \cdot kg^{-1} \cdot min^{-1}$ infusion of human regular insulin (Eli Lilly) was started, and the plasma glucose was allowed to fall to 50 mg/dl (~2.8 mmol/l), where it was maintained for 90 min using a variable rate 20% dextrose infusion (based on frequent [−5 min] plasma glucose determinations). Samples for measurement of the hormones epinephrine, norepinephrine, glucagon, insulin, and C-peptide were taken at −30, 0, 60, and 90 min.

Analytical procedures. Plasma glucose was measured by the glucose oxidase method (Beckman, Fullerton, CA). Catecholamine analysis was performed by high-performance liquid chromatography using electrochemical detection (ESA, Acton, MA); plasma insulin and glucagon were measured by radioimmunoassay (Linco, St. Charles, MO). All data are expressed as the means ± SE and were compared using Student's two-tailed t test. (Prism 4.0; GraphPad Software, San Diego, CA).

RESULTS

SUR-1 KCO delivery amplifies the epinephrine response to hypoglycemia in a dose-dependent manner. Overnight-fasted rats were given intravenous bolus injections of NN414 in doses of 6, 0.6, and 0.06 mg/kg or vehicle 30 min before performing a hypoglycemic clamp study ($n = 6–10$ rats in each group). Additional studies were performed with 0.006 mg/kg NN414 ($n = 4$), but these data did not differ significantly from the control studies (data not shown). Basal ($t = -30$ min), after NN414 injection ($t = 0$ min), and hypoglycemia ($t = 90$ min) levels of glucose, C-peptide, and insulin are shown in Table 1. Of

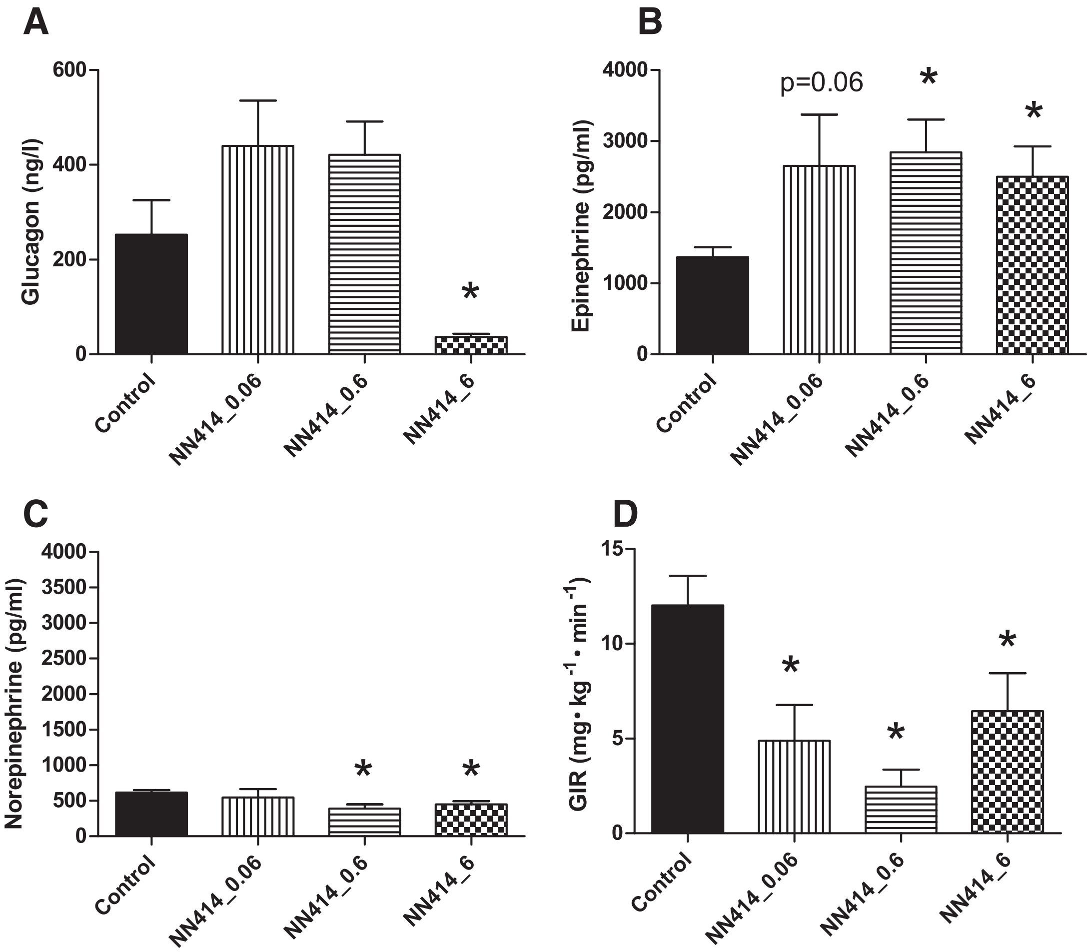

FIG. 1. Systemic delivery of the SUR-1–selective KCO NN414 amplifies the glucose counterregulatory response to acute hypoglycemia in nondiabetic rodents. Peak glucagon (*A*), peak epinephrine (*B*), peak norepinephrine (*C*), and GIRs (*D*) required to maintain the hypoglycemic clamp are shown for control (■), 0.06 mg/kg NN414 (▥), 0.6 mg/kg NN414 (▤), and 6 mg/kg NN414 (▩) studies. *$P < 0.05$ vs. control.

note, only the higher dose of NN414 (6 mg/kg) induced a significant fall in C-peptide before initiation of the hypoglycemic clamp study (308 ± 53 to 75 ± 16 pmol/l; $P < 0.05$). In addition, mean plasma glucose achieved during the clamp procedure was slightly but significantly higher in those rats that had been given 0.6 and 0.06 mg/kg NN414.

During the basal period, mean counterregulatory hormone levels did not differ between groups and were not affected by injection of NN414 or control (data not shown). Hypoglycemia induced significant rises in all counterregulatory hormones in both control and NN414 groups, with the exception of high-dose NN414, in which glucagon failed to rise during hypoglycemia, despite the marked reduction in C-peptide (Fig. 1*A*). In contrast, NN414 had a marked and significant effect on plasma epinephrine during hypoglycemia (Fig. 1*B*). NN414 given at 0.6 mg/kg produced an ~108% increase in the peak plasma epinephrine over the control group. The rise in plasma norepinephrine (Fig. 1*C*) was substantially less than the rise in epinephrine, and peak levels were lower in rats treated with 0.6 and 6 mg/kg NN414. However, the overall glucose counterregulatory response was significantly amplified with NN414, with all doses resulting in a clear reduction in the amount of exogenous glucose (glucose infusion rate [GIR]; Fig. 1*D*) required to maintain the hypoglycemic clamp. After 0.6 mg/kg NN414, this represented an ~80% reduction in the GIR.

To explore the utility of the nonselective KCO diazoxide, an identical hyperinsulinemic hypoglycemia study was performed in Sprague-Dawley rats. Diazoxide (6 mg/kg; $n = 6$) delivered systemically significantly amplified the mean (± SE) peak epinephrine (2,307 ± 342 vs. 1,479 ± 205 pg/ml, diazoxide vs. control, respectively; $P < 0.05$) but not the peak norepinephrine (507 ± 89 vs. 539 ± 29 pg/ml; NS) or peak glucagon (354 ± 38 vs. 252 ± 82 ng/l; $P = 0.2$) response to hypoglycemia. Diazoxide-treated rats also required less glucose during the hypoglycemic clamp study (4.9 ± 1.6 vs. 10.5 ± 1.8 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $P < 0.05$). C-peptide levels fell after diazoxide injection (476 ± 64 to 313 ± 43 pmol/l; $P < 0.05$) and during the hypoglycemic clamp study (34 ± 6 pmol/l).

Antecedent delivery of SUR-1 KCO amplifies epinephrine response to subsequent hypoglycemia. To determine whether the SUR-1 KCO NN414 would have a persisting effect on counterregulatory responses to hypoglycemia, chronically catheterized Sprague-Dawley rats were injected once daily intravenously with 0.6 mg/kg NN414 ($n = 7$) or vehicle ($n = 7$) for 3 consecutive days. On day 4, the overnight-fasted rats underwent a hypoglycemic clamp study. Mean (± SE) plasma glucose (47 ± 3 vs. 44 ± 3 mg/dl; NS), insulin (4,347 ± 723 vs. 4,876 ± 642 pmol/l; NS) and C-peptide (39 ± 3 vs. 52 ± 11 pmol/l; NS) during hypoglycemia did not differ between antecedent control or NN414-injected groups, respectively. However, during hypoglycemia, antecedent NN414 resulted in an

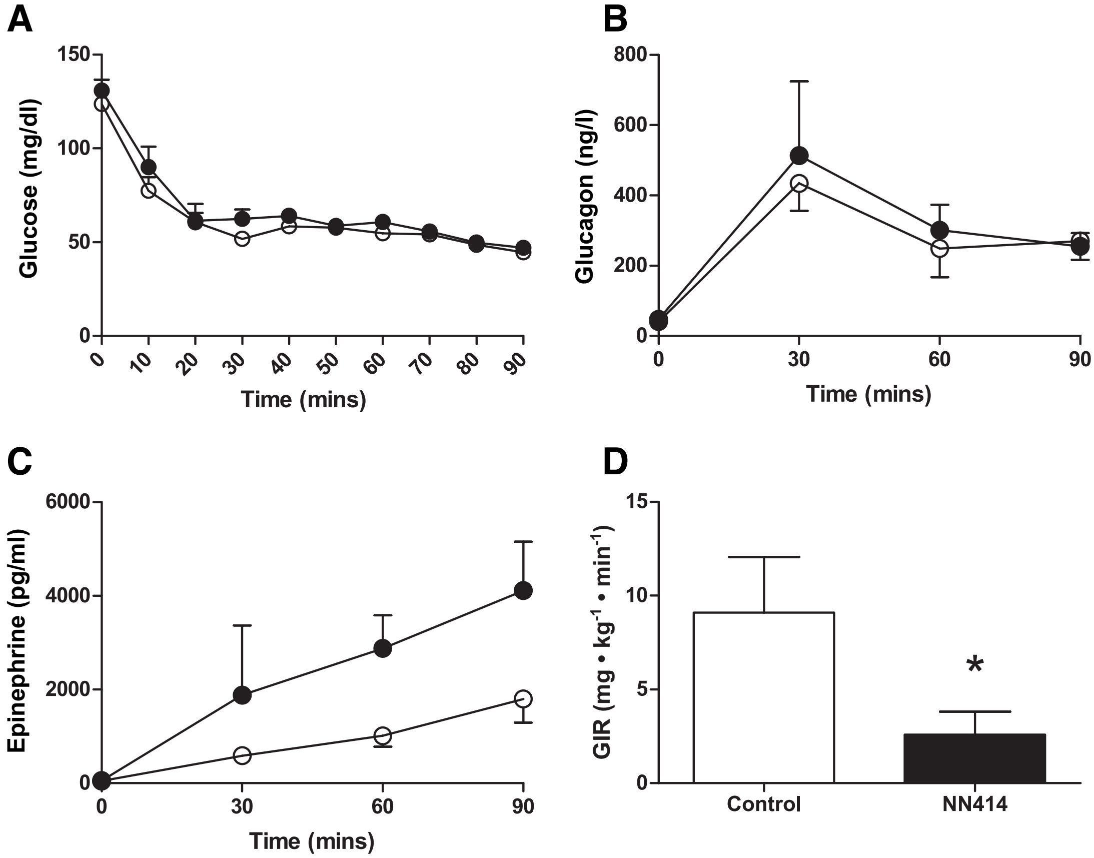

FIG. 2. Antecedent delivery of SUR-1 KCO amplifies epinephrine response to subsequent hypoglycemia. Graphs show plasma glucose (*A*), plasma glucagon (*B*), plasma epinephrine (*C*), and GIR (*D*) during hyperinsulinemic hypoglycemia in rats injected once daily on 3 prior consecutive days with either control (○, □) or 0.6 mg kg^{-1} NN414 (●, ■). Values are shown as means ± SE. *$P < 0.05$.

~54% increase in the peak epinephrine response and an ~71% reduction in the GIR required to maintain the hypoglycemic clamp (Fig. 2*A*–*D*).

VMH K_{ATP} channel blockade reverses the action of systemic SUR-1 KCO. To determine whether the systemic effects of the KCO NN414 were potentially mediated through an action on the VMH, a key glucose-sensing region, rats were systemically administered with 0.6 mg/kg NN414 30 min before performing a hyperinsulinemic-hypoglycemic clamp study as above. In addition, 5 min before the start of the clamp, the rats were bilaterally microinjected to the VMH with the K_{ATP} channel blocker, glibenclamide (12.4 ng in 0.25 μl; $n = 10$), or control solution (vehicle; $n = 11$). Mean plasma glucose, insulin, and C-peptide did not differ between groups under basal or hypoglycemic conditions (data not shown) nor did basal levels of the counterregulatory hormones (Fig. 3*A*–*C*). In control studies, systemic NN414 combined with VMH-vehicle microinjection produced a similar stimulus to epinephrine and glucagon as that seen in the dose-response studies. This effect was significantly blunted when systemic NN414 (KCO) was combined with VMH-glibenclamide (K_{ATP} channel closer) (Fig. 3*A*–*C*). VMH-glibenclamide resulted in a 52% reduction in peak plasma epinephrine (4,287 ± 807 vs. 2,074 ± 316 pg/medio-lateral; control vs. glibenclamide, respectively; $P < 0.05$) and a 90% increase in mean GIR during hypoglycemia (4.2 ± 1.0 vs. 8.0 ± 1.2 mg · kg^{-1} · min^{-1}, respectively; $P < 0.05$). As in the previous studies, no effect was seen on peak glucagon (290 ± 48 vs. 321 ± 51 ng/l; NS) or norepinephrine (546 ± 55 vs. 573 ± 122 pg/ml; NS).

Systemic SUR-1 KCO delivery amplifies the epinephrine response to hypoglycemia in nondiabetic and diabetic BB rats exposed to prior hypoglycemia. To determine whether NN414, given systemically, would improve the counterregulatory responses to hypoglycemia in a rodent model of hypoglycemia-associated autonomic failure (HAAF), hyperinsulinemic-hypoglycemic clamp studies were performed in nondiabetic rodents who had been exposed to three consecutive, once-daily episodes of 10 mU/kg insulin-induced hypoglycemia. We have previously reported that this model induces defective counterregulatory responses to subsequent hypoglycemia (35,37). As in the previous studies, rats received 0.6 mg/kg NN414 or vehicle delivered intravenously 30 min before the clamp procedure. Basal levels of glucose, insulin, and counterregulatory hormones did not differ between groups nor did levels of glucose and insulin during the clamp procedure. Plasma C-peptide fell in both groups from baseline values of 218 ± 45 and 264 ± 39 to 34 ± 6 and 28 ± 4 pmol/l (control vs. NN414, respectively; NS). Consistent with the earlier studies, no effect of NN414 was seen on peak plasma glucagon (Fig. 4*A*), whereas the peak epinephrine response during the subsequent hypoglycemic challenge was increased by ~114% (Fig. 4*B*; $P < 0.05$). The increased glucose counterregulatory response was reflected in a 70% reduction in the GIR required to maintain the hypoglycemic clamp (Fig. 4*D*; $P < 0.01$).

Subsequently, we examined the effect of systemic NN414 in diabetic BB rats, a rodent model of type 1 diabetes. The diabetic BB rats were also subjected to 3-day antecedent hypoglycemia to induce further defects in the

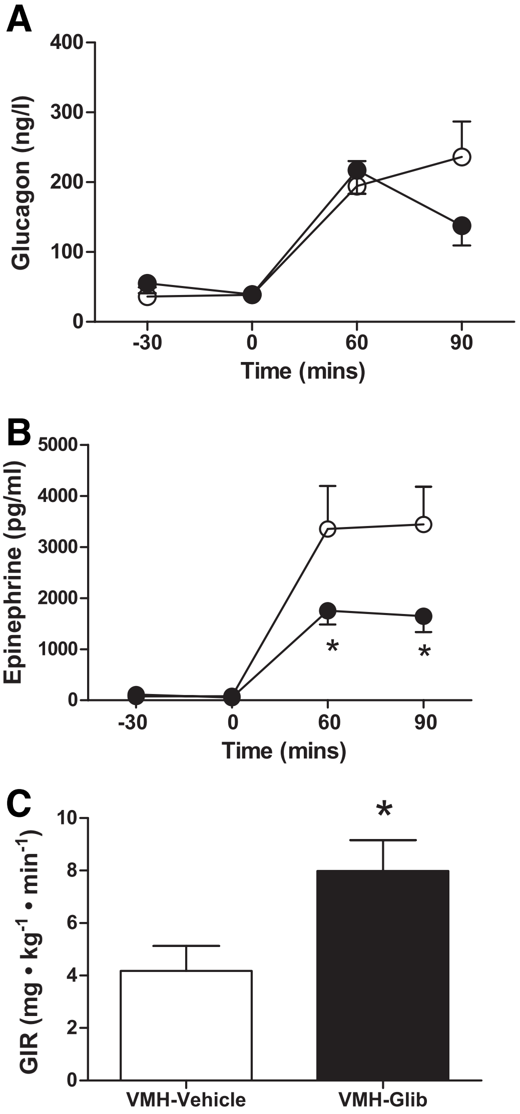

FIG. 3. Local VMH K_{ATP} channel blockade blunts the effect of systemic KCOs to amplify the glucose counterregulatory response to hypoglycemia. Plasma glucagon (*A*), plasma epinephrine (*B*), and GIR (*C*) during hyperinsulinemic hypoglycemia in rats pretreated with systemic NN414 and with VMH microinjection of glibenclamide (●, ■) or systemic NN414 and VMH microinjection of vehicle (○, □). Values are shown as means ± SE. *$P < 0.005$ vs. control study.

counterregulatory response. Basal levels of glucose, insulin, and counterregulatory hormones did not differ between groups nor did levels of glucose and insulin during the clamp procedure. As expected, plasma C-peptide was low under basal (42 ± 10 and 36 ± 3 pmol l^{-1}) and hypoglycemic (36 ± 1 and 29 ± 3 pmol/l) conditions and did not differ between groups (control [$n = 6$] vs. NN414 [$n = 6$], respectively). In the diabetic BB rats, no significant rise in plasma glucagon from baseline was seen during hypoglycemia in either group, and peak plasma glucagon during hypoglycemia also did not differ between groups (Fig. 5*A*). However, as in the nondiabetic rats, systemic NN414 produced a marked stimulus to epinephrine secretion (~200% increase; Fig. 5*B*; $P < 0.05$), although absolute epinephrine levels remained lower than those seen in the nondiabetic rats. In addition, NN414 significantly amplified the norepinephrine response during hypoglycemia (Fig. 5*C*; $P < 0.05$), the overall effect being to reduce GIR by ~60% (Fig. 5*D*; $P < 0.01$).

DISCUSSION

In the present study, systemically delivered SUR-1–selective KCO was shown to amplify the glucose counterregulatory response to acute hypoglycemia in normal and recurrently hypoglycemic nondiabetic rats and in diabetic BB rats exposed to recurrent hypoglycemia (a rodent model of type 1 diabetes with markedly impaired glucose counterregulation). Moreover, the SUR-1–selective KCO was effective when given acutely or when delivered for 3 consecutive days before the hyperinsulinemic-hypoglycemic clamp study. Taken together, these studies in the rat suggest that SUR-1–selective KCOs may have therapeutic potential for the treatment of HAAF in type 1 diabetic humans.

The K_{ATP} channel consists of pore-forming Kir6.x subunits that associate with different types of regulatory sulfonylurea receptor subunits: SUR-1, SUR-2A, and SUR-2B. SUR-2 channels are predominantly expressed in cardiac/skeletal muscle (SUR-2A) and vascular smooth muscle (SUR-2B) (39), activation of which can cause vasodilation and effect cardiac muscle contractility. Diazoxide activates both SUR-1 and SUR-2B regulatory subunits. When delivered directly to the VMH, diazoxide can amplify the counterregulatory response to hypoglycemia (35), and in the current study, when given systemically at a dose of 6 mg/kg, diazoxide can also amplify the counterregulatory response (the higher dose required consistent with the reduced potency of diazoxide to activate SUR-1 compared with NN414 [38]). However, through its action on the SUR-2B subunit of the K_{ATP} channel, the use of diazoxide is limited by its potential to cause vasodilation, reflex tachycardia, and hirsuitism (also thought to be SUR2 mediated). The current studies used a compound, NN414, developed by Novo Nordisk, as a selective SUR-1 KCO. In vitro studies have shown that NN414 is highly selective for the Kir6.2/SUR-1 channel, with no significant activation of SUR-2A and -2B channels (38). NN414, even at high doses, has been shown to have no effect on blood pressure (40). Thus, for an effective long-term therapy for individuals with type 1 diabetes, a SUR-1–selective agent is liable to be both more effective in its action on glucose sensing and less likely to be associated with these adverse effects.

Our findings contrast with those of Raju and Cryer (41), who examined the effect of oral diazoxide (6 mg/kg) on the counterregulatory responses to hypoglycemia in 14 nondiabetic humans. Diazoxide, given orally, suppressed basal C-peptide and suppressed the glucagon response to hypoglycemia induced 2 h later while having no effect on epinephrine, norepinephrine, or neurogenic symptoms (41). Similarly, Bingham et al. (42) reported no effect of oral diazoxide (5 mg/kg) on the hormonal counterregulatory response in 10 nondiabetic humans. In our rodent study, diazoxide given intravenously 30 min before hypoglycemia also suppressed C-peptide but resulted in an amplified epinephrine response during hypoglycemia. The glucagon response was also increased but not significantly so. These differences may reflect species differences in Kir6.2/SUR-1 channel expression and function, and in addition, the higher systemic diazoxide levels that were probably achieved in the rodent may have induced a degree of hypotension (not measured in the present study) that may have contributed to the epinephrine response. However, this would not explain the NN414 findings. Furthermore, in pilot studies we found that oral NN414 (1.2

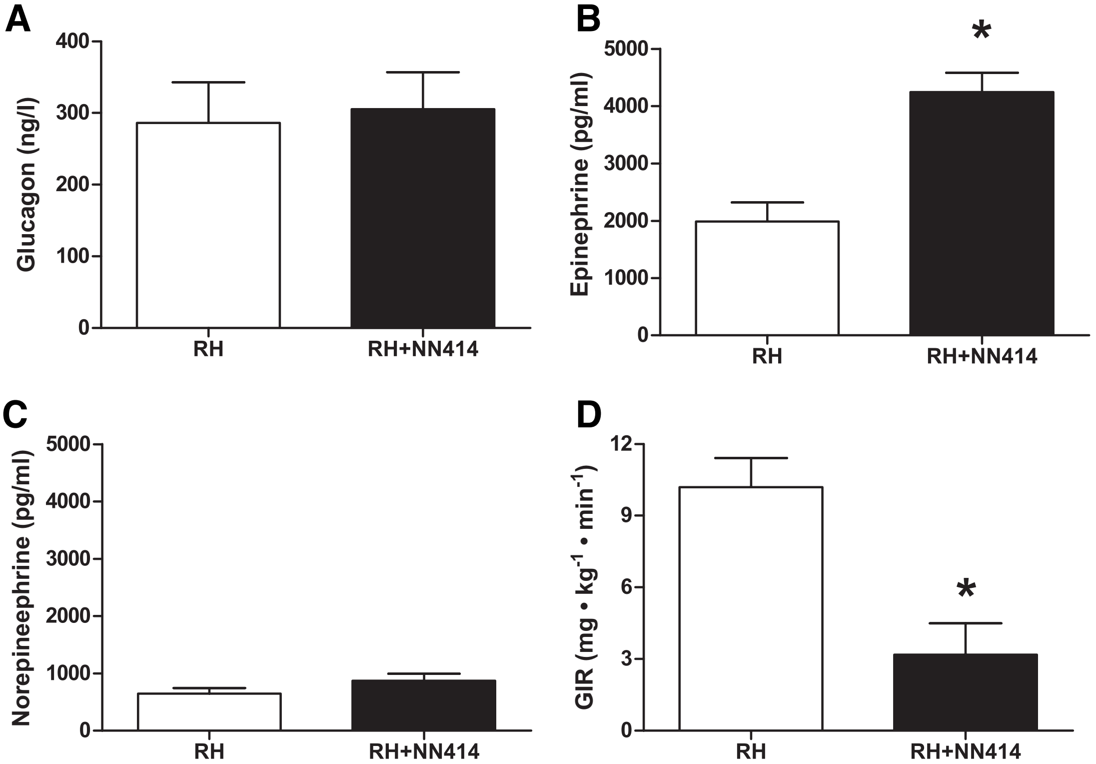

FIG. 4. Systemic delivery of a SUR-1–selective KCO (0.6 mg kg^{-1} NN414) amplified the glucose counterregulatory response to acute hypoglycemia in nondiabetic rodents with defective counterregulation after exposure to three episodes of antecedent recurrent hypoglycemia (RH). Peak glucagon (*A*), peak epinephrine (*B*), peak norepinephrine (*C*), and GIRs (*D*) during subsequent hyperinsulinemic hypoglycemia are shown. □, Recurrent hypoglycemia control rats; ■, NN414-treated recurrent hypoglycemia rats. Values are shown as means ± SE. *P < 0.005 vs. control study.

mg/kg) has the same effect to amplify counterregulation during hypoglycemia (data not shown). The consistent effect of NN414 to amplify the epinephrine counterregulatory response suggests that higher systemic levels of diazoxide may be necessary to induce a SUR-1–mediated action on the catecholaminergic response to hypoglycemia.

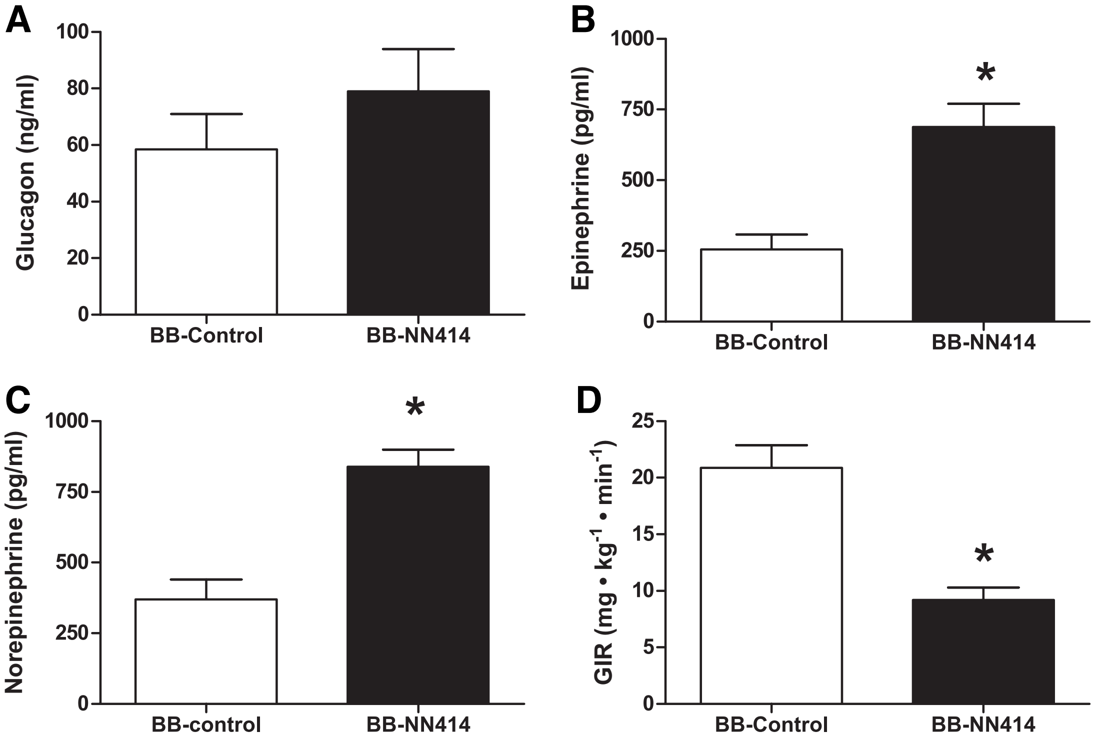

FIG. 5. Systemic delivery of a SUR-1–selective KCO (0.6 mg kg^{-1} NN414) amplified the glucose counterregulatory response to acute hypoglycemia in diabetic rats (BB) with defective counterregulation. Peak glucagon (*A*), peak epinephrine (*B*), peak norepinephrine (*C*), and GIRs (*D*) during subsequent hyperinsulinemic hypoglycemia. □, BB control rats; ■, NN414-treated BB rats. Values are shown as means ± SE. *P < 0.005 vs. control study.

It was notable in the present study that high-dose NN414 markedly suppressed glucagon secretion during hypoglycemia. Before induction of the hypoglycemic clamp, plasma C-peptide was also more markedly suppressed by high-dose NN414. This latter finding is consistent with the intraislet insulin hypothesis, in which defective glucagon secretion during hypoglycemia is thought to arise through a failure of intraislet insulin levels to fall (41). Alternatively, it may represent a direct action of NN414 on K_{ATP} channels in the pancreatic α-cells. It has been shown in α-cells isolated from SUR-$1^{-/-}$ mice, but not wild-type mice, that both tolbutamide and glucose fail to produce membrane depolarization and that diazoxide reduces glucagon secretion to the same extent as an elevation of glucose (43). This effect might arise from the distinct electrophysiological properties of α-cells.

It was also notable that norepinephrine responses during hypoglycemia did not parallel those of epinephrine. During hypoglycemia, systemic norepinephrine has not proven discriminatory in previous clamp studies in rodents by our group (34,35). Norepinephrine is released primarily from sympathetic nerve terminals, and so, systemic levels provide only a weak index of local sympathetic neural activity. Plasma norepinephrine is also both lower than epinephrine (~10–25%) and a less potent agonist of adrenergic receptors, so small differences between groups in systemic levels are not informative. It was also of note that the absolute effect of NN414 was less in diabetic BB rats than in nondiabetic rats. This suggests that additional mechanisms, not reversible through KCO therapy, may contribute to defective counterregulation in diabetes.

It is interesting to speculate on where the systemically delivered KCO might act. Although the Kir6.x pore-forming unit is widely expressed in the body, the expression of SUR-1 is predominantly limited to brain and pancreas (44). Within the brain, SUR-1 and Kir6.x are expressed both in regions known to be involved in glucose sensing and in other brain regions (45). Glucose-sensing neurons in the VMH are known to express SUR-1 (30). Our data demonstrating that the action of systemic NN414 could be reversed after the direct application of a K_{ATP} channel blocker to the VMH suggest this is one potential site that mediates the effect of NN414. That being said, it is also possible that the changes seen were the net result of K_{ATP} channel opening at a different site and an independent action of K_{ATP} channel closure in the VMH. NN414, if acting centrally, could equally be acting on glucose-sensing neurons in other brain regions such as the hindbrain. Sulfonylurea-like compounds are not generally thought to cross the blood-brain barrier (46), although recent studies of KCOs in ischemic preconditioning in the brain provide some evidence that they might act centrally (47). Unfortunately, a radiolabeled form of NN414 is not available to directly test this question. Another possibility is that NN414 is interacting with portal-mesenteric vein (PMV) glucose sensors. PMV glucose sensors play a role in the modulation of counterregulatory responses to hypoglycemia. However, PMV glucose sensors are thought to play a greater role in slow-fall rather than the rapid induction of hypoglycemia induced in the present study (48), and a role for SUR-1 in these sensors has not been established. Another peripheral glucose sensor is located in the carotid body, but dogs with carotid body resections showed blunted glucagon and cortisol secretion during hypoglycemia, with epinephrine and norepinephrine release being unaffected (49). Finally, SUR-1 and Kir6.2 are coexpressed with glucagon-like peptide 1 (GLP-1) in intestinal L- and K-cells (50), which raises the possibility of an indirect action through altered GLP-1 secretion. However, we have previously shown in human subjects that glucose ingestion while hypoglycemia is maintained leads to an amplification of the epinephrine response (51), which would suggest that K_{ATP} closure, rather than opening, within L-cells mediates this effect. Moreover, GLP-1 secretion is liable to be minimal during hypoglycemia.

One other notable finding in the present study was that 3 days of NN414 given as a single intravenous bolus resulted in an amplified epinephrine response to a subsequent controlled hypoglycemic challenge. The ability of a compound to amplify counterregulatory responses to a subsequent episode of hypoglycemia and not just when given during the hypoglycemic episode would be important for type 1 diabetic individuals in whom hypoglycemia does usually occur at predictable times. Why this effect should occur after 3 days of NN414 therapy is unknown. NN414 given to human subjects at a similar dose (0.625 mg/kg) has a half-life of only 1.2 h (40), and so it seems unlikely to represent a persisting direct action on the Kir6.2/SUR-1 channel. This finding therefore raises the possibility that NN414 could through secondary effects render the glucose-sensing pathway more sensitive to a subsequent hypoglycemic challenge.

In summary, in the current study, we have been able to demonstrate in a variety of rodent models that the SUR-1/Kir6.2–selective KCO when given systemically is able to amplify epinephrine responses to hypoglycemia. These studies provide the first evidence of a potential therapeutic intervention for HAAF in type 1 diabetes. The dose of NN414 that we used in these rodent studies compares with that of trials in human subjects assessing pharmacokinetics after single-dose NN414 (0.625–12.5 mg/kg) (40); however, no human studies using this compound during hypoglycemia have as yet been performed. Future studies in rodent models designed to establish the best strategies for delivering these agents and in human subjects to validate the rodent data are now required.

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