Original Article

Activation of ATP-Sensitive K^+ Channels in the Ventromedial Hypothalamus Amplifies Counterregulatory Hormone Responses to Hypoglycemia in Normal and Recurrently Hypoglycemic Rats

Rory J. McCrimmon,[1] Mark L. Evans,[1] Xiaoning Fan,[1] Ewan C. McNay,[1] Owen Chan,[1] Yuyan Ding,[1] Wanling Zhu,[1] Dorte X. Gram,[2] and Robert S. Sherwin[1]

The mechanism(s) by which glucosensing neurons detect fluctuations in glucose remains largely unknown. In the pancreatic β-cell, ATP-sensitive K^+ channels (K_{ATP} channels) play a key role in glucosensing by providing a link between neuronal metabolism and membrane potential. The present study was designed to determine in vivo whether the pharmacological opening of ventromedial hypothalamic K_{ATP} channels during systemic hypoglycemia would amplify hormonal counterregulatory responses in normal rats and those with defective counterregulation arising from prior recurrent hypoglycemia. Controlled hypoglycemia (~2.8 mmol/l) was induced in vivo using a hyperinsulinemic (20 mU · kg^{-1} · min^{-1}) glucose clamp technique in unrestrained, overnight-fasted, chronically catheterized Sprague-Dawley rats. Immediately before the induction of hypoglycemia, the rats received bilateral ventromedial hypothalamic microinjections of either the potassium channel openers (KCOs) diazoxide and NN414 or their respective controls. In normal rats, both KCOs amplified epinephrine and glucagon counterregulatory responses to hypoglycemia. Moreover, diazoxide also amplified the counterregulatory responses in a rat model of defective hormonal counterregulation. Taken together, our data suggest that the K_{ATP} channel plays a key role in vivo within glucosensing neurons in the ventromedial hypothalamus in the detection of incipient hypoglycemia and the initiation of protective counterregulatory responses. We also conclude that KCOs may offer a future potential therapeutic option for individuals with insulin-treated diabetes who develop defective counterregulation. *Diabetes* 54:3169–3174, 2005

From the [1]Department of Internal Medicine and Endocrinology, Yale University School of Medicine, New Haven, Connecticut; and [2]Pharmacology Research 3, Novo Nordisk, Mālløv, Denmark.

Address correspondence and reprint requests to Rory J. McCrimmon, MD, Yale University School of Medicine, Section of Endocrinology, 300 Cedar St., P.O. Box 208020, New Haven, CT 06520-8020. E-mail: rory.mccrimmon@yale.edu.

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aECF, artificial extracellular fluid; AUC, area under the curve; GIR, glucose infusion rate; K_{ATP} channel, ATP-sensitive K^+ channel; KCO, potassium channel opener; VMH, ventromedial hypothalamus.

The importance of glucose as a fuel, especially for the brain, ensures that numerous homeostatic mechanisms have evolved that serve to maintain the blood glucose within a relatively narrow physiological range. In type 1 diabetes, supraphysiological insulin replacement therapy and defective glucose counterregulatory mechanisms combine to disrupt normal glucose homeostasis and significantly increase the risk of hypoglycemia (1). As clinicians strive to lower average blood glucose levels further in an attempt to reduce complications related to chronic hyperglycemia, the risk of moderate to severe hypoglycemia increases further (2). Severe hypoglycemia is understandably feared by individuals with type 1 diabetes (3) and as a result has emerged as the major factor limiting effective insulin therapy. To therapeutically intervene to reduce the risk of hypoglycemia, a greater understanding is required of the mechanisms that have evolved to detect incipient hypoglycemia and to trigger a counterregulatory response.

Specialized neurons whose activity appears to be directly linked to fluctuations in the glucose concentration to which they are exposed have to date been found in both the brain (4–17) and periphery (18–20). Within the brain, glucosensing neurons have been localized to the ventromedial hypothalamus (VMH), which includes ventromedial and arcuate nuclei (5,6,14,21–23). Glucosensing neurons use glucose as a signaling molecule to alter their firing rate. The two predominant glucosensing neuronal subtypes in the brain are glucose-excited neurons, whose firing rate increases, and glucose-inhibited neurons, whose firing rate decreases, as ambient glucose levels rise (14,17,24).

ATP-sensitive K^+ channels (K_{ATP} channels) provide a link between neuronal metabolism and membrane potential in many tissues (25,26). 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.x) (26,27). Skeletal muscle and cardiac K_{ATP} channels comprise SUR-2A and Kir6.2, whereas the pancreatic β-cell K_{ATP} channel, the prototype glucosensing cell, comprises SUR-1 and Kir6.2 (25–27). 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 (28,29). In this system, the K_{ATP} channel indi-

rectly senses glucose fluctuations through changes in the intracellular ratio of ATP and ADP (28,29).

K_{ATP} channels have been demonstrated throughout the brain, including in hypothalamic regions thought to be involved in glucosensing (21,30–34). Examination of gene expression in glucosensing neurons using single-cell RT-PCR amplification of cytoplasm harvested at the end of fura-2 Ca^{2+} imaging studies has identified mRNA for SUR-1 and Kir6.2 in ventromedial hypothalamic neurons (23). Electrophysiological studies of rat (35–37) and mouse brain-slice preparations (38) 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 (38), and we have recently shown in vivo that pharmacological closure of the K_{ATP} channel in the VMH via direct microinjection of glibenclamide suppressed hormonal counterregulatory responses to systemic insulin-induced hypoglycemia (39).

The present study was designed to answer the perhaps more clinically relevant question; namely, would pharmacological opening of ventromedial hypothalamic K_{ATP} channels during systemic hypoglycemia amplify the hormonal counterregulatory response? Furthermore, we also sought to determine whether we could reverse the counterregulatory hormone defect that ensues from recurrent antecedent hypoglycemia through pharmacological opening of ventromedial hypothalamic K_{ATP} channels during a subsequent episode of systemic hypoglycemia.

RESEARCH DESIGN AND METHODS

Three separate studies were performed: *1*) an examination of the effect of acute microinjection of the potassium channel opener (KCO), diazoxide, to the VMH on counterregulatory responses to hypoglycemia; *2*) an examination of the effect of acute microinjection of the SUR-1 selective KCO, NN414 (40), to the VMH on counterregulatory responses to hypoglycemia; and *3*) an examination of the effect of acute microinjection of the KCO, diazoxide, to the VMH on counterregulatory responses to hypoglycemia in rats that had experienced recurrent episodes of insulin-induced hypoglycemia (as described below).

Male Sprague-Dawley rats (means ± SE, weight 305 ± 4 g) 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. The animal care and experimental protocols were reviewed and approved by the Yale Animal Care and Use Committee.

One week before each study, all animals were anesthetized with an intraperitoneal injection (1 ml/kg) of a mixture of xylazine (AnaSed, 20 mg/ml; Lloyd Laboratories, Shenandoah, IA) and ketamine (Ketaset, 100 mg/ml; Aveco, Fort Dodge, IA) in a ratio of 1:2 (vol:vol) before undergoing vascular surgery for the implantation vascular catheters in a carotid artery and jugular vein. Following this, microinjection guide cannulas were bilaterally inserted into the VMH, targeting the ventromedial nucleus (coordinates from bregma: AP −2.6 mm, ML ±3.8 mm, and DV −8.3 mm at an angle of 20 [0]), as described previously (6,22).

Recurrent hypoglycemia protocol. Each rat underwent surgery, as described above, on day 1. On days 4–6 at 0900, each rat was injected intraperitonially (10 units/kg) with human regular insulin (Eli Lilly, Indianapolis, IN). Following microinjection, food was withheld from the rats so that they experience ~3 h of hypoglycemia (tail vein glucose 1.7–2.2 mmol/l [30–40 mg/dl]). This model of recurrent hypoglycemia (3 days) has been previously reported in detail and has been shown to induce suppression of epinephrine responses to subsequent hypoglycemia (41). Each rat then underwent a hyperinsulinemic-hypoglycemic clamp procedure with bilateral ventromedial hypothalamic microinjection the following day (day 7).

Microinjection. The microinjection procedure was the same in both experiments. 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 bilaterally inserted through the guide cannula into each ventromedial hypothalamus. The study rat was then microinjected over 2 min at a rate of 0.1 μl/min with diazoxide (231 ng in 0.5% DMSO/artificial extracellular fluid [aECF]) or vehicle (CON-1; 0.5% DMSO in aECF) or NN414 (58 ng in aECF) or vehicle (CON-2; aECF), using a CMA-102 infusion pump (CMA Microdialysis, Chelmsford, MA). Following microinjection, the needles were left in place for 3 min before being removed. At the end of the study, the rats were killed, and the probe position was confirmed in all rats histologically.

Diazoxide was initially dissolved in DMSO and then diluted in aECF to produce a solution containing diazoxide, aECF, and 0.5% DMSO. NN414 was dissolved in basic aECF, which was then pH-adjusted to 7.4. The control solutions (CON-1 and CON-2) for each group of rats were made in the same way but without the addition of KCO. The doses used were based on the results of pilot studies in smaller groups of rats.

Infusion protocol. In all experiments, the same infusion protocol was used. All animals were fasted overnight. On the morning of the study, the vascular catheters were opened and maintained patent by a slow infusion of saline (20 μl/min). During the first 90 min, animals were allowed to settle and recover from any stress of handling. Immediately before the commencement of the hyperinsulinemic glucose clamp, each animal was microinjected with diazoxide, NN414, or vehicle as described above. Thereafter, a hyperinsulinemic-hypoglycemic clamp technique, as adapted for the rat (42), was used to provide a standardized hypoglycemic stimulus. At $t = 0$, a 90-min 20 mU · kg^{-1} · min^{-1} infusion of human regular insulin (Eli Lilly) was begun. The plasma glucose was allowed to fall to ~2.8 mmol/l (50 mg/dl) and was then maintained at this level for 90 min using a variable-rate 20% dextrose infusion based on frequent plasma glucose determinations. Samples for measurement of the hormones epinephrine, norepinephrine, glucagon, and insulin were taken at −10, 45, 60, 75, and 90 min.

Analytical procedures. Plasma levels of glucose were 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 means ± SE. Area under the curve (AUC) for each hormone was calculated for each study and then divided by time of study (90 min). Means from each group were then compared using a *t* test (SPSS 11.0 for Windows; SPSS).

RESULTS

Effect of ventromedial hypothalamic microinjection of the KCO, diazoxide, on counterregulatory responses to acute hypoglycemia. In the first study, the effect of bilateral microinjection of the KCO, diazoxide ($n = 11$), in comparison with vehicle-injected rats (CON-1; $n = 11$), on counterregulatory responses to acute hypoglycemia was examined. Plasma glucose profiles under the two study conditions did not significantly differ (mean glucose 60–90 min: 2.9 ± 0.1 vs. 2.8 ± 0.1 mmol/l, diazoxide vs. CON-1, respectively). In contrast, the glucose infusion rates (GIRs) from 60 to 90 min required to maintain hypoglycemia were reduced by ~45% following VMH diazoxide (9.9 ± 1.9 vs. 17.6 ± 2.6 mg · kg^{-1} · min^{-1} for diazoxide vs. control, respectively; $P < 0.05$; Fig. 1*A*). The reduction in GIR required to maintain hypoglycemia with diazoxide was accompanied by a significant amplification of plasma epinephrine (AUC/time 7.4 ± 1.4 vs. 3.4 ± 0.6 nmol/l for diazoxide vs. CON-1, respectively; $P < 0.05$; Fig. 1*B*) and glucagon (AUC/time 179.8 ± 29.1 vs. 84.2 ± 17.7 ng/l; $P < 0.05$; Fig. 1*C*) but not norepinephrine (1.4 ± 0.2 vs. 1.3 ± 0.2 nmol/l; P = NS) responses to hypoglycemia. Plasma insulin did not differ between groups during the clamp procedure (AUC/time 2,993 ± 500 vs. 3,457 ± 495 pmol/l; P = NS).

Effect of ventromedial hypothalamic microinjection of the SUR-1 selective KCO, NN414, on counterregulatory responses to acute hypoglycemia. NN414 is a novel KCO that selectively activates Kir6.2/SUR-1 (40). We also compared the effect of bilateral ventromedial hypothalamic microinjection of NN414 ($n = 7$) with control rats (CON-2; $n = 6$) on counterregulatory responses to hyperinsulinemic hypoglycemia. Mean plasma glucose during each hypoglycemic plateau did not significantly differ

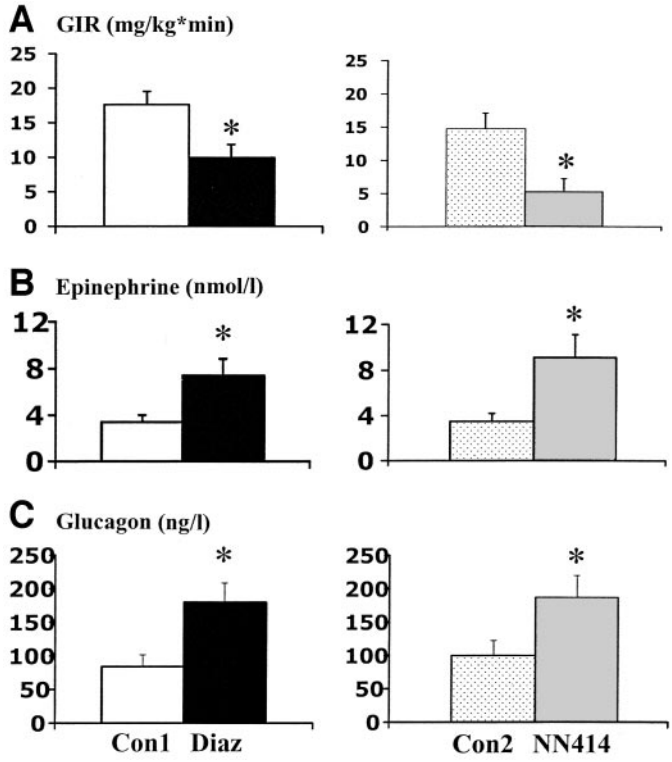

FIG. 1. GIRs (*A*), plasma epinephrine (*B*), and plasma glucagon (*C*) following ventromedial hypothalamic microinjection in normal Sprague-Dawley rats under each study condition. ■, diazoxide group; ▨, NN414; and controls (CON-1 [□] and CON-2 [▨]). Values are shown as means ± SE and represent AUC/time. *$P < 0.05$ vs. control study.

(mean plasma glucose 60–90 min: 2.7 ± 0.1 vs. 2.7 ± 0.1 mmol/l for NN414 vs. CON-2, respectively). As with the diazoxide study, we found that NN414 ventromedial hypothalamic–microinjected rats required significantly less exogenous glucose (~65%) to maintain equivalent hypoglycemia (5.2 ± 2.0 vs. 14.7 ± 2.3 mg · kg^{-1} · min^{-1}; $P < 0.05$; Fig. 1*A*). NN414-injected rats also demonstrated significant increases in plasma epinephrine (AUC/time 9.1 ± 2.0 vs. 3.5 ± 0.7 nmol/l; $P < 0.05$; Fig. 1*B*) and glucagon (AUC/time 186.5 ± 32.9 vs. 100.0 [22.1] ng/l; $P < 0.05$; Fig. 1*C*) but not norepinephrine (1.8 ± 0.4 vs. 1.6 ± 0.3 nmol/l; P = NS) responses to hypoglycemia in comparison with control rats. Plasma insulin did not differ between groups during the clamp procedure.

Effect of ventromedial hypothalamic microinjection of the KCO, diazoxide, on counterregulatory responses to acute hypoglycemia in rats who had experienced recurrent episodes of insulin-induced hypoglycemia. Plasma glucose profiles during the hyperinsulinemic-hypoglycemic clamp studies in recurrently hypoglycemic Sprague-Dawley rats did not differ between the diazoxide (n = 10) or control (n = 14) rats (mean glucose 60–90 min: 2.9 ± 0.1 vs. 2.9 ± 0.1 mmol/l, respectively; P = NS). However, once again, ventromedial hypothalamic microinjection of diazoxide resulted in a significant reduction in the GIR required to maintain the hypoglycemic plateau (11.1 ± 2.2 vs. 21.0 ± 2.1 mg · kg^{-1} · min^{-1} in controls; $P < 0.05$; Fig. 2*A*). The reduction in GIR following diazoxide was of a similar magnitude to that seen in the normal rats (~45%). This was accompanied by significant increases in epinephrine (AUC/time: 4.4 ± 0.7 vs. 1.6 ± 0.3 nmol/l; $P < 0.05$; Fig. 2*B*) and glucagon (173.2 ± 28.6 vs. 77.3 ± 15.2 ng/l; $P < 0.05$; Fig. 2*C*) but not norepinephrine (2.3 ± 0.4 vs. 1.9 ± 0.3 nmol/l; P = NS) secretory responses during subsequent hypoglycemia. Plasma insulin levels did not differ between groups during the clamp procedure in this experiment.

Comparison of our two control groups in the diazoxide studies showed that the recurrent hypoglycemia protocol had resulted in a significant impairment of the epinephrine (3.4 ± 0.6 vs. 1.6 ± 0.3 nmol/l; normal control vs. recurrently hypoglycemic control; $P < 0.05$) but not the glucagon (88.1 ± 17.9 vs. 77.3 ± 15.2 ng/l; P = NS) response to the study hypoglycemia (Table 1). VMH diazoxide in recurrently hypoglycemic rats restored the counterregula-

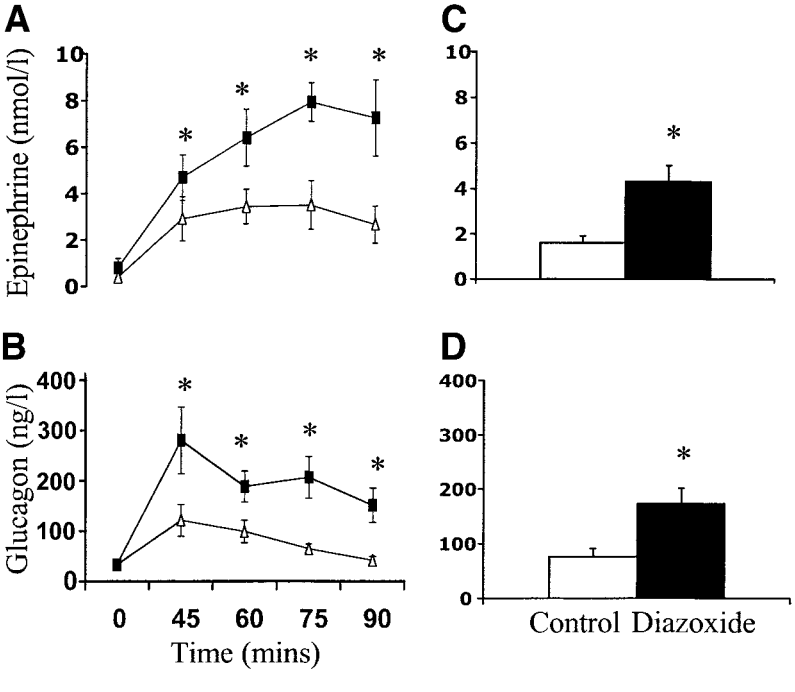

FIG. 2. Epinephrine and glucagon levels during hypoglycemia in recurrently hypoglycemic Sprague-Dawley rats following ventromedial hypothalamic microinjection of diazoxide or control. Results shown as means ± SE plasma values at each sampling time point (*A* and *B*: ■, diazoxide; △, control) and as AUC/time. *C* and *D*: ■, diazoxide; □, control. *$P < 0.05$ vs. control.

TABLE 1
Comparison of GIR, glucagon (AUC/time), and epinephrine (AUC/time) during hypoglycemia in normal control rats and recurrently hypoglycemic control and diazoxide rats

	Normal	Recurrent hypoglycemia	
	Control	Control	Diazoxide
GIR (mg · kg^{-1} · min^{-1})	17.6 ± 2.6	21.0 ± 2.1	11.1 ± 2.2*†
Glucagon (ng/l)	84.2 ± 17.7	77.3 ± 15.2	173.2 ± 28.6*†
Epinephrine (nmol/l)	3.4 ± 0.6	1.6 ± 0.3†	4.4 ± 0.7*

Data are means ± SE. *$P < 0.05$ vs. recurrent hypoglycemia control; †$P < 0.05$ vs. normal control.

tory responses to levels above those seen in the normal control rats (Table 1).

DISCUSSION

There is substantial evidence in vitro (10,14,21,33,35,42–44) and in vivo (38,39) indicating a key role for the K_{ATP} channel in glucosensing in the hypothalamus and, in particular, the VMH. This evidence includes *1*) demonstration of K_{ATP} channels in brain, including the VMH (33,43); *2*) RT-PCR amplification of cytoplasm harvested at the end of fura-2 Ca^{2+} imaging studies identifying SUR-1 and Kir6.2 in ventromedial hypothalamic neurons (23); *3*) electrophysiological studies in brain-slice preparations showing ventromedial hypothalamic K_{ATP} channel activity that is responsive to both changes in extracellular substrate and SUR-1 ligands and moreover that K_{ATP} responses to substrate can be modified by SUR-1 ligands (14,17,21,24,38); and *4*) the in vitro demonstration that ventromedial hypothalamic neurons in Kir6.2$^{-/-}$ mice are unresponsive to changes in extracellular glucose and SUR-1 modulation (38). In keeping with these observations, the current study together with our previous report provide data demonstrating that in vivo delivery of agents that either open or close the K_{ATP} channel within the VMH of the rat have converse effects on the normal hormonal counterregulatory response to acute hypoglycemia. These in vivo studies extend earlier work by providing the specificity that limits interpretation of data from the study of transgenic mice where there is a more generalized defect in the target gene and from the in vitro study of brain-slice preparations or cells in culture where normal interneuronal connectivity is disrupted.

K_{ATP} channels consist of pore-forming Kir6.x subunits that associate with different kinds of regulatory sulfonylurea receptor subunits: SUR-1, SUR-2A, and SUR-2B. Diazoxide acts predominantly through Kir6.2/SUR-1; however, it can also act on SUR-2B regulatory subunits found on vascular smooth muscle fibers, which suggests that under certain conditions it will have a vasodilatory action. To investigate whether the action of diazoxide in the VMH to amplify counterregulatory responses to acute hypoglycemia might have resulted from an alteration in local cerebral blood flow through activation of Kir6.2/SUR-2B, we chose to perform a further series of in vivo studies using a second potassium channel activator, NN414. NN414 has been shown to selectively activate KATP channels of the Kir6.2/SUR-1 type (40). Dabrowski et al. (40) compared the effects of NN414 and diazoxide on whole-cell K^+ currents in an HEK293 cell line stably expressing the pancreatic β-cell–type K_{ATP} channel Kir6.2/SUR-1 and reported an EC_{50} for NN414 of 0.45 ± 0.1 μmol/l and for diazoxide 31 ± 5 μmol/l. In contrast, NN414 had no activating effect on oocytes expressing either Kir6.2/SUR-2A or Kir6.2/SUR-2B channels. Interestingly, when the investigators examined Kir6.2/SUR-2A and Kir6.2/SUR-2B channels in inside-out membrane patches, they found no significant effect of NN414 when the channels were preblocked with 100 μmol/l MgATP or preactivated with 100 μmol/l MgADP, but, in the absence of nucleotide, NN414 actually had an inhibitory effect on these channels with an IC50 for SUR-2A and SUR-2B of 10 ± 2 and 7.1 ± 0.8 μmol/l, respectively. We found that microinjection of NN414 bilaterally to the VMH also amplified counterregulatory responses to acute hypoglycemia, an effect that was greater in magnitude to that seen following diazoxide microinjection. Taken together, these studies provide compelling evidence that the Kir6.2/SUR-1 form of K_{ATP} channel is involved in the glucosensing mechanism used by neurons in the VMH.

While in vivo microinjection certainly provides a greater specificity by targeting specific brain regions, it is not possible to completely exclude effects outside a region of interest. The small volume of injection (0.2 μl) and rapid fall in drug concentration from the injection site suggest that a primary action in other central glucosensing regions (e.g., hindbrain) is unlikely. We also considered the possibility of nonspecific effects resulting from microinjection of diazoxide. We think this is unlikely because we were able to replicate the diazoxide study with an alternate KCO (NN414) and because our previous study showed that microinjection of a KCC had the opposite effect on hormonal counterregulation. DMSO, used as a vehicle to dissolve diazoxide, could potentially have independent effects on neuronal activity. However, no significant differences were apparent when we compared counterregulatory responses between the controls in the acute diazoxide study (CON-1) with those of the controls in the acute NN414 study (CON-2), where DMSO was not present in the solution. This suggests that any potential independent effect of the DMSO is unlikely to have had a significant impact on our findings.

Taken together, the acute studies support the view that modulation of the K_{ATP} channels in the VMH has a direct effect on neuronal responses to changing extracellular glucose. Recent studies (35,45,46) implicating glucokinase in hypoglycemia sensing provide support for the hypothesis that the mechanism by which specialized glucosensing neurons within the VMH detect a change in extracellular glucose is similar to that used by the pancreatic β-cell. It is unlikely, however, that this is the sole mechanism used, given that not all glucosensing neurons express glucokinase or Kir6.2 (23), and there may be other potential sensing mechanisms, e.g., AMP-activated protein kinase (47). However, overall the data to date indicate the presence of at least one signaling system in the VMH for detecting a falling glucose that uses glucokinase and the K_{ATP} channel as key regulatory steps.

Recurrent severe hypoglycemia is a risk associated with, and a primary limitation to, intensive insulin therapy (48). Single (49) or multiple (50) episodes of acute hypoglycemia induce defective counterregulation in individuals with (51) and without (50) type 1 diabetes. The mechanism(s) by which this defect is induced is not yet known, although current data suggest that the defect may, directly or indirectly, arise as a consequence of hypothalamo-pituitary-adrenal axis activation during acute hypoglycemia (41,52). Given that we had demonstrated an acute effect of KCO to amplify counterregulatory responses, we

sought to determine whether we could also restore counterregulatory responses in an animal model of defective hormonal counterregulation through the direct application of a KCO to the VMH. Normal male Sprague-Dawley rats were subjected to three consecutive daily episodes of acute hypoglycemia before undergoing a hyperinsulinemic-hypoglycemic clamp study. Consistent with a previous report (41), this model induced a defective epinephrine counterregulatory response as assessed by the hyperinsulinemic-hypoglycemic clamp (Table 1). Ventromedial hypothalamic microinjection of diazoxide produced an amplification of hormonal counterregulatory responses, and a reduction in the amount of exogenous glucose required to maintain the hyperinsulinemic-hypoglycemic clamp, in rats with defective counterregulation secondary to recurrent hypoglycemia. The responses generated were in fact greater than those seen in the control rats that had not undergone the recurrent hypoglycemia protocol. It is of note that recurrent hypoglycemia had only a small effect on glucagon secretion in the control rats (comparison of the two control groups). This may be a reflection of the model we chose; it is likely that factors such as depth of hypoglycemia, its duration, and the frequency of induced episodes all have an impact on hormone counterregulatory responses. In our experience, it takes a more chronic exposure to recurrent once-daily hypoglycemia to induce a glucagon secretory defect in normal rats (41,42). This may reflect the evidence now accruing that abnormalities in glucagon secretion during hypoglycemia primarily result from the failure of intraislet insulin levels to fall in type 1 diabetes (53,54). Despite this, the fact that we saw an amplification of the glucagon secretory response to hypoglycemia in both normal and recurrently hypoglycemic rats underscores the importance of the autonomic nervous system in determining the magnitude of the glucagon secretory response to acute hypoglycemia. Our data indicate that providing an additional pharmacological stimulus to open K_{ATP} channels in the VMH of rats who have experienced recurrent hypoglycemia markedly enhances both epinephrine and glucagon responses to a subsequent episode of hypoglycemia and that the defect induced by recurrent episodes of hypoglycemia may operate in a different way on those circuits effecting epinephrine and glucagon secretion.

The clinical applications of diazoxide, the only commercially available KCO in clinical use, are limited because it lacks sufficient specificity, strongly activating β-cell and smooth muscle K_{ATP} channels but additionally having a weak stimulatory effect on cardiac and vascular K_{ATP} channels (40). Because of this, diazoxide has many undesired side effects (e.g., vasodilation and hirsuitism). Moreover, although research in this area is scarce, very little diazoxide is thought to cross the blood-brain barrier (55), and hence effects on central glucosensing systems may be limited. However, the different composition, tissue expression patterns, and functional roles of the K_{ATP} channel subtypes offer a potential means of developing novel therapies for specific conditions. Our data would suggest that a SUR-1–specific KCO that is able to cross the blood-brain barrier would amplify counterregulatory responses to insulin-induced hypoglycemia. As such, as proof of concept, our study offers the first in vivo demonstration of the potential use of KCOs in the treatment of individuals with type 1 diabetes who develop the complication of defective hypoglycemia counterregulation.

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