

Hypothalamic ATP-sensitive K⁺ Channels Play a Key Role in Sensing Hypoglycemia and Triggering Counterregulatory Epinephrine and Glucagon Responses

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It has been postulated that specialized glucose-sensing neurons in the ventromedial hypothalamus (VMH) are able to detect falling blood glucose and trigger the release of counterregulatory hormones during hypoglycemia. The molecular mechanisms used by glucose-sensing neurons are uncertain but may involve cell surface ATP-sensitive K⁺ channels (K_{ATP} channels) analogous to those of the pancreatic β -cell. We examined whether the delivery of sulfonylureas directly into the brain to close K_{ATP} channels would modulate counterregulatory hormone responses to either brain glucopenia (using intracerebroventricular 5-thioglucose) or systemic hypoglycemia in awake chronically catheterized rats. The closure of brain K_{ATP} channels by global intracerebroventricular perfusion of sulfonylurea (120 ng/min glibenclamide or 2.7 μ g/min tolbutamide) suppressed counterregulatory (epinephrine and glucagon) responses to brain glucopenia and/or systemic hypoglycemia (2.8 mmol/l glucose clamp). Local VMH microinjection of a small dose of glibenclamide (0.1% of the intracerebroventricular dose) also suppressed hormonal responses to systemic hypoglycemia. We conclude that hypothalamic K_{ATP} channel activity plays an important role in modulating the hormonal counterregulatory responses triggered by decreases in blood glucose. Our data suggest that closing of K_{ATP} channels in the VMH (much like the β -cell) impairs defense mechanisms against glucose deprivation and therefore could contribute to defects in glucose counterregulation. *Diabetes* 53:2542–2551, 2004

The benefits of lowering average blood glucose levels in diabetes to reduce the risk of long-term complications are well established. In clinical practice, the degree to which this can be achieved is often limited by the increased risk of hypoglycemia that accompanies intensified glucose-lowering regimens (1,2). When healthy, blood glucose levels are normally maintained within relatively narrow limits. A fall in blood glucose is rapidly detected and a series of compensatory homeostatic responses are triggered that tend to prevent or limit hypoglycemia and to restore euglycemia. These responses include secretion of the counterregulatory hormones glucagon and epinephrine, which promote endogenous glucose production and limit tissue utilization of glucose and the generation of typical warning symptoms. These protective responses against hypoglycemia are disrupted in diabetes. Within 5 years of diagnosis, almost all patients with type 1 diabetes will develop defective secretion of the counterregulatory hormone glucagon during hypoglycemia (3). This leaves epinephrine as the major hormonal counterregulatory defense against low blood glucose. However, a significant number of patients will also develop additional deficiencies in epinephrine and other neurohumoral responses to hypoglycemia associated with the loss of symptomatic awareness of hypoglycemia (3). The combination of impaired counterregulation and hypoglycemia unawareness significantly increases the risk of suffering severe episodes of hypoglycemia (4,5).

To trigger protective counterregulatory neurohumoral responses, hypoglycemia must first be detected. Although low blood glucose may be detected, at least in part, by peripheral sensors (6–9), much evidence suggests that hypoglycemia is sensed predominantly by the brain (10–13) by glucose-sensing neurons in the ventromedial hypothalamus (VMH), with ventromedial and arcuate nuclei probably playing a key role (14–16). Glucose-sensing neurons may be either stimulated (glucose excited) or inhibited (glucose inhibited) by a rise in glucose (17). The mechanisms used by specialized glucose excited and inhibited neurons to sense changes in glucose remain undetermined, but recent in vitro evidence suggests that there may be parallels with pancreatic β -cell glucose

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5TG, 5-thioglucose; aECF, artificial extracellular fluid; K_{ATP} channel, ATP-sensitive K⁺ channel; KIR, pore-forming subunit; SUR, sulfonylurea receptor subunit; VMH, ventromedial hypothalamus.

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sensing (18,19). In the classical pathway of β -cell glucose sensing, a fall in blood glucose leads, via a fall in intracellular ATP, to the opening of K_{ATP} channels and thus cell membrane hyperpolarization (leading to decreased insulin secretion). K_{ATP} channels thus allow β -cells to transduce the metabolic signal of altered ambient glucose levels into changes in cell membrane electrical charge, eventually leading to altered insulin secretion. K_{ATP} channels are present in brain (20), and electrophysiological studies of rat brain slices have demonstrated that sulfonylureas (drugs that act to inhibit and in turn close K_{ATP} channels) can stimulate the firing of glucose-excited neurons (18,21,22).

Our hypothesis was that hypothalamic K_{ATP} channels play a key role in the sensing of hypoglycemia in brain. We examined this in an in vivo model, measuring the effects of direct delivery of agents acting on K_{ATP} channels into rat brain during both brain glucopenia and controlled systemic hypoglycemia. Our results support a key role for brain K_{ATP} channels in the detection of low blood glucose and the triggering of counterregulatory hormonal responses to hypoglycemia.

RESEARCH DESIGN AND METHODS

Male Sprague-Dawley rats were purchased from Charles River Laboratories (Wilmington, MA). The animals were housed in the Yale Animal Care Facility in an environmentally controlled room with a 12-h light/dark cycle and fed a standard ad libitum rat diet comprised of 22% protein, 5% fat, and 51% carbohydrate (Agway Prolab 3000; Agway, Syracuse, NY). The protocol was reviewed and approved by the Yale Animal Care and Use Committee.

Study 1: effects of intracerebroventricular glibenclamide on responses to brain glucopenia. Rats were anesthetized with intraperitoneal ketamine (100 mg/kg) and xylazine (20 mg/kg) and placed in a stereotaxic frame (Kopf Instruments). A single-guide cannula (Plastics One, Roanoke, VA) was inserted into a lateral ventricle under stereotaxic control (coordinates from bregma -0.9 mm anteroposterior, 1.4 mm lateral, 3 mm dorsoventral) and cemented in place with anchoring screws also placed into the skull to ensure stability. Seven days later, the rats underwent a second surgery for insertion of a vascular catheter into the superior vena cava via the right jugular vein, as previously described (23). The catheter was flushed with 42 units/ml heparin and 1.7 g/ml polyvinylpyrrolidone solution, tunneled subcutaneously, and externalized behind the nape of the neck. Postoperatively, analgesia was routinely provided and only those animals that had recovered and showed no signs of infection were studied. Animals were studied 7 days after vascular surgery after an overnight fast (16 h). On the study morning, catheters were reopened and, after a period of at least 90 min equilibration, an intracerebroventricular cannula was inserted for continuous perfusion of test substances. One group of animals ($n = 6$) received a primed ($3 \mu\text{l}/\text{min}$ for 3 min followed by $0.5 \mu\text{l}/\text{min}$) infusion of glibenclamide. In control studies ($n = 5$), animals were treated with an otherwise identical infusion of vehicle. After 120 min, during which time blood glucose, insulin, and baseline hormones were measured, intracerebroventricular infusions were briefly interrupted in order to deliver a bolus into the lateral ventricle of $50 \mu\text{g}$ 5-thiogluconate (5TG) in $5 \mu\text{l}$ artificial extracellular fluid (aECF) over 5 min. Glibenclamide or vehicle infusions were then restarted and continued for an additional 90 min. The dose of glibenclamide was determined from a pilot study using a $25 \text{ ng}/\text{min}$ infusion of glibenclamide ($n = 4$) that had shown inconsistent suppression of glucose counterregulation in response to intracerebroventricular 5TG. The studies reported here were performed using $120 \text{ ng}/\text{min}$ glibenclamide dissolved in a vehicle consisting of aECF/0.5% DMSO (glibenclamide).

Plasma glucose was monitored at 15- to 30-min intervals throughout using a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Additional volumes of blood were drawn at baseline, just before the 5TG bolus and at 60 and 90 min, and then after the 5TG bolus for later measurement of epinephrine, glucagon, insulin, and C-peptide. Following these studies, the animals were killed and cannula positions checked by injection of dye.

Study 2: effects of intracerebroventricular tolbutamide on responses to brain glucopenia. Surgeries and studies were performed as above, with the exception that tolbutamide was used in place of glibenclamide, delivered at $2.7 \mu\text{g}/\text{min}$ ($0.5 \mu\text{l}/\text{min}$) dissolved in 5% DMSO/aECF for 120 min (tolbutamide 120; $n = 6$) or 240 min (tolbutamide 240; $n = 5$) before delivery of the

bolus of 5TG. Control animals received vehicle infusions (5% DMSO/aECF) for 120 min ($n = 5$) but were otherwise treated identically.

Study 3: effects of intracerebroventricular glibenclamide on responses to systemic hypoglycemia. Surgeries and preparations were performed as described above for study 1. A continuous glibenclamide ($120 \text{ ng}/\text{min}$, $n = 9$) or vehicle ($n = 10$) infusion was commenced as in study 1 and continued throughout the study. After 60 min of euglycemia, a primed-continuous intravascular infusion of $10 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ regular insulin (Humulin R; Eli Lilly, Indianapolis, IN) was started together with a variable infusion of 10% dextrose. To maintain euglycemia for an additional 60 min, the dextrose infusion was adjusted in response to 5- to 10-min bench-top measurements of plasma glucose. At this time, the dextrose infusion rate was decreased and the plasma glucose allowed to fall rapidly to a target value of $2.8 \text{ mmol}/\text{l}$. The dextrose infusion was then adjusted to maintain this level for an additional 45 min. In addition to monitoring plasma glucose, additional volumes of blood were drawn 15 min (for glucagon) and 45 min (for epinephrine, glucagon, insulin, and C-peptide) after the onset of hypoglycemia.

Study 4: effects of VMH glibenclamide on responses to systemic hypoglycemia. The animals underwent surgery for placement of vascular catheters as described above. In addition, under the same anesthesia, guide cannulae for microinjections were placed bilaterally into the VMH (coordinates from bregma -2.6 mm anteroposterior, ± 3.8 mm lateral, 9.5 mm at 20° dorsoventral). Animals were studied 7 days after an overnight fast (16 h). Microinjection cannulae (designed to extend 1 mm beyond the guide cannula tip) were inserted through the guide cannulae and 12.4 ng glibenclamide injected bilaterally ($0.25 \mu\text{l}$ in volume to each side). The total dose of glibenclamide delivered into the VMH represented $\sim 0.1\%$ of the dose delivered into the brain by intracerebroventricular infusion in studies 1 and 3 above. Fifteen minutes after the VMH injections, baseline blood samples were taken and a $10 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ insulin infusion was started together with a variable infusion of 10% dextrose, as described in study 3. On this occasion, plasma glucose was lowered in two steps, aiming for a plateau of $3.3 \text{ mmol}/\text{l}$ between 30 and 60 min, with a second step of $2.5 \text{ mmol}/\text{l}$ between 80 and 110 min.

Lab measurements. Epinephrine was measured by high-performance liquid chromatography with electrochemical detection (ESA, Acton, MA) and glucagon, C-peptide, and insulin by radioimmunoassay (Linco, St. Charles, MO).

Statistical analysis. Epinephrine responses in studies 1–3 and insulin/C-peptide data in study 2 were not normally distributed and were thus analyzed by nonparametric analysis (Mann-Whitney unpaired test) and presented in figures and/or text as median \pm interquartile range. All other data were normally distributed and analyzed using parametric analysis (repeated-measures ANOVA with post hoc t test) and presented in figures and text as means \pm SE. Data were analyzed using SPSS for Windows (version 10.0.0; SPSS, Chicago, IL). There were no baseline differences between groups where incremental hormone responses are shown in the figures.

RESULTS

Study 1: effects of intracerebroventricular glibenclamide on responses to brain glucopenia. In vehicle-infused rats, intracerebroventricular delivery of 5TG resulted in a rise in systemic plasma glucose, a response that was largely and significantly attenuated by intracerebroventricular glibenclamide (Fig. 1A). Insulin levels fell slightly in vehicle infusions during the first 120 min but then rose in response to hyperglycemia following the bolus of 5TG (Fig. 1B). In contrast, during the initial 120 min of intracerebroventricular infusion of glibenclamide, insulin levels were maintained at baseline levels. Insulin levels failed to rise in response to brain glucopenia in glibenclamide animals. Plasma C-peptide responses mirrored those of insulin, rising in vehicle and falling in glibenclamide studies following the bolus of 5TG (Fig. 1B). In vehicle studies, we observed a significant rise in both plasma epinephrine and glucagon in response to brain glucopenia induced by 5TG (Fig. 1C). In contrast, glibenclamide infusion markedly suppressed epinephrine and glucagon responses to brain glucopenia.

Study 2: effects of intracerebroventricular tolbutamide on responses to brain glucopenia. In our intracerebroventricular tolbutamide studies, we observed a

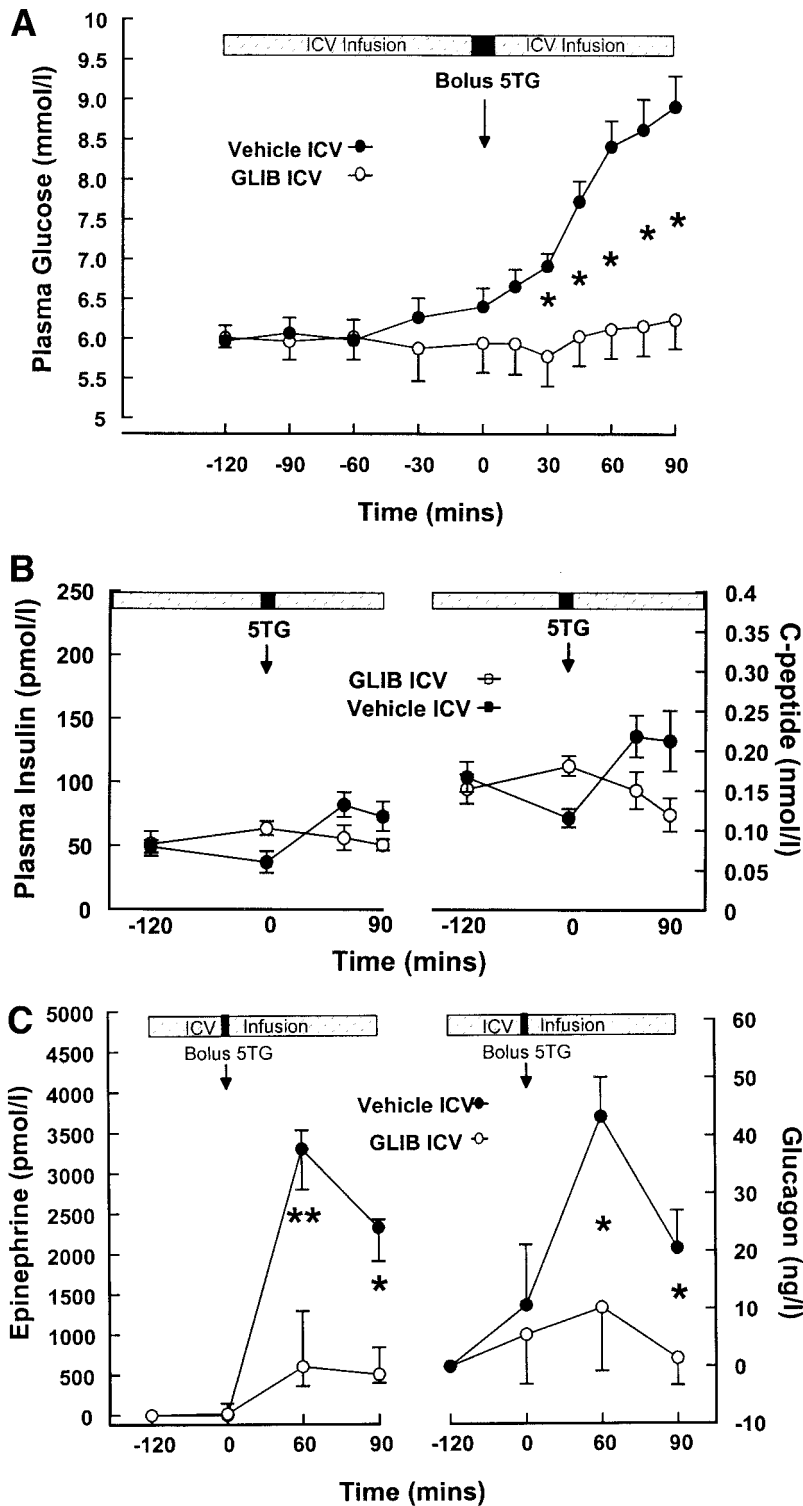


FIG. 1. Effects of intracerebroventricular glibenclamide on responses to brain glucopenia (study 1). Rats received a 2-h intracerebroventricular (ICV) infusion of either glibenclamide (GLIB; ○) or vehicle (●) followed by an intracerebroventricular bolus of 5TG to create brain glucopenia. Intracerebroventricular glibenclamide or vehicle was then continued for an additional 90 min. Plasma glucose (A), plasma insulin (B), and C-peptide (C) and incremental epinephrine and glucagon responses are shown. * $P < 0.05$ glibenclamide vs. vehicle.

similar pattern to that seen with intracerebroventricular glibenclamide. The rise in plasma glucose in response to brain glucopenia in vehicle studies was significantly attenuated by the 4-h intracerebroventricular infusion of tolbutamide 240 (Table 1). In keeping with this finding, plasma insulin and C-peptide responses did not change in response to 5TG in tolbutamide 240 studies (Table 1). In tolbutamide 120 rats, basal C-peptide levels were lower in tolbutamide 120 than in vehicle or tolbutamide 240 animals. These levels were taken before any brain

injections or infusions consistent with a chance finding. Although levels of C-peptide and insulin in tolbutamide 120 animals were significantly higher than basal values at 60 and 90 min after the bolus of 5TG, the levels seen were statistically indistinguishable from those seen in vehicle and tolbutamide 240 groups. As in study 1, counterregulatory hormonal responses to brain glucopenia were suppressed by intracerebroventricular tolbutamide, with epinephrine and glucagon responses to 5TG being largely and significantly suppressed in tolbu-

TABLE 1
Effect of intracerebroventricular tolbutamide on glucose, insulin, and C-peptide responses to 5TG

	Vehicle	Tolbutamide 120	Tolbutamide 240
Glucose (mmol/l)			
Basal	6.1 ± 0.1	5.5 ± 0.1	5.6 ± 0.1
0 min	6.1 ± 0.2	5.8 ± 0.3	5.5 ± 0.1
60 min	7.8 ± 0.5*	6.4 ± 0.2	5.8 ± 0.2†
90 min	8.1 ± 0.5*	7.0 ± 0.3*	5.8 ± 0.2†
Insulin (pmol/l)			
Basal	82 ± 12	53 ± 11	52 ± 17
0 min	87 ± 9	71 ± 17	65 ± 20
60 min	99 ± 13	116 ± 19*	112 ± 60
90 min	92 ± 13	105 ± 10*	57 ± 7
C-peptide (nmol/l)			
Basal	0.13 ± 0.02	0.06 ± 0.01‡	0.13 ± 0.03
0 min	0.13 ± 0.01	0.13 ± 0.02	0.15 ± 0.03
60 min	0.18 ± 0.03	0.18 ± 0.03*	0.14 ± 0.01
90 min	0.19 ± 0.02	0.17 ± 0.03*	0.14 ± 0.01

Data are means ± SE. Values shown are basal (prior to starting ICV infusion), 0 min (prior to intracerebroventricular 5TG injection), and 60 and 90 min after 5TG injection. * $P > 0.05$ vs. basal, † $P > 0.05$ vs. vehicle, ‡ $P > 0.05$ vs. vehicle and tolbutamide 240.

tamide 240 studies when compared with vehicle studies (Fig. 2).

Study 3: effects of intracerebroventricular glibenclamide on responses to hypoglycemia. The vehicle and glibenclamide groups were exposed to similar plasma glucose profiles and a similar degree of hyperinsulinemia, with C-peptide secretion reflecting endogenous insulin secretion equally suppressed in both treatment groups (Fig. 3A and B). The time taken to fall to hypoglycemic nadir was identical in both groups (12 ± 2 min). In keeping with our data from studies 1 and 2, epinephrine responses to systemic hypoglycemia during intracerebroventricular glibenclamide were reduced to approximately one-half of the values seen in vehicle studies (Fig. 3C). Glucagon rose in response to hypoglycemia in both vehicle and glibenclamide rats, with no statistically significant difference in response between the two treatment groups. Consistent with the deficit in counterregulatory epinephrine responses, the amount of exogenous dextrose that was

necessary to maintain the plasma glucose at the target level during hypoglycemia was significantly greater during the glibenclamide studies (Fig. 3D).

Study 4: effects of VMH glibenclamide on responses to hypoglycemia. Baseline glucose values before the VMH injection were similar in both the vehicle (5.9 ± 0.2 mmol/l) and glibenclamide (5.7 ± 0.2 mmol/l) groups ($P = \text{NS}$). Fifteen minutes after the VMH injection, plasma glucose values had risen slightly in vehicle but not VMH animals (vehicle 6.1 ± 0.2 mmol/l vs. glibenclamide 5.7 ± 0.1 mmol/l, $P < 0.05$). Thereafter, both groups were exposed to similar hypoglycemic challenges with two sequential steps of 3.3 and 2.5 mmol/l (Fig. 4A). As above, baseline insulin levels were similar in both groups, and a similar degree of hyperinsulinemia was achieved in both vehicle and glibenclamide rats (Fig. 4B). We observed a similar subsequent suppression of C-peptide during insulin infusion in both treatment groups (Fig. 4B). A small difference in basal (preinjection) C-peptide was seen between the animal groups (vehicle 0.16 ± 0.01 nmol/l vs. glibenclamide 0.08 ± 0.01 nmol/l, $P < 0.05$). As expected, vehicle-infused rats displayed marked rises in epinephrine and glucagon in response to the hypoglycemic challenge. These counterregulatory responses were significantly suppressed by a prior injection of glibenclamide directly into the VMH (Fig. 4C). In keeping with this counterregulatory deficit, dextrose infusion rates were significantly greater in glibenclamide rats (Fig. 4D).

DISCUSSION

In keeping with our hypothesis, our data showed that counterregulatory hormonal responses to both brain glucopenia and systemic hypoglycemia in free moving conscious rats were attenuated by brain delivery of sulfonylurea to close brain K_{ATP} channels. Although there is considerable evidence from our laboratory and others that the VMH plays a key role in sensing hypoglycemia, there are undoubtedly glucose-sensing neurons in other areas of the brain. Our strategy was therefore to initially examine the effects of sulfonylureas on whole-brain hypoglycemia sensing by delivering the intracerebroventricular infusion in studies 1–3. We then examined the role of hypothalamic K_{ATP} channels by directly injecting gliben-

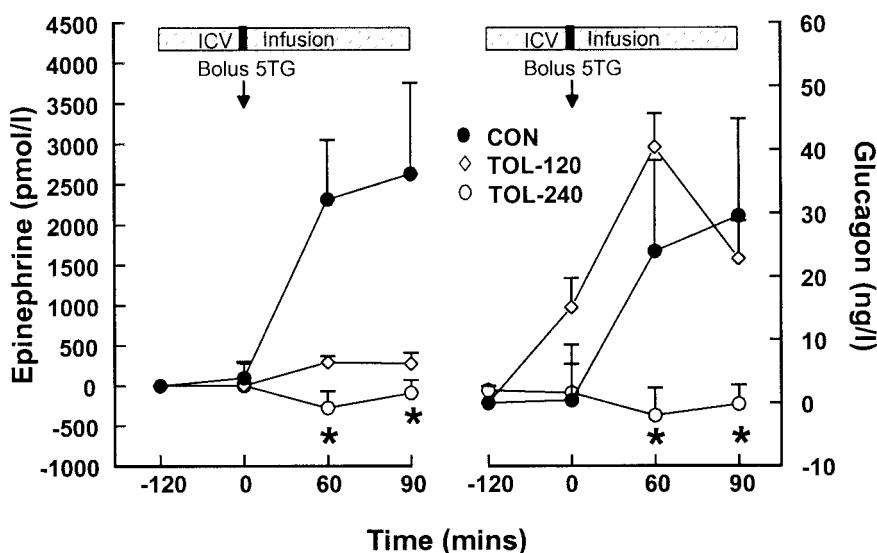


FIG. 2. Effects of intracerebroventricular tolbutamide on hormonal counterregulatory responses to brain glucopenia (study 2). Rats received infusions of either intracerebroventricular (ICV) vehicle (CON; ●), intracerebroventricular tolbutamide 120 (TOL-120; ◇), or tolbutamide 140 (TOL-240; ○) followed by an intracerebroventricular bolus of 5TG to create brain glucopenia. Intracerebroventricular vehicle or tolbutamide was then continued for an additional 90 min. Incremental epinephrine and glucagon responses are shown. * $P < 0.05$ tolbutamide 240 vs. vehicle.

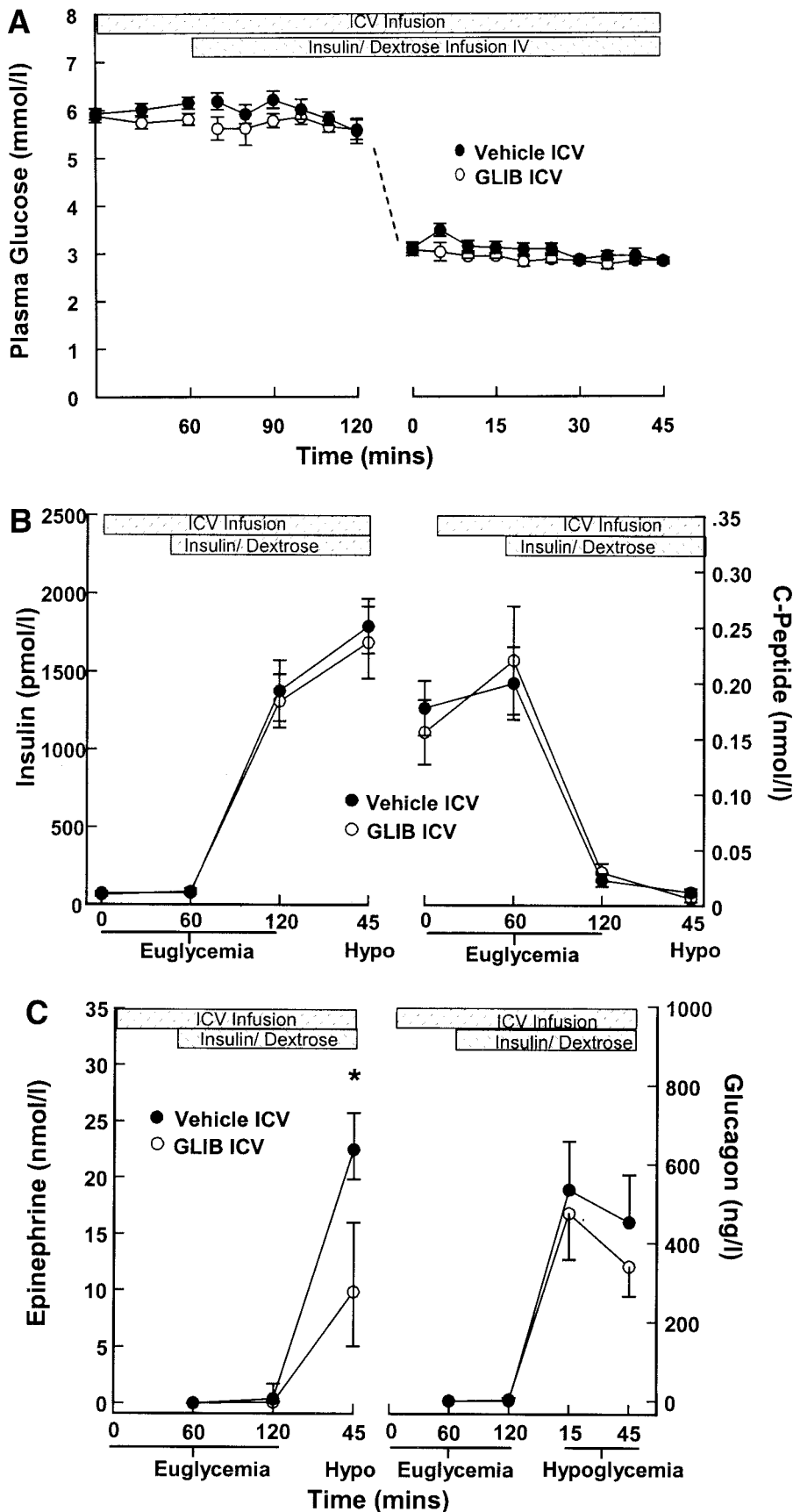


FIG. 3. Effects of intracerebroventricular GLIB on responses to hypoglycemia (study 3). Rats received an intracerebroventricular (ICV) infusion of either glibenclamide (GLIB; \circ) or vehicle (\bullet). Sixty minutes after starting intracerebroventricular infusion, a $10\text{-mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ hyperinsulinemic clamp was started with sequential euglycemic and hypoglycemic steps. Plasma glucose (A), plasma insulin (B), and C-peptide (C) and epinephrine and glucagon responses and dextrose infusion rates (D) are shown. $*P < 0.05$, $**P < 0.01$ glibenclamide vs. vehicle.

clamide into the VMH in study 4. We found that epinephrine responses to either brain glucopenia or systemic hypoglycemia were suppressed by either the delivery of

sulfonylureas into the brain ventricles or direct instillation into the VMH. Glucagon responses to brain glucopenia were also suppressed by intracerebroventricular delivery

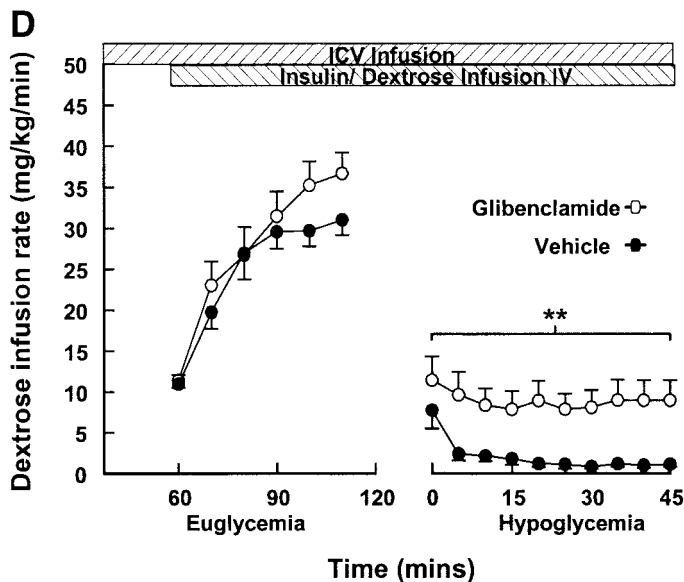


FIG. 3—continued.

of sulfonylureas. Moreover, we observed suppression of glucagon responses to systemic hypoglycemia by injecting glibenclamide directly into the VMH, suggesting that this area of the hypothalamus is important in generating the glucagon response to low blood glucose in keeping with previous reports from our laboratory (14–16).

K_{ATP} channels are composed of two different components: pore-forming (KIR) and sulfonylurea receptor (SUR) subunits arranged in a 4:4 stoichiometry (24). Different types of KIR and SUR proteins are found in K_{ATP} channels in different tissues. Pancreatic β -cells contain a combination of KIR6.2 with SUR1. In most of our studies, we used glibenclamide because of its greater potency compared with other sulfonylureas (25). However, glibenclamide may also act on SUR2-containing K_{ATP} channels (26). Therefore, in study 2, we examined the actions of the selective SUR1 sulfonylurea tolbutamide (25). Inasmuch as tolbutamide displayed similar actions as glibenclamide, it is likely that brain K_{ATP} channels in glucose-sensing neurons contain SUR1s analogous to those seen in pancreatic β -cells.

Although subnormal glucagon responses to hypoglycemia have been reported in KIR6.2 knockout mice by Miki et al. (31), our data suggest that brain K_{ATP} channels have a more extensive role in counterregulatory defenses, with epinephrine responses, the major hormonal defense against hypoglycemia in type 1 diabetes, also being dependent on hypothalamic K_{ATP} channel opening. Epinephrine responses to hypoglycemia in KIR6.2 knockout mice were reported to be intact, similar to those in control wild-type mice. However, closer examination of the data reported by Miki et al. suggests that the glucose nadir was unmatched, being much deeper in the knockout animals, and it is possible that this may have masked any differences between the groups. Baseline epinephrine levels were also significantly elevated in this murine study, indicating a significant study stress, per se, that may have overwhelmed any differences in responses to hypoglycemia. A major advantage of our protocol of using rats is that indwelling vascular catheters allowed blood sampling for epinephrine measurement with little or no stress to the

animals. Circumstantial evidence supporting our data also exists from a study in which insulin bolus hypoglycemia was examined in rats in which glibenclamide was injected directly into rat VMH. Blood glucose recovery was delayed in rats receiving VMH glibenclamide, suggesting a counterregulatory deficit, although the study was limited in that there was no measurement of counterregulatory hormones, insulin, or C-peptide (35).

Previous work (27,28) has demonstrated that glucagon responses to hypoglycemia can be suppressed by systemic administration of sulfonylureas, which act directly on the pancreas to create intraislet hyperinsulinemia. One possibility that we considered, therefore, was that the suppression of glucagon responses to brain glucopenia/hypoglycemia that we saw was simply a consequence of sulfonylureas leaching out of the brain and acting peripherally to create intraislet hyperinsulinemia. Careful examination of our data suggests that this is not the explanation for our findings. In the brain glucopenic studies, insulin and C-peptide levels were actually lower in the intracerebroventricular sulfonylurea-treated animals following the 5TG bolus in study 1 (glibenclamide) and similar in study 2 (tolbutamide), suggesting that the absence of a rise in glucagon was not simply a consequence of elevated intraislet insulin. During the hyperinsulinemic clamp studies (3 and 4), both groups of animals were exposed to identical high-circulating levels of insulin. In addition, both groups of rats displayed identical falls in C-peptide secretion, a marker of endogenous insulin secretion, suggesting that intraislet levels of insulin were also matched between groups.

K_{ATP} channels are widely distributed in the brain (20). They are found not only in putative areas but also in other brain areas not identified as playing a role in glucose sensing. In the latter brain areas, K_{ATP} channels may have a role in neuroprotection under such conditions as severe ischemia (29). Our in vivo data are consistent with a number of in vitro electrophysiological studies suggesting a role for K_{ATP} channels in glucose-sensing neurons. A number of different subtypes of glucose-sensing neurons have been identified in the VMH (17,30), with at least a proportion of the neurons that are excited by glucose also being stimulated by sulfonylureas (18,21). Examination of gene expression in glucose-sensing neurons using single-cell RT-PCR amplification of cytoplasm harvested at the end of electrophysiological studies has identified mRNA for SUR1 and KIR6.1 and -6.2 in VMH neurons (31,32).

It is possible that the differences we have observed following direct microinjection into intracerebroventricular and VMH resulted from non- K_{ATP} channel effects rather than a specific effect of our agents on the K_{ATP} channel. We feel this is unlikely given the failure of lower doses of sulfonylurea in our pilot studies to fully suppress glucose counterregulation. Moreover, preliminary data from studies using K_{ATP} channel openers support this view. In these experiments, microinjection of the K_{ATP} channel opener diazoxide directly into the VMH of Sprague-Dawley rats that additionally had experienced 3 consecutive days of insulin-induced hypoglycemia resulted in an amplification of counterregulatory hormonal responses of hypoglycemia (R.J.M., unpublished observations). The fact that pharmacological agonism and antagonism of VMH K_{ATP} channels resulted in amplification or

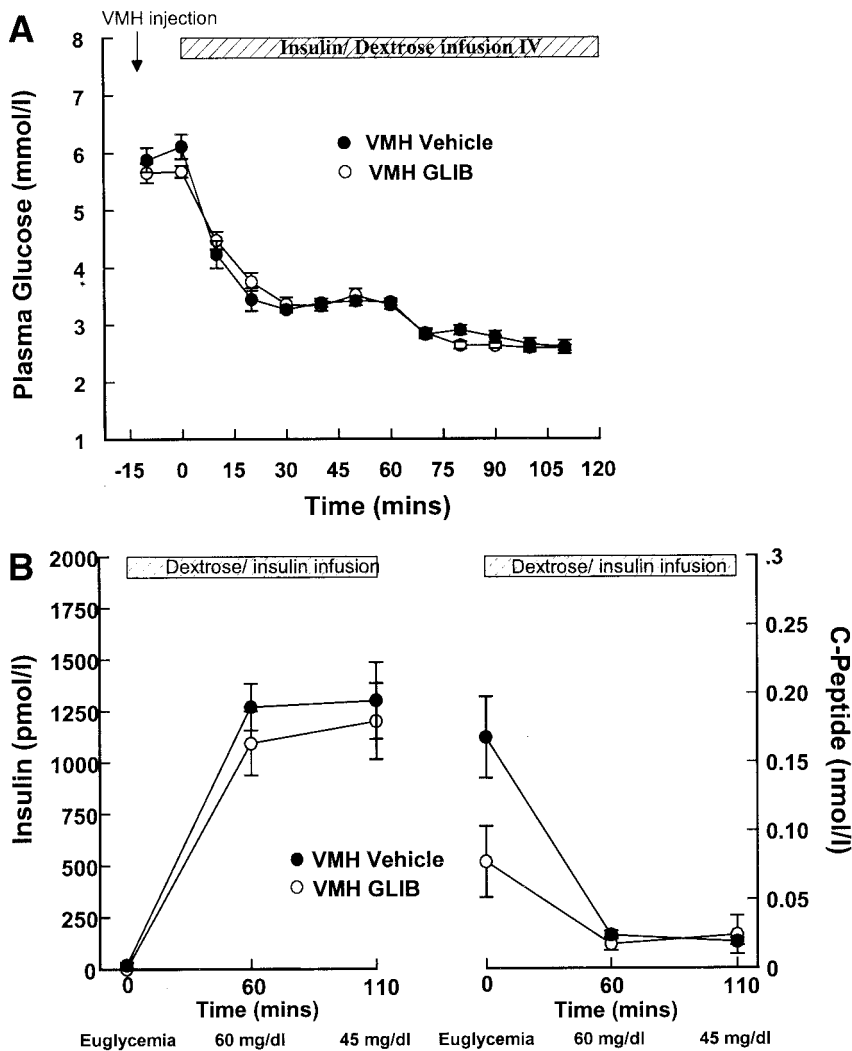


FIG. 4. Effects of VMH glibenclamide on responses to hypoglycemia (study 4). Rats received a VMH microinjection of either glibenclamide (GLIB; ○) or vehicle (●). After 15 min, a $10\text{-mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ hyperinsulinemic clamp was started with plasma glucose lowered in two steps to 60 and 45 mg/dl. Plasma glucose (A), plasma insulin (B), and C-peptide (C) and epinephrine and glucagon responses and dextrose infusion rates (D) are shown. * $P < 0.05$, ** $P < 0.01$ glibenclamide vs. vehicle.

suppression, respectively, of hormonal counterregulatory responses to hypoglycemia provides support for a selective effect of these interventions on the K_{ATP} channel. It is also possible that in our intracerebroventricular infusion studies, sulfonylureas were not only acting on the VMH but also on other glucose-sensing areas such as those located in the brain stem (12,13). A number of electrophysiological studies have demonstrated neurons in the brain stem with the ability to respond to changes in glucose (33,34), at least some of which respond to sulfonylureas (22). Other hypothalamic areas outside of the VMH also contain neurons that can sense changes in ambient glucose, leading us to consider whether glibenclamide injected directly into the VMH might be leaking into adjacent brain areas to mediate effects. Although this possibility cannot be excluded, we think that the VMH is likely to be the primary site of action of glibenclamide in the VMH studies given the previous data from our lab supporting the importance of the VMH in hypoglycemia sensing (14–16) and the ex vivo data from other labs in which K_{ATP} channels containing glucose-sensing neurons have been identified in the VMH (17,18).

In these whole-body studies, we cannot determine which glucose-sensing neurons mediate the effects of sulfonylureas that we observed. It is tempting, however, to spec-

ulate about which neurons may be involved in light of published electrophysiological data. Song et al. (17) demonstrated a subtype of hypothalamic neurons that was directly excited by glucose and which probably contains cell surface K_{ATP} channels. Glucose-excited neurons become less active during hypoglycemia, with this decrease in activity being prevented by sulfonylureas. If these glucose-excited neurons are the primary site of action of sulfonylureas seen in our studies, it suggests that hypoglycemia is detected by the brain through a mechanism that involves predominantly electrical silencing of glucose-sensing neurons, presumably allowing the release of a tonic inhibition on other neurons that can trigger counterregulatory responses.

It is not clear why glucagon responses to systemic hypoglycemia were not significantly affected by intracerebroventricular glibenclamide, a finding that was in contrast to the marked suppression of glucagon responses to brain glucopenia with intracerebroventricular glibenclamide and to systemic hypoglycemia with VMH glibenclamide. This pattern is also different from that seen with epinephrine, where responses to both glucopenia and hypoglycemia were suppressed by both intracerebroventricular and VMH delivery of sulfonylureas. The differences seen between the effects of intracerebroventricular sulfonylureas on glucagon responses to brain glucopenia

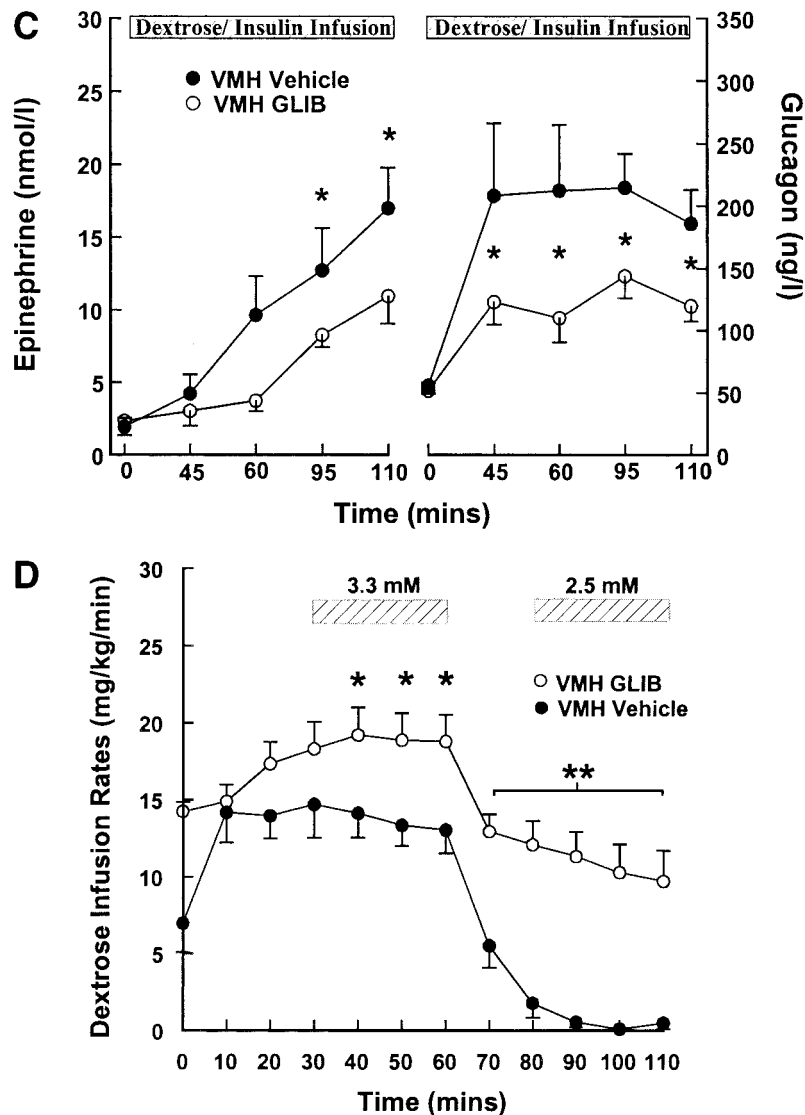


FIG. 4—continued.

and systemic hypoglycemia may be explained at least in part by the multiple mechanisms contributing to glucagon release. Glucagon release during hypoglycemia is almost certainly mediated by both central and peripheral mechanisms, with local pancreatic sensing of hypoglycemia being complemented by both neural and humoral signals to α -cells so that brain K_{ATP} closure may negate only part of the signal to increase glucagon release (41). Our VMH studies suggest that the effects of these other mechanisms to stimulate glucagon release can still be suppressed by closing hypothalamic K_{ATP} channels. As previously discussed, sulfonylurea-delivered intracerebroventricular may also be acting at a number of brain glucose-sensing areas, including those around the brain stem (12,13,22,33,34). Glucagon and epinephrine responses to hypoglycemia may be controlled differentially by different hypoglycemic-sensing areas of brain and/or by different subtypes of neurons within these areas. It may also be the case that epinephrine responses are more sensitive than glucagon responses to brain K_{ATP} channel closure.

One obvious question arising from our results is

whether the sulfonylureas used clinically to treat type 2 diabetes might gain access to brain glucose-sensing areas, suppress counterregulation, and thus increase the risk of hypoglycemia. Little is known about the kinetics of sulfonylurea penetration into brain. Short-term studies in nondiabetic subjects have shown either no effect or only a minimal effect on counterregulatory hormones (36–38). In the U.K. Prospective Diabetes Study, sulfonylurea therapy was associated with a lower incidence of hypoglycemia than insulin treatment but a higher risk than metformin therapy (39). Peacey et al. (40) examined a small cohort of patients from the U.K. Prospective Diabetes Study in more detail, finding no difference in hormonal responses to a controlled hypoglycemic challenge between sulfonylurea- and insulin-treated groups. In practice, this is a difficult question to answer since any direct effects of a blood glucose-lowering treatment on counterregulatory mechanisms are difficult to separate from the effects of glycemic exposure, per se, particularly because an HbA_{1c} measurement may not reflect antecedent exposure to hypoglycemia. Similarly, there appears to be no compelling evidence at

present to link the clinical use of sulfonylureas with a direct effect to suppress counterregulation (independent of any effect mediated by iatrogenic hypoglycemia itself), but this is clearly an important area for further work.

In summary, our results demonstrate that closing hypothalamic K_{ATP} channels during hypoglycemia prevents the triggering of protective hormonal counterregulatory responses. Our data also imply that the activated state of hypothalamic K_{ATP} channels during hypoglycemia plays a key role in the capacity of brain glucose-sensing neurons to detect falling blood glucose and provoke subsequent activation of physiological defense mechanisms against glucose deprivation. In particular, our data show for the first time that activation of epinephrine responses to hypoglycemia, a major defense system against hypoglycemia in most patients with type 1 diabetes who have lost glucagon defenses, is dependent on brain K_{ATP} channels. Our work supports the broad hypothesis that analogies exist between the glucose-sensing pathways in pancreatic β -cells and those used by glucose-sensing neurons in specialized brain areas. These data also have potential clinical implications, as they may contribute to our understanding of how counterregulation becomes defective in some patients with diabetes. Finally, this pathway may even offer novel therapeutic targets for restoring or amplifying counterregulatory responses to hypoglycemia.

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