Effect of Recurrent Hypoglycemia on Spatial Cognition and Cognitive Metabolism in Normal and Diabetic Rats

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The effects of recurrent hypoglycemia (RH) on cognition in human subjects remain controversial, perhaps in part due to difficulty in completely controlling previous hypoglycemic history. We used a model of RH in nondiabetic and diabetic rats to examine the effects of short-term (3 h daily for 3 days) RH on subsequent hippocampally dependent spatial memory, tested either at euglycemia or under acute hypoglycemia. Hippocampal metabolism was simultaneously measured using microdialysis. Antecedent RH improved task performance (79 ± 2% alternation in nondiabetic RH animals vs. 63 ± 3% in controls; $P < 0.001$) at euglycemia, accompanied by reversal of the task-associated dip (20 ± 1% below baseline) in hippocampal extracellular fluid (ECF) glucose seen in control animals. RH rats also had a larger rise in hippocampal ECF glucose, after intraperitoneal glucose injection, than did controls. However, RH animals tested at acute hypoglycemia (~2.8 mmol/l) performed significantly worse than control animals. Results were similar in diabetic and nondiabetic rats. Our data suggest that RH causes improvement in subsequent cognitive performance at euglycemia, accompanied by alterations in cognitive metabolism. When glucose availability is limited, complex cognitive functioning seems to be adversely effected in RH animals, perhaps to better maintain and preserve basic brain functions. *Diabetes* 53:418–425, 2004

Hypoglycemia remains the major obstacle to achieving the established benefits of intensive insulin therapy in individuals with type 1 diabetes (1); in the Diabetes Control and Complications Trial, severe hypoglycemia was increased threefold in intensively treated patients (2). As a result, hypoglycemia is viewed as the most feared complication of such treatment (3). Extensive evidence shows that acute hypoglycemia impairs cognitive performance. Moreover, recurrent hypoglycemia may also alter brain function, brain glucose supply, and/or the ability to meet cognitive challenges. Reports differ regarding whether recurrent hypoglycemia (RH) may further worsen (4–6), have no effect on (7–11), or protect (12–18) cognitive performance during a period of acute hypoglycemia, although the question is generally posed as one of whether beneficial adaptation occurs (3). Furthermore, many studies report variable effects across their test batteries.

There have been few studies of the effects of RH on subsequent cognitive performance when euglycemic, which will be the case for the majority of the time even in patients with type 1 diabetes. The limited literature suggests that RH may cause slight deterioration, especially in challenging and/or hippocampally mediated tasks (19–21). The hippocampus plays a critical role in learning and memory and seems to be the most sensitive brain region to a variety of insults, including hypoglycemia (19,22). Although not unanimous (18), the literature suggests that cognitive effects of acute hypoglycemia are also most readily seen on relatively demanding tasks (3,7,17,23,24).

During elevated cognitive demand, glucose supply to the hippocampus of even euglycemic, nondiabetic animals is insufficient to meet the demands placed by cognitive testing (25); administration of exogenous glucose both enhances performance and reverses the decrease seen in brain extracellular fluid (ECF) glucose (25), a decrease that is correlated with the difficulty of task used. Antecedent hypoglycemia causes increased blood-brain barrier glucose transporter availability, and chronic hypoglycemia may also cause increased neuronal GluT3 and blood-brain barrier GluT1 expression (26–28). Cerebral blood flow may also be affected by both acute and chronic hypoglycemia, but reports are conflicting (18,29–31). Whether these changes result in increased brain glucose uptake is also controversial (18,30,32,33) and may depend on the exact parameters of antecedent hypoglycemia. However, increased glucose delivery to the brain after RH might lead to improved cognitive performance, provided that tasks of sufficient difficulty are used.

This study examined the effects of RH, in both nondiabetic and streptozotocin (STZ)-induced diabetic rats, on subsequent cognitive performance, using a hippocampally dependent task known to be sensitive to glucose availability (25). Throughout testing, we measured local metabolism in the hippocampus by microdialysis. We hypothesized that RH might act to improve task performance by increasing hippocampal glucose supply, in keeping with data on the effects of glucose in modulating human cognition, particularly when tasks of sufficient difficulty are used (34). However, an alternative, consistent with many studies to date (e.g., (8,10,13,24)), is that adaptation to RH might preferentially support more basic brain functions, particularly when glucose supply is limited, thereby preserving neuronal health at the expense of more complex cognitive function.

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ECF, extracellular fluid; RH, recurrent hypoglycemia; STZ, streptozotocin.

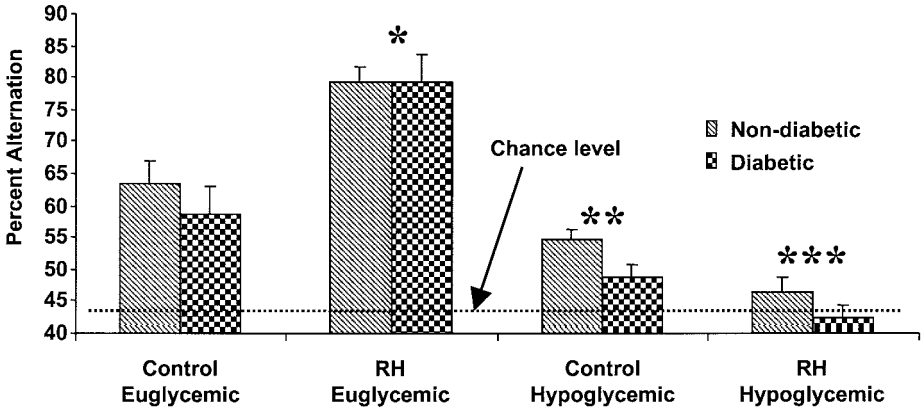

FIG. 1. Alternation performance of animals, both diabetic and nondiabetic. Control animals are those that have not previously been made hypoglycemic; RH animals have been made recurrently hypoglycemic on each of the preceding 3 days. "Euglycemic" and "Hypoglycemic" denote the state of animals at the time of testing. Asterisks indicate significantly different performance levels; groups marked with *, **, or * are significantly different from control, euglycemic animals, and from each other. See text for full details.**

RESEARCH DESIGN AND METHODS

A total of 125 male Sprague-Dawley rats (Charles River, Wilmington, MA), 3 months of age, were studied. Rats were individually housed, with food and water available ad libitum, on a 12:12-h light:dark schedule (lights on 0700). All procedures were approved by the Institutional Animal Care and Use Committee. Some animals (n = 41) were given intraperitoneal STZ (65 mg/kg) to induce experimental diabetes. One month after injection, they were screened after a 24-h fast; a fasting plasma glucose of 150 mg/dl or greater (higher than seen in any control animal) was accepted as diabetic. Nine animals did not meet this criterion and were not included in analyses. However, these animals were tested as if they had met the blood glucose criterion, allowing evaluation of any nonspecific effects of STZ administration. None differed from the appropriate nondiabetic group in performance, blood glucose, or hippocampal measures.

Surgery. Rats received atropine sulfate (0.2 ml of 540-mg/ml solution, intraperitoneally) 10 min before anesthesia with a ketamine:xylazine mix. Sterile stereotaxic procedures were used to implant microdialysis guide cannulae (CMA/Microdialysis; outer diameter 0.8 mm) aimed at the hippocampus, as described previously (35). The nose bar was set at 5.0 mm above the interaural line, and coordinates were 3.8 mm posterior from bregma, 5.0 mm lateral from midline, and 4.5 mm ventral from dura. Rats were allowed to recover for at least 1 week, during which time all animals were handled individually for a minimum of 5 min each day. Approximately 5 days before testing, indwelling vascular catheters were implanted as described previously (36); in some animals, the two surgeries were combined.

Groups. Animals were randomly assigned to RH or control. RH animals were made hypoglycemic on each of the 3 days immediately before testing: food was removed and insulin (6–10 units/kg) administered intraperitoneally. This dose of insulin reduced blood glucose to ~50 mg/dl (data not shown; in line with previous work [36]) for 3 h, after which food was given and any animal not eating or showing signs of torpor received 1 g of glucose intraperitoneally. Animals were observed to ensure absence of seizures. Control animals received saline injections.

Twenty-four hours before testing, a microdialysis probe was inserted through the guide cannula, left in place for 5 min, then replaced with the dummy stylet. We and others have shown this to produce optimal conditions for measurement of glucose during testing the following day (25,37–39). On the day of cognitive testing, animals were further randomly assigned to one of three conditions: *1*) euglycemia (EU), *2*) acute hypoglycemia (HYPO), or *3*) acute glucose-injected (GLC). STZ animals were not tested under the GLC condition, given their chronic hyperglycemia, giving a total of 10 groups of animals (6 nondiabetic and 4 STZ diabetic).

HYPO animals (RH n = 7, control n = 8, STZ-RH n = 8, STZ-control n = 8) received intraperitoneal insulin (6–10 units/kg) 20 min before maze testing. This reduced their plasma glucose to ~50 mg/dl at the midpoint of the test period. EU animals (RH n = 14, control n = 11, STZ-RH n = 9, STZ-control n = 7) received a control injection of saline. GLC animals (RH n = 12, control n = 14) received 250 mg/kg glucose intraperitoneally 30 min before testing (this dose and timing have been previously shown to enhance maze performance [25]).

An additional 18 animals were not tested for maze performance but were used to determine the impact of RH on blood and hippocampal ECF glucose after an intraperitoneal glucose bolus. These were split into two groups: RH-bolus (n = 8) and control-bolus (n = 10).

Microdialysis procedures. A fresh microdialysis probe was inserted on the morning of testing, connected via a liquid swivel (Instech), and animals were allowed to acclimatize for 2 h. The dialysis membrane (CMA12; CMA/Microdialysis) was 3 mm long and thus sampled across several regions of the hippocampal formation. Rats were allowed to move freely, minimizing possible alteration of brain or plasma glucose as a result of restraint stress. Probes were perfused at 1.5 μl/min with artificial cerebrospinal fluid, as previously described (35). All reagents were obtained from Sigma Chemicals (St. Louis, MO).

Blood sampling. On the day of testing, the vascular catheters were opened and plasma glucose samples (100 μl) were taken at 10-min intervals. Samples were taken at the beginning of the corresponding microdialysis sample and so measured at discrete time points rather than being an average over the 10-min period in the way that microdialysis samples are. Additional samples (200 μl) were taken during baseline and immediately after testing for analysis of circulating plasma epinephrine and glucagon. Several animals did not have patent catheters; their performance and microdialysis data were included in analyses.

Sample analysis. Microdialysis samples were assayed for glucose and lactate using a CMA600 analyzer designed for small aliquots. Glucose and lactate concentration in the microdialysis samples was corrected for in vivo probe recovery (33%) using the slope of a hippocampal ECF zero-net-flux plot for glucose under the same experimental conditions (see 35 for details; the slope of a zero-net-flux plot gives a direct measure of probe recovery). In vitro pilot experiments showed probe recovery for glucose and lactate to be very similar. Plasma glucose was measured by the glucose oxidase method (Beckman, Fullerton, CA). Catecholamine analysis was performed by HPLC (ESA, Acton, MA).

Behavioral procedures. Rats were placed into the center of a four-arm maze and allowed to explore for 20 min. Samples were collected continuously before, during, and after the test period. Rats spontaneously alternate between maze arms, using spatial working memory to retain knowledge of arms previously visited. Spontaneous alternation has been used extensively as a spatial working memory task (40–43). Specifically, the measure of memory performance used was percent 4/5 alternation. An alternation is counted when the rat visits all four arms within a span of five arm choices. Chance level on this measure is 44% (25). Motor activity was measured by total arm entries, although cognitive performance on this task has been shown not to be correlated with motor activity (25). Bolus-group animals were not placed on the maze but remained in the control chamber.

Histology. Rats were killed by overdose of sodium pentobarbital. Brains were placed in a 30% sucrose/10% formalin solution for 3 days, then frozen at −20°C and mounted on a cryostat (Leica). Sections (40 μm) were taken through the hippocampus and stained with cresyl violet for confirmation of probe placement; two animals' microdialysis data were excluded on the basis of incorrect placements.

Statistical analysis. Plasma and ECF glucose during the euglycemic and hypoglycemic protocols were analyzed using a three-way (pretest condition, diabetes status, time) repeated measures covariance pattern model. The unstructured covariance pattern provided the best fit based on likelihood ratio tests. Simple effects were examined using post hoc contrasts with appropriate Bonferroni adjustments. Changes in epinephrine levels from baseline to posttesting were compared using paired t tests and ANOVA. Plasma and ECF glucose after glucose bolus were analyzed using area-under-curve t tests. Maze performance and motor activity were compared using three-way (pretest condition, diabetes status, acute glycemic status) ANOVA. Comparisons between the two groups that received glucose used unpaired t tests. Error variances were compared using Levene's test. An α level of 0.05 was set for significance. Means are expressed ± SE.

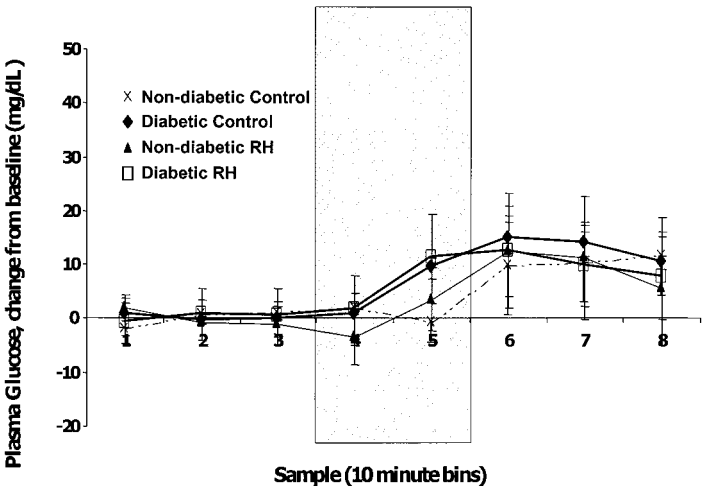

FIG. 2. Change in plasma glucose levels in animals tested at euglycemia, throughout testing procedure. No significant intergroup differences were seen. Maze testing period is shown by the light gray box and covers samples 4 and 5.

RESULTS

Studies at euglycemia

Maze performance. Performance of all groups is shown in Fig. 1. Acute hypoglycemia alone ($F_{1,71} = 114.41$, $P < 0.0001$), RH alone ($F_{1,71} = 6.94$, $P = 0.01$), and their interaction ($F_{1,71} = 37.45$, $P < 0.0001$) affected performance. Post hoc tests among euglycemic groups revealed that RH improved performance in both nondiabetic ($P < 0.001$) and diabetic ($P < 0.01$) animals. Diabetes did not affect performance either alone or in interaction (all $F < 0.7$, all $P > 0.4$). Task performance in both euglycemic RH groups approached ceiling levels (note that 100% alternation is not optimal but rather indicates abnormal perseveration of turning in a given direction [40]). No main or interaction effects on motor activity (number of arms entered) were found between any euglycemic groups (group means all between 19.3 and 23.6 arms entered; all $F < 2.0$, all $P > 0.15$).

Plasma glucose. Plasma glucose was significantly higher for diabetic animals (355 ± 20 mg/dl vs. 126 ± 1 mg/dl in nondiabetics; $F_{1,21} = 18.45$, $P < 0.001$). In all groups, plasma glucose rose gradually ($F_{7,21} = 3.12$ for main effect of time, $P = 0.02$; Fig. 2); however, post hoc comparisons of baseline to recovery plasma glucose within each group did not reach statistical significance. RH had no significant effect.

Hippocampal glucose. ECF glucose was higher in diabetic animals (2.01 ± 0.11 vs. 1.05 ± 0.02 mmol/l in nondiabetics; $F_{1,29} = 514.0$, $P < 0.0001$). There was a main effect of time ($F_{7,29} = 12.32$, $P < 0.0001$); however, this effect was dependent on RH ($F_{7,29} = 3.23$ for RH*time interaction, $P = 0.01$). Post hoc tests revealed significant decreases in ECF glucose in the first maze sample (time 4) in both diabetic and nondiabetic control groups ($P < 0.01$ and 0.001, respectively; Fig. 3). The task-associated dip was similar in the two groups (0.15 ± 0.04 mmol/l in nondiabetic controls, 0.15 ± 0.03 mmol/l in diabetic control animals), although this was a smaller percentage drop in diabetic animals given their higher baseline (7.7 vs. 14%). In contrast, no significant changes from baseline were observed in either the diabetic or nondiabetic RH animals ($P = 0.82$ and 0.26, respectively). After testing, ECF glucose rebounded in control animals, showing a significant increase from time 4 to the recovery period ($P < 0.0001$ and 0.01 for diabetic and nondiabetics, respectively).

Hippocampal lactate. Baseline lactate levels were slightly but significantly higher in diabetic animals (1.60 ± 0.01 vs. 1.40 ± 0.02 mmol/l in nondiabetics; $F_{1,24} = 8.42$, $P < 0.01$). RH did not affect lactate levels. The pattern of hippocampal ECF lactate in all groups is shown in Fig. 4.

Peripheral epinephrine. Small and marginally signifi-

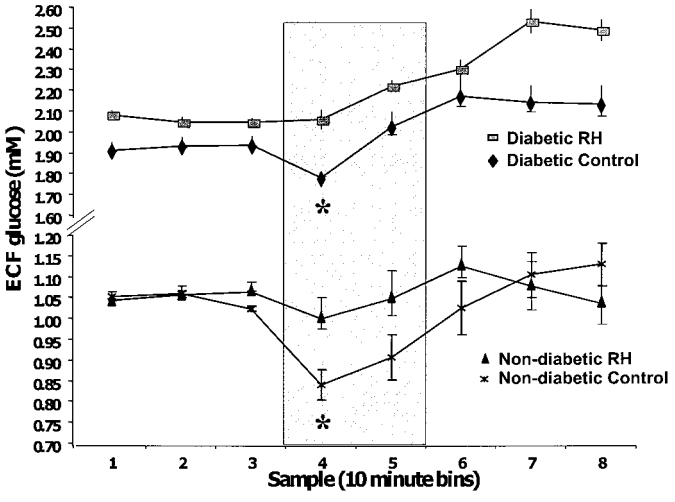

FIG. 3. Hippocampal ECF glucose levels before, during, and after maze testing, marked by light gray box. Asterisks indicate significant differences from baseline for sample 4.

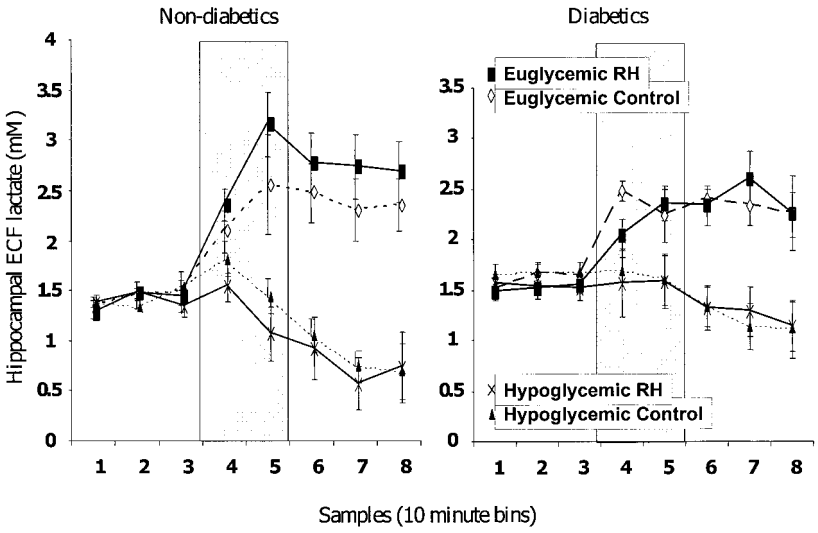

FIG. 4. Hippocampal ECF lactate levels, both diabetic and nondiabetic, throughout maze testing (denoted by light gray boxes). Legend applies to both parts of figure.

cant ($P < 0.10$) rises were observed in posttesting samples, compared with baseline levels (Table 1). The magnitude of response was not significantly affected by diabetes or RH group. Furthermore, no animal had an epinephrine level that would suggest stress.

ECF glucose response to intraperitoneal glucose bolus—no maze testing. To examine the effects of RH on delivery of glucose from the periphery, we administered glucose (250 mg/kg intraperitoneally) to control and RH animals, without any further testing, and measured subsequent glucose levels in the blood and hippocampal ECF. As shown in Fig. 5, there was a small rise in plasma glucose levels, after glucose injection, which did not differ between the two groups [area under curve $t(10) = 0.23$, $P > 0.8$]. In contrast, ECF glucose showed a striking effect of RH, with the RH-bolus group showing a three- to fourfold greater increase in hippocampal ECF glucose [$t(14) = 3.44$, $P < 0.005$; Fig. 5].

Maze performance after exogenous glucose. If the enhanced performance seen at euglycemia after RH was due (at least in part) to increased hippocampal glucose supply, allowing optimal function, then RH animals might not show any further benefit from administration of glucose (which enhances performance in control animals [25]). Hence, we tested additional animals on maze performance after an intraperitoneal glucose bolus. STZ-diabetic animals were not tested with exogenous glucose because of their baseline hyperglycemia. As in the bolus injection study above, plasma glucose rose identically in control and RH animals (peak of 154 ± 4.8 mg/dl in control and 157 ± 8.3 mg/dl in RH animals; data not shown). The control-GLC group showed a significant improvement in alternation performance versus the control-EU group [80 ± 3% vs. 64 ± 3%, $t(20) = 3.97$, $P < 0.05$]. However, glucose administration did not affect performance in the RH animals [77 ± 2% in GLC animals vs. 79 ± 2% in EU animals, $t(20) = 0.80$, $P > 0.4$]. Glucose administration did not alter the number of arms entered compared with euglycemic animals; group means for control-GLC and RH-GLC animals were 21.5 and 23.6 arms entered, respectively.

Hippocampal glucose. The rise in ECF glucose after glucose injection was greater in RH animals than in control animals [137 ± 6.4% of baseline in RH animals vs. 123 ± 2.2% in control animals, $t(17) = 2.2$, $P < 0.05$]. Neither group showed a dip in ECF glucose levels below baseline during testing.

TABLE 1
Plasma epinephrine (pg/ml)

	Basal	After maze
Euglycemic		
Nondiabetic		
Control	58 ± 4	110 ± 20
RH	63 ± 3	142 ± 32
Diabetic		
Control	51 ± 6	160 ± 28
RH	49 ± 8	124 ± 21
Hypoglycemic		
Nondiabetic		
Control	65 ± 5	4,986 ± 539*
RH	75 ± 13	1,611 ± 363*
Diabetic		
Control	72 ± 7	3,010 ± 695*‡
RH	83 ± 12	890 ± 293*†‡

Plasma epinephrine concentrations, taken both at baseline (basal) and immediately after completion of maze testing. *Significant increase in after-maze versus basal samples; †significant effect of RH (epinephrine decreased versus controls); ‡significant effect of diabetes (epinephrine decreased versus nondiabetics).

Studies at hypoglycemia

Plasma glucose. At baseline, plasma glucose was significantly elevated in the diabetic compared with nondiabetic groups ($F_{1,21} = 28.9$, $P < 0.0001$), but no difference was present during testing ($F_{1,21} = 3.61$, $P = 0.21$) or recovery ($F_{1,21} = 1.85$, $P = 0.54$). Plasma glucose was reduced close to 50 mg/dl during the testing period (group means during testing: nondiabetic control 49 ± 6, nondiabetic RH 49 ± 4, STZ-control 58 ± 6, STZ-RH 56 ± 4) (Fig. 6). No significant main or interactive effects involving RH were present.

Performance. As noted above, acute hypoglycemia alone, RH alone, and their interaction significantly affected performance. Post hoc tests among hypoglycemic groups revealed that, in contrast to the results at euglycemia, RH impaired performance in both nondiabetic ($P = 0.01$) and diabetic ($P = 0.04$) animals. Hypoglycemia markedly reduced motor activity ($F_{1,71} = 9.27$, $P < 0.01$), in line with

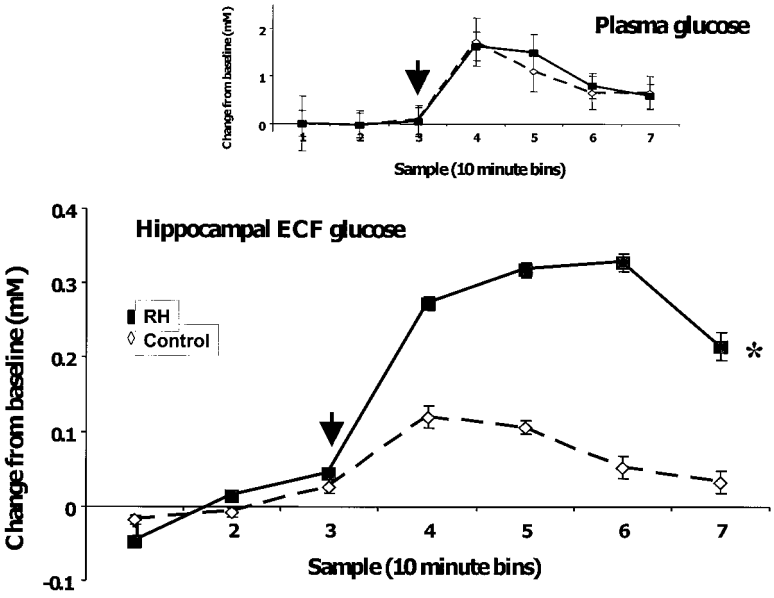

FIG. 5. Response to intraperitoneal glucose bolus (250 mg/kg), given at time of arrows. Main figure shows response of hippocampal ECF glucose in control and RH animals. Asterisk indicates significant intergroup differences for total area under the curve. Inset figure shows plasma glucose response, with same legend; no intergroup difference was seen.

human data (7), but no other effects were found (group means between 14.1 and 15.3 arms entered).

Hippocampal glucose. Hippocampal glucose decreased in all groups after induction of hypoglycemia ($F_{7,24} = 112$, $P < 0.0001$; Fig. 7), and this was dependent on both diabetes and RH status ($F_{7,24}$ for three-way interaction = 5.84, $P < 0.001$). Diabetic animals demonstrated greater percentage and absolute reductions (50 vs. 32% in controls, 63 vs. 55% in RH animals). Despite this, diabetic animals demonstrated consistently higher ECF glucose than nondiabetics ($F_{1,24} = 59.05$, $P < 0.0001$). Furthermore, we conducted two-way repeated measures analyses within diabetics and nondiabetics separately. In diabetics, a significant RH × time interaction was observed ($F_{7,14} = 18.26$, $P < 0.0001$). Post hoc tests revealed lower ECF glucose in the RH group at times 3 and 4 ($P < 0.0001$ and 0.01, respectively), indicating a more rapid fall in ECF glucose among the RH group. In nondiabetics, ECF glucose tended to be lower in the RH group ($F_{1,10} = 3.2$, $P = 0.10$), but neither this nor the interaction between RH and time ($F_{7,10} = 2.3$, $P = 0.23$) was statistically significant.

Peripheral epinephrine. Significant increases in circulating epinephrine after maze testing (hence at hypoglycemia) were observed in all groups ($P < 0.05$ in each group). The magnitude of epinephrine response was dependent on diabetes and RH status, with diabetic and RH groups showing significantly blunted responses ($F_{1,18} = 4.45$, $P = 0.05$ and $F_{1,18} = 14.14$, $P < 0.001$, respectively) (Table 1).

DISCUSSION

Our data form a somewhat complex picture. Three days of RH enhanced performance in animals that were tested the following day when euglycemic. However, in animals that were tested when hypoglycemic, RH resulted in a decrement in performance. Euglycemic control animals showed a task-associated dip in hippocampal ECF glucose, replicating our previous findings (25), suggesting that under control conditions, hippocampal glucose supply may not fully meet the demands of cognitive processing on the relatively difficult task used here. RH reversed this dip, a finding consistent with the hypothesis that RH might

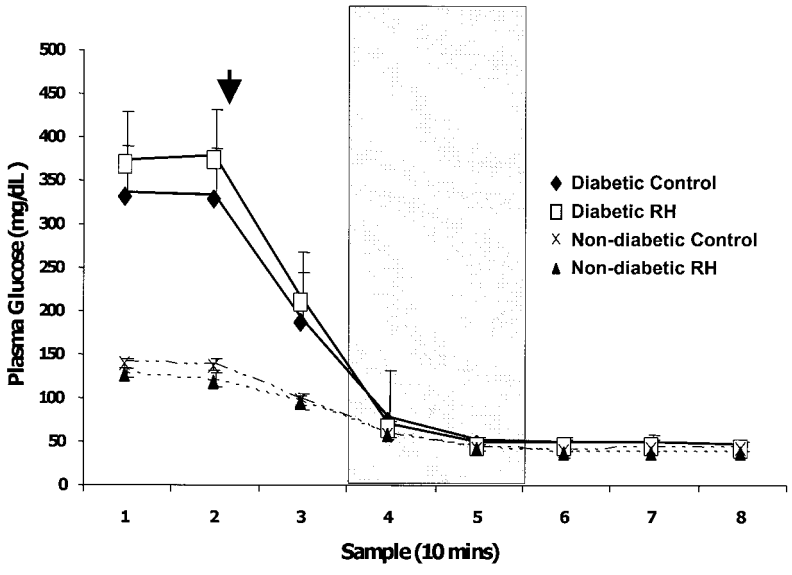

FIG. 6. Plasma glucose levels in animals tested at hypoglycemia, throughout testing procedure (light gray box denotes time of testing). Intraperitoneal insulin bolus given to all groups at time of arrow. No significant intergroup differences were seen during or after the maze period.

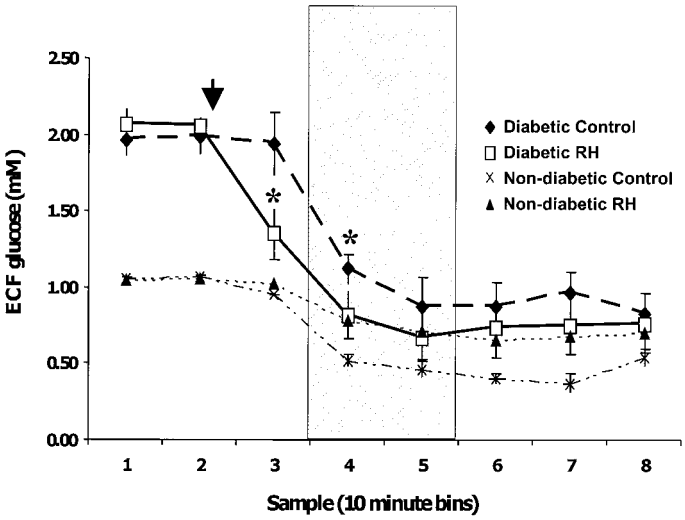

FIG. 7. Hippocampal ECF glucose levels in animals tested at hypoglycemia, throughout testing procedure (marked by light gray box). Intraperitoneal insulin bolus given to all groups at time of arrow. Asterisks mark significant differences between control and RH diabetic groups at times 3 and 4.

facilitate hippocampal delivery of extra glucose to meet metabolic demands of cognitive challenge.

Alternative mechanisms of action for RH exist, however. For instance, RH might alter local glucose metabolism, requiring less glucose to meet the task's demands and/or making increased use of lactate as a fuel (because ECF lactate levels were not affected by RH, more efficient glucose usage is perhaps more likely). Such effects might occur in addition to an increase in glucose transport—they are not exclusive. Another possibility is that RH causes hippocampal cell loss (22) and hence reduced glucose demand, although this would be inconsistent with improved hippocampal function. Nonspecific leakage of glucose across the blood-brain barrier seems unlikely, given the lack of any change in baseline ECF glucose. The finding that ECF glucose rose more in RH animals, in response to an intraperitoneal glucose bolus, is consistent with the suggestion of increased glucose delivery but does not rule out the possibility of additional effects. In any case, the net effect of RH at subsequent euglycemia seems to be to allow improved hippocampal functioning.

Diabetes did not affect performance. If RH improves cognitive performance, at least in part, by increasing glucose delivery to the hippocampus, then one might question why RH should enhance the performance of diabetic animals, with their elevated levels of blood and ECF glucose, and why diabetic control animals do not perform better than do nondiabetic controls. However, our data, showing that diabetic control animals had a task-associated dip in hippocampal ECF glucose (reversed by RH) similar to that of nondiabetic controls, suggest that despite higher basal ECF glucose, hippocampal glucose supply is insufficient for optimal task performance just as in nondiabetic animals. Prolonged hyperglycemia may lead to such an insufficiency of functional glucose supply at times of increased demand; a reduction in, e.g., glucose transport across the blood-brain barrier is consistent with the fact that although diabetic plasma glucose was roughly threefold higher in diabetic animals, ECF glucose was only approximately twofold higher. Alternatively, diabetic animals may have adapted hippocampal metabolism to rely on high levels of circulating glucose for even basal cognitive function.

At moderate hypoglycemia, in contrast to euglycemia, RH significantly diminished cognitive performance in both diabetic and nondiabetic animals. Hippocampal glucose began to decrease in diabetic RH animals immediately after insulin administration, whereas ECF glucose did not begin to decrease in diabetic control animals until the following sample; a similar although nonsignificant trend was seen in nondiabetic animals. Such a more rapid translation of change in peripheral glucose to change in ECF glucose (as a result of altered blood-brain barrier transport?) might have contributed to the adverse effect of RH in the setting of acute hypoglycemia. The reason for the difference between diabetics and nondiabetics is not clear, but (as suggested above) the diabetic hippocampus may adapt to high circulating glucose, with increased susceptibility to reductions in glucose availability. The occurrence of such adaptation is further supported by examination of the ECF glucose data in control, hypoglycemic, diabetic animals, in which the glucose level does not reach the nadir seen in control, euglycemic, nondiabetic animals, yet the performance of the hypoglycemic diabetics is much worse, suggesting diminished hippocampal functioning at normal ECF glucose levels.

Additional mechanisms may contribute to the performance deficit in RH animals during hypoglycemia. For example, suppression of circulating epinephrine in hypoglycemic RH animals might reduce cognitive performance. Peripheral epinephrine enhances memory (44) but has traditionally been thought to act via elevation of circulating glucose (45), a mechanism not consistent with the current data. However, epinephrine may also affect memory via modulation of vagal input (46). RH is known to blunt other counterregulatory responses to further hypoglycemic episodes (47), and several of these hormones (in particular, glucocorticoids) are also known to affect hippocampal cognitive processing (48–51).

An alternative possibility is that RH causes increased glucose supply at hypoglycemia as well as at euglycemia but that this supply is targeted, at times of acute hypoglycemia, to support basic brain and cognitive functions at the expense of more complex cognitive faculties. This possibility is consistent with literature on human hypoglycemic cognitive function (8,10,13,23,24), as well as with recent functional imaging data suggesting that brain glucose uptake during hypoglycemia may be enhanced by

previous RH (32), and is compatible with an additional effect of circulating hormone levels; the two are not mutually exclusive. The hippocampus is a major target of neuronal loss after hypoglycemia (22), so adaptation to RH might adaptively preserve cellular function and health at the expense of complex cognitive functioning. Indeed, Cranston et al. (32) noted explicitly that their data would be explicable by alterations in metabolic rate, rather than necessarily being purely due to transport effects; a lower cerebral metabolic rate in RH animals would be in line with suggestions of protective adaptation to recurrent hypoglycemia. Preferential support of more basic brain functions after RH would reconcile much of the seemingly contradictory data on the impact of RH on human cognitive function, as well as the observation that auditory brainstem function is not diminished after RH in rats (52). Caution is needed in extending the present data beyond the hippocampus, however, as the hippocampus has a relatively high basal rate of glucose metabolism.

Our data show that RH diminishes ability to meet the demands of a relatively demanding cognitive challenge during hypoglycemia. For diabetic patients who are being treated intensively with insulin, this poses a concern. Not only may they be more vulnerable to hypoglycemia as a result of hypoglycemia-associated autonomic failure (53), but also the deleterious cognitive effects of acute hypoglycemia in such patients may reduce ability to respond appropriately, further delaying any action to restore euglycemia, in a vicious cycle.

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