Severe Hypoglycemia–Induced Fatal Cardiac Arrhythmias Are Augmented by Diabetes and Attenuated by Recurrent Hypoglycemia

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We previously demonstrated that insulin-mediated severe hypoglycemia induces lethal cardiac arrhythmias. However, whether chronic diabetes and insulin deficiency exacerbates, and whether recurrent antecedent hypoglycemia ameliorates, susceptibility to arrhythmias remains unknown. Thus, adult Sprague-Dawley rats were randomized into four groups: *1*) nondiabetic (NONDIAB), *2*) streptozotocin-induced insulin deficiency (STZ), *3*) STZ with antecedent recurrent (3 days) hypoglycemia (~40–45 mg/dL, 90 min) (STZ+RH), and *4*) insulin-treated STZ (STZ+Ins). Following treatment protocols, all rats underwent hyperinsulinemic (0.2 units · kg^{-1} · min^{-1}), severe hypoglycemic (10–15 mg/dL) clamps for 3 h with continuous electrocardiographic recordings. During matched nadirs of severe hypoglycemia, rats in the STZ+RH group required a 1.7-fold higher glucose infusion rate than those in the STZ group, consistent with the blunted epinephrine response. Second-degree heart block was increased 12- and 6.8-fold in the STZ and STZ+Ins groups, respectively, compared with the NONDIAB group, yet this decreased 5.4-fold in the STZ+RH group compared with the STZ group. Incidence of third-degree heart block in the STZ+RH group was 5.6%, 7.8-fold less than the incidence in the STZ group (44%). Mortality due to severe hypoglycemia was 5% in the STZ+RH group, 6.2-fold less than that in the STZ group (31%). In summary, severe hypoglycemia–induced cardiac arrhythmias were increased by insulin deficiency and diabetes and reduced by antecedent recurrent hypoglycemia. In this model, recurrent moderate hypoglycemia reduced fatal severe hypoglycemia–induced cardiac arrhythmias.

People with type 1 diabetes experience an average of two episodes of symptomatic hypoglycemia each week and at least one episode of temporarily disabling severe hypoglycemia each year (1). Symptoms of hypoglycemia range from mild to severe and can include anxiety, palpitations, tremor, hunger, sweating, cognitive dysfunction, seizures, and coma (2). When severe, hypoglycemia can cause brain damage and even death (3–6). Up to 10% of deaths among young people with type 1 diabetes are caused by hypoglycemia (7). The "dead in bed syndrome" describes the sudden, unexplained death of young people with type 1 diabetes (6,8). Case reports have confirmed hypoglycemia associated with sudden death (6,7), but how severe hypoglycemia causes sudden death is not well understood. Our previous research in a rat model suggests that cardiac arrhythmias induced by severe hypoglycemia precede sudden death (3).

The risk of severe hypoglycemia is increased in patients who experience repeated episodes of hypoglycemia. This increased risk is thought to be due to a blunted counterregulatory response induced by recurrent hypoglycemia and lack of awareness of hypoglycemia (4,9–12). While recurrent hypoglycemia can be considered maladaptive, our laboratory and others (13) have advanced the notion that the adaptive response to recurrent hypoglycemia can be considered beneficial in that it reduces brain damage and cognitive dysfunction induced by a subsequent episode of severe hypoglycemia (4). However, whether recurrent hypoglycemia is also beneficial during severe hypoglycemia to reduce fatal cardiac arrhythmias is unknown. We therefore wished to test, using a rodent model of streptozotocin-induced insulin deficiency, the hypothesis that the adaptive response to recurrent moderate hypoglycemia could reduce the incidence of severe hypoglycemia–induced fatal cardiac arrhythmias. Mechanistically we sought to explore the possible

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contribution of insulin therapy per se, versus insulin-induced hypoglycemia, in mediating susceptibility to arrhythmias. In addition, because the effect of diabetes on severe hypoglycemia–induced cardiac arrhythmias remains unexplored, we tested the hypothesis that diabetic rats have an increased susceptibility to hypoglycemia-induced cardiac arrhythmias when compared with nondiabetic rats.

RESEARCH DESIGN AND METHODS

Animals

Adult male Sprague-Dawley rats (weight, 250–300 g; Charles River Laboratories, Malvern, PA) were housed individually in temperature- and light-controlled environments and fed ad libitum a standard chow diet and water. All studies were done in accordance with and approved by the Animal Studies Committee at Washington University School of Medicine and the University of Utah School of Medicine.

Surgery

All four groups of rats underwent surgery for carotid artery (blood glucose and hormone sampling) and jugular vein (insulin and glucose infusions) and electrocardiogram (ECG) lead placement, as previously described (3) (Fig. 1*A* and *B*).

Induction of Diabetes

Two days after cannulation, three groups of rats received intraperitoneal injections of streptozotocin (65 mg/kg; Sigma, St. Louis, MO) to induce diabetes (n = 64); a fourth group of rats received sodium citrate buffer and acted as a control (NONDIAB group; n = 28). Blood glucose was measured from the tail vein (Ascensia Contour; Bayer HealthCare, LLC, Mishawaka, IN).

Insulin Treatment

Two days after streptozotocin injection, rats in one group were implanted with subcutaneous insulin pellets (2 units/day; LinPlant; Lin Shin, Toronto, ON, Canada) (STZ+Ins group; n = 12). To avoid the possible confounding effects of recurrent hypoglycemia in these insulin-treated rats, a glycemic goal of 200–300 mg/dL was chosen. Glucose levels were checked via the tail vein twice daily.

Recurrent Hypoglycemia

Approximately 2 weeks after streptozotocin injection, insulin-deficient rats were randomized to one of two groups: *1*) insulin deficiency with recurrent saline (STZ group; n = 32) or *2*) insulin deficiency with recurrent hypoglycemia (STZ+RH group; n = 20). The rats with recurrent hypoglycemia underwent recurrent moderate hypoglycemia (40–45 mg/dL for 90 min) for three consecutive days, with subcutaneous insulin injections (22–25 units/kg; Humulin R; Eli Lilly, Indianapolis, IN). The STZ group was injected with saline for 3 days. Food was withheld after injections and blood glucose was measured every half hour via the tail vein. To terminate hypoglycemia, rats were subcutaneously administered 50% dextrose (Hospira, Lake Forest, IL) and allowed free access to food. Hyperinsulinemic–severe hypoglycemic clamps were performed on day 16 (i.e., after the preceding 3 days of treatment with recurrent hypoglycemia or saline).

Hyperinsulinemic–Severe Hypoglycemic Clamp

All four groups of rats, which had been fasted overnight and were awake and unrestrained, were subjected to hyperinsulinemic (0.2 units $\cdot$ kg^{-1} $\cdot$ min^{-1}; Humulin R), severe hypoglycemic (10–15 mg/dL) clamps with continuous ECGs for 3 h, as previously described (3).

Arterial blood samples were obtained throughout the clamp in order to measure blood glucose, electrolytes, and gases (pHOx Plus C arterial blood gas analyzer; Nova Biomedical, Waltham, MA). Epinephrine was measured by ELISA (Abnova, Taipei, Taiwan). Insulin was also measured by ELISA (Crystal Chem Inc., Downers Grove, IL). Respiration was determined by counting visible breaths. ECGs were recorded and arrhythmias assessed using PowerLab 26T software (LabChart; ADInstruments, Colorado Springs, CO), as previously described (3).

Statistical Analyses

All data are represented as means ± SEMs. ANOVA was used to determine significance, unless otherwise indicated. Two-way repeated-measures ANOVA was used to compare glucose infusion rates. A Fisher exact test with

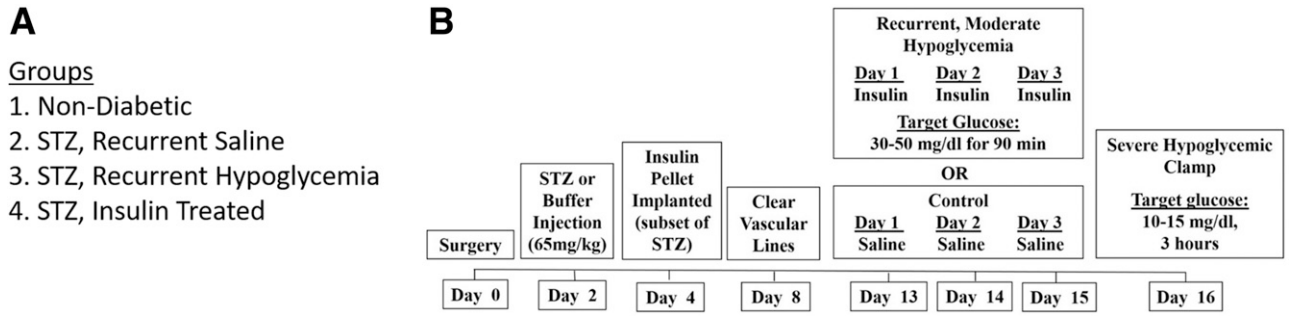

Figure 1—Experimental protocol. *A*: Rats were divided into four groups: *1*) NONDIAB (n = 28), *2*) STZ (n = 32), *3*) STZ+RH (n = 20), and *4*) STZ+Ins (n = 12). *B*: All rats underwent surgery to place catheters and ECG leads (day 0). Rats were injected 2 days later with either STZ or sodium citrate buffer. On day 4, one group of rats was treated with subcutaneous insulin pellets. On day 8, vascular lines were externalized, cleared, and replaced under the skin. On days 13–15, one group of rats underwent recurrent hypoglycemia (STZ+RH) while the other groups of rats were given saline. On day 16, all rats underwent hyperinsulinemic/severe hypoglycemic (10–15 mg/dL) clamps.

Freeman-Halton extension was used to determine significance for mortality and incidence of arrhythmias. $P < 0.05$ was considered significant.

RESULTS

For 2 weeks after the initial randomization, rats had a mean glucose of 107 ± 1, 528 ± 7, 523 ± 9, and 308 ± 13 mg/dL in the NONDIAB, STZ, STZ+RH, and STZ+Ins groups, respectively (Fig. 2*A*). Body weight increased among rats in the NONDIAB group during the experiment, but it did not change in the insulin-deficient (STZ, STZ+RH) or insulin-treated (STZ+Ins) groups (Fig. 2*B*). The STZ+RH-treated rats, which underwent 90-min periods of recurrent hypoglycemia on days 13, 14, and 15, exhibited glucose levels of 44 ± 2, 43 ± 4, and 43 ± 2 mg/dL, respectively (Fig. 2*C*). During these 3 days before the severe hypoglycemic clamp, saline-injected rats (STZ group) had glucose values of 528 ± 17, 517 ± 8, and 522 ± 19 mg/dL, respectively; rats in the NONDIAB group had glucose values of 108 ± 7, 109 ± 3, and 106 ± 1 mg/dL, respectively; and the rats in the STZ +Ins group, recurrently treated with insulin, had glucose levels of 398 ± 47, 233 ± 48, and 335 ± 40 mg/dL, respectively (Fig. 2*C*).

Severe Hypoglycemic Clamp

Glucose levels during the 3-h severe hypoglycemic clamp were evenly matched among the four groups (NONDIAB, 12 ± 0.2 mg/dL; STZ, 13 ± 0.3 mg/dL; STZ+RH, 13 ± 0.5 mg/dL; STZ+Ins, 13 ± 0.5 mg/dL; Fig. 3*A*). Insulin was similar among the groups during the clamp (data not shown). The mean glucose infusion rates for these four groups were 5.5 ± 0.5, 1.7 ± 0.3, 2.7 ± 0.4 and 0.3 ± 0.1 mg/kg/min, respectively (Fig. 3*B*). Epinephrine levels were similar among the groups during the basal period (before insulin infusion). During severe hypoglycemia, epinephrine increased in all groups; however, this response was blunted in the STZ +RH group (NONDIAB, 3,124 ± 81 pg/mL; STZ, 3,508 ± 387 pg/mL; STZ+RH, 1,856 ± 348 pg/mL; STZ+Ins, 2,695 ± 336 pg/mL; $P < 0.03$) (Fig. 3*C*).

Mortality due to severe hypoglycemia was not significantly different among the NONDIAB (14%), STZ (31%), or STZ+Ins (33%) groups. However, treatment with recurrent hypoglycemia decreased mortality 6.2-fold to just 5% in the STZ+RH group ($P < 0.035$, Fisher exact test; Fig. 4*A*).

Severe hypoglycemia–induced cardiac arrhythmias were consistently increased in rats in the STZ and STZ+Ins groups, whereas recurrent hypoglycemia reduced these arrhythmias. First-degree heart block was increased in the STZ (1.6 ± 0.8/min) and STZ+Ins (0.6 ± 0.2/min) groups compared with the NONDIAB group (0.009 ± 0.007/min; $P < 0.05$) (Fig. 4*B*). Second-degree heart block was similarly increased in the STZ (18 ± 2/min) and STZ+Ins (10 ± 4/min) groups compared with the NONDIAB group (1.5 ± 0.7/min; $P < 0.05$) (Fig. 4*C*). Antecedent recurrent hypoglycemia virtually eliminated first-degree heart block (0.004 ± 0.003/min; $P < 0.007$) and reduced second-degree heart block by 82% (3 ± 0.8/min; $P < 0.001$). As shown in Fig. 5, rats in the STZ and STZ+Ins groups had an increased frequency of second-degree heart block compared with rats in both the NONDIAB and STZ+RH groups. The incidence of third-degree heart block was 20%, 44%, and 42% in the NONDIAB, STZ, and STZ+Ins groups, respectively, but this was reduced to just 5.6% in the STZ+RH group ($P < 0.04$, Fisher exact test) (Fig. 4*D*). Nonsustained ventricular tachycardia (Vtach; defined as four or more premature ventricular contractions in a row) was present in 50% of the rats in the STZ+Ins

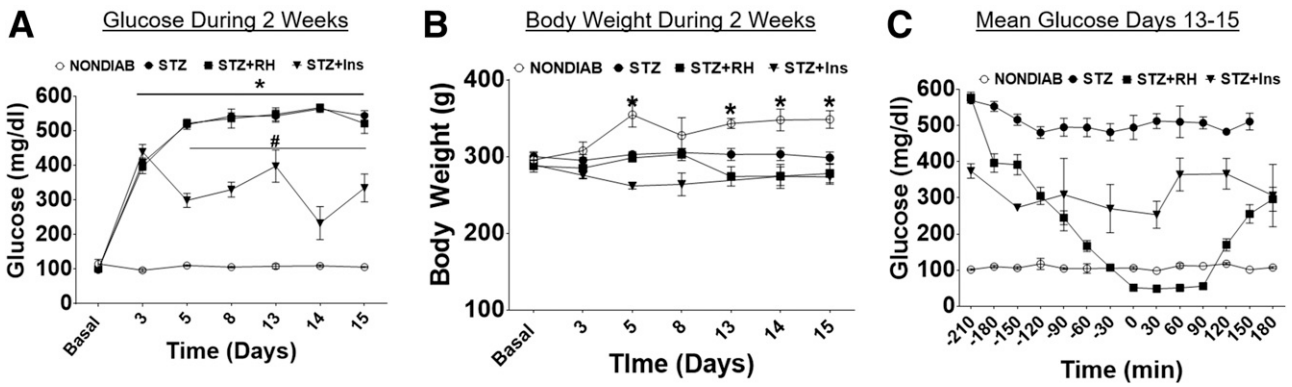

Figure 2—Glucose and body weight. *A*: All rats had normal glucose before STZ injection (Basal). After STZ injection (day 3), glucose levels were increased and remained elevated for the duration of the study in both the STZ (black circles) and STZ+RH (black squares) groups, whereas STZ rats receiving insulin treatment (black triangles) had lower glucose levels. Nondiabetic rats injected with buffer (white circles) had normal glucose for the duration of the study. *$P < 0.001$, NONDIAB vs. STZ, STZ+RH, and STZ+Ins; #$P < 0.001$, STZ+Ins vs. STZ and STZ+RH. *B*: Body weight increased from basal to the end of the study for the NONDIAB group. Body weight did not change in the STZ, STZ+RH, and STZ+Ins groups throughout the experiment. *$P < 0.05$, NONDIAB vs. STZ, STZ+RH, and STZ+Ins. *C*: Rats in the NONDIAB, STZ, and STZ+Ins groups were given saline injections on days 13, 14, and 15, and blood glucose levels remained stable throughout those days. The STZ+RH group underwent recurrent moderate hypoglycemia. Glucose levels steadily declined after insulin injection, and rats were hypoglycemic (~40–45 mg/dL) for 90 min before recovering with i.p. glucose injection and free access to food. Shown are the mean glucose values on days 13, 14, and 15 for each group. Data are means ± SEMs.

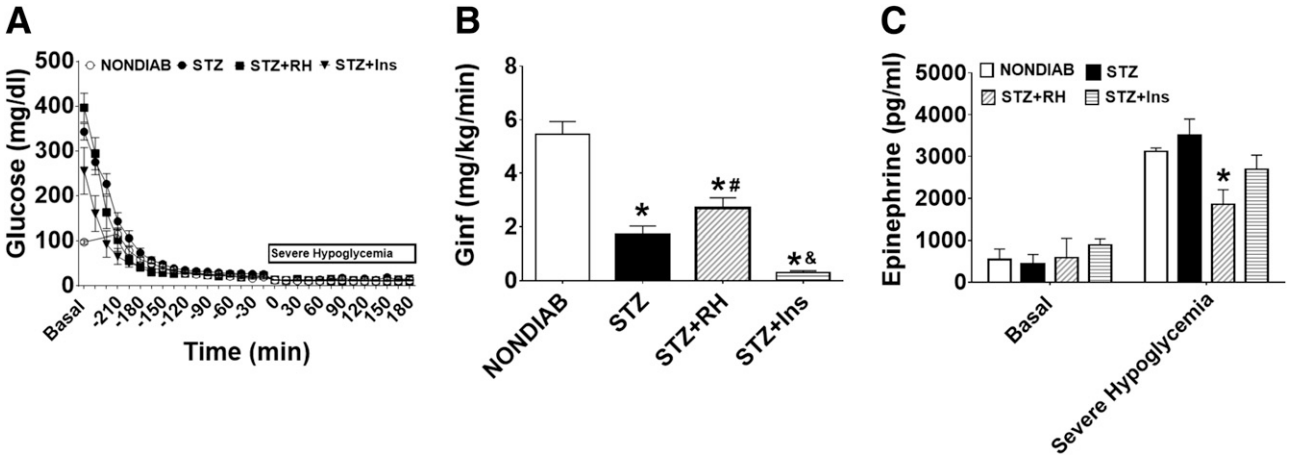

Figure 3—Glucose parameters during the hyperinsulinemic–severe hypoglycemic clamp. *A*: Glucose levels during the clamp were evenly matched among the NONDIAB (white circles), STZ (black circles), STZ+RH (black squares), and STZ+Ins (black triangles) groups. It took 3.5 h, on average, to reach 15 mg glucose/dL. Time 0 is the start of severe hypoglycemia (<15 mg/dL). Rats were clamped at 10–15 mg/dL for 3 h. *B*: Mean glucose infusion (Ginf) rates during severe hypoglycemia were lower in rats in the STZ groups than in those in the NONDIAB group, but rats in the STZ+RH group required a significantly higher glucose infusion rate than those in the STZ and STZ+Ins groups in order to maintain a similar glucose level. *$P < 0.05$, vs. NONDIAB; #$P < 0.05$, vs. STZ and STZ+Ins; &$P < 0.05$, vs. STZ and STZ+RH. *C*: Basal epinephrine levels were similar among the groups. During severe hypoglycemia, epinephrine was blunted in the STZ+RH group (1,856 ± 348 pg/mL) compared with values in the NONDIAB (3,124 ± 81 pg/mL), STZ (3,508 ± 387 pg/mL), and STZ+Ins (2,695 ± 336 pg/mL) groups. *$P < 0.05$. Data are means ± SEMs.

group ($P < 0.05$, Fisher exact test), whereas the NONDIAB (10.5%), STZ (32%), and STZ+RH (12.5%; $P < 0.05$, vs. STZ+Ins) groups had a similar incidence of nonsustained Vtach (Fig. 4*E*). Premature ventricular contractions were similar among the NONDIAB, STZ, and STZ+RH groups, whereas the STZ+Ins group had a higher number of premature ventricular contractions ($P < 0.05$, ANOVA) (Fig. 4*F*).

Sensitivity, specificity, and positive predictive value to predict mortality were 100%, 90%, and 71%, respectively, for third-degree heart block, and 64%, 58%, and 26%, respectively, for second-degree heart block. First-degree heart block and nonsustained Vtach were highly specific, but not sensitive, predictors of mortality.

Heart rate was variable throughout the clamp (Fig. 6*A*). At baseline, rats in the STZ (301 ± 8 bpm) and STZ+RH (357 ± 8 bpm; $P < 0.01$) groups had lower heart rates than rats in the NONDIAB group (367 ± 4 bpm). Insulin treatment for 12 days in STZ-treated rats increased baseline heart rate (STZ+Ins, 406 ± 6 bpm) to levels greater than those in the NONDIAB group. As blood glucose levels declined with insulin infusion, heart rate decreased in all groups (NONDIAB, 294 ± 2 bpm; STZ, 246 ± 8 bpm; STZ+RH, 265 ± 6 bpm; STZ+Ins, 273 ± 6 bpm).

The corrected QT interval was increased at baseline in all STZ-treated groups (STZ, 160 ± 5 ms; STZ+RH, 167 ± 3 ms; STZ+Ins, 167 ± 3 ms) compared with that in the NONDIAB group (123 ± 5 ms; $P < 0.05$) (Fig. 6*B*). QTc increased during the clamp in all groups. The mean QTc during severe hypoglycemia was 175 ± 1, 172 ± 3, 186 ± 2, and 180 ± 3 ms in the NONDIAB, STZ, STZ+RH, and STZ+Ins groups, respectively.

Respiration (Fig. 6*C*), oxygen saturation, carbon dioxide, and pH levels were similar among the groups throughout the duration of the clamp (data not shown). Only after fatal cardiac arrhythmias did respiration, oxygen, and pH levels decline and carbon dioxide levels increase. Potassium levels decreased to a similar extent during severe hypoglycemia in all groups (NONDIAB, 3.4 ± 0.2 mmol/L; STZ, 3.3 ± 0.1 mmol/L; STZ+RH, 3.6 ± 0.2 mmol/L).

DISCUSSION

Recurrent episodes of hypoglycemia in people with type 1 diabetes are traditionally considered harmful because the adapted brain elicits a reduced counterregulatory response and has a reduced awareness of hypoglycemia, thereby increasing the risk for severe hypoglycemia (1,6,8). This study demonstrates that in rats, hypoglycemia-induced cardiac arrhythmias are exacerbated by type 1 diabetes. Consistent with our previous studies indicating that the adaptive response to recurrent hypoglycemia may be beneficial (4), recurrent hypoglycemia diminished fatal cardiac arrhythmias in this rat model.

Various types of cardiac arrhythmias were observed during severe hypoglycemia, including all forms of heart block (first, second, and third degree), which were increased in insulin-deficient rats (in the STZ groups) compared with rats in the NONDIAB group. Interestingly, insulin treatment of streptozotocin-treated rats (as a model of insulin-treated type 1 diabetes) had no effect on the severity of cardiac arrhythmias during severe hypoglycemia. However, recurrent antecedent hypoglycemia significantly reduced these fatal cardiac arrhythmias. It was noted that high-grade atrioventricular block led to sudden death during severe hypoglycemia, consistent with previous findings (3). As

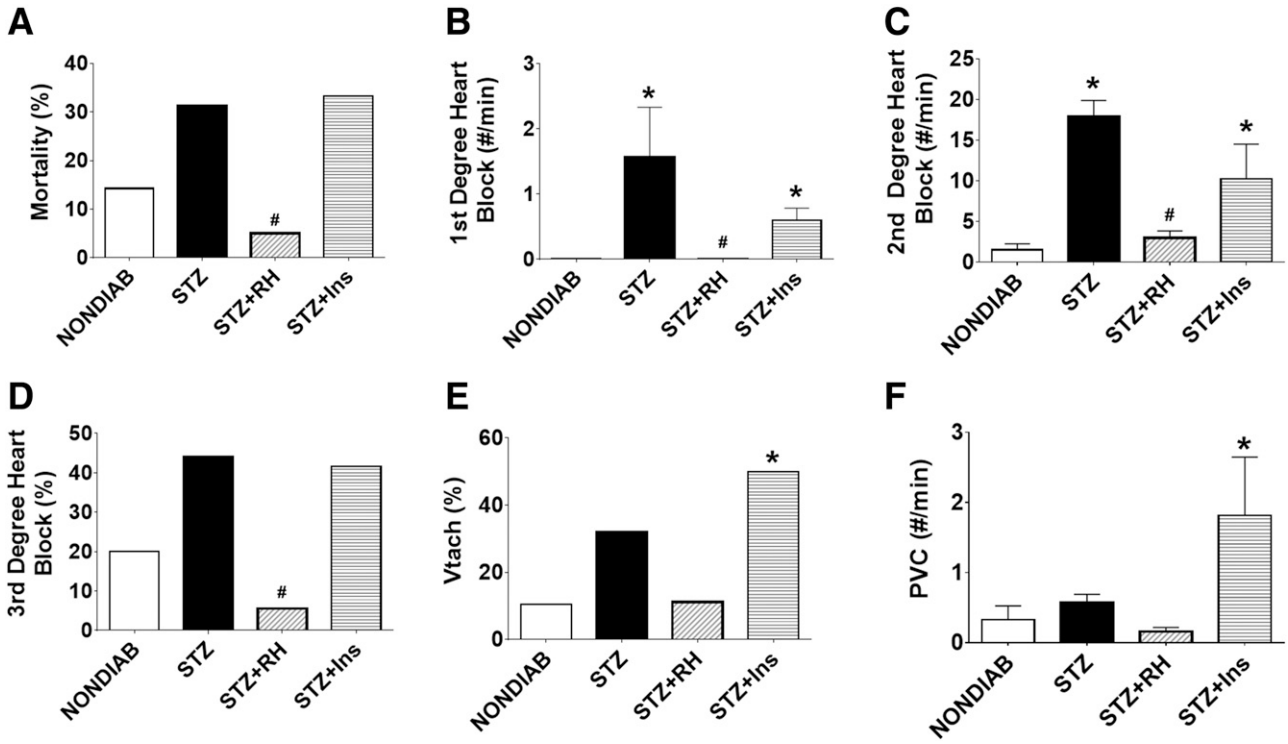

Figure 4—Mortality and cardiac arrhythmias during severe hypoglycemia. *A*: Mortality due to severe hypoglycemia was 31% in the STZ group (black), 33% in the STZ+Ins group (horizontal lines), and 14% in the NONDIAB group (white) (*P* = nonsignificant). Recurrent hypoglycemia reduced mortality to just 5% in the rats in the STZ+RH group (diagonal lines). #*P* < 0.035, vs. STZ. *B*: First-degree heart block was nearly absent in rats in the NONDIAB (0.009 ± 0.007/min) and STZ+RH (0.004 ± 0.003/min) groups, whereas rats in the STZ (1.6 ± 0.8/min) and STZ+Ins (0.6 ± 0.19/min) groups experienced more first-degree heart block. *C*: Second-degree heart block was significantly increased in rats in the STZ (18 ± 2/min) and STZ+Ins (10 ± 4/min) groups compared with rats in the NONDIAB group (1.5 ± 0.7/min), and recurrent hypoglycemia significantly reduced second-degree heart block (STZ+RH group: 3 ± 0.8/min). *D*: Incidence of third-degree heart block was 44% in the STZ, 42% in the STZ+Ins, and 20% in the NONDIAB group (*P* = nonsignificant). Recurrent hypoglycemia decreased third-degree heart block to 5.6% in the STZ+RH group. #*P* < 0.04, vs. STZ and STZ+Ins. *E*: Incidence of nonsustained Vtach was similar in the NONDIAB (10%), STZ (32%), and STZ+RH (11%) groups but was higher in the STZ+Ins group (50%). *F*: Premature ventricular contractions (PVCs) were increased in the STZ+Ins group (1.8 ± 0.9/min; *P* < 0.05) compared with the NONDIAB (0.33 ± 0.2/min), STZ (0.56 ± 0.1/min), and STZ+RH (0.17 ± 0.1/min) groups. **P* < 0.05, vs. NONDIAB; #*P* < 0.05, vs. STZ, ANOVA. Data are means ± SEMs (*n* = 12–32 rats/group).

indicated, second- and third-degree heart blocks were highly sensitive and specific in predicting mortality. In addition, detailed temporal analysis revealed that respiratory arrest consistently followed fatal cardiac arrhythmias, thus revealing that respiratory arrest is a consequence, not a cause, of fatal arrhythmias. These data indicate that *1*) insulin deficiency and hyperglycemia increase severe hypoglycemia–induced cardiac arrhythmias; *2*) insulin treatment in streptozotocin-treated rats, in order to model type 1 diabetes, has no effect on severe hypoglycemia–induced cardiac arrhythmias; and *3*) antecedent recurrent hypoglycemia in the STZ model significantly reduces fatal arrhythmias during subsequent severe hypoglycemia.

In spite of the increased arrhythmias in the STZ and STZ+Ins groups, the associated trend for increased mortality in these two groups did not reach statistical significance. This study may not have been sufficiently powered to detect a mortality difference the STZ and NONDIAB groups. In a previous study with higher power, severe hypoglycemia–induced mortality was increased in diabetic rats (*n* = 95) and reduced in nondiabetic rats that underwent preconditioning with recurrent hypoglycemia (*n* = 27) (3).

The mechanisms of how antecedent recurrent hypoglycemia reduces severe hypoglycemia–induced fatal cardiac arrhythmias remains to be established. It is hypothesized that the blunting of the counterregulatory response, particularly epinephrine, may reduce arrhythmias. Our previous research showed that nonselective β-adrenergic receptor blockade prevents mortality resulting from severe hypoglycemia (3). As previously noted by our laboratory and others, 3 days of recurrent moderate hypoglycemia leads to a blunted epinephrine response during hypoglycemia on the fourth day (4,9–11) (Fig. 3). Consistent with this blunted counterregulatory response to hypoglycemia in rats that underwent antecedent recurrent hypoglycemia, the glucose infusion rate in the STZ+RH group was 1.7-fold higher than that in the STZ group. It should be noted that epinephrine levels were similar in the NONDIAB, STZ, and STZ+Ins groups, whereas arrhythmia frequencies were significantly greater in the STZ and STZ+Ins groups. Thus

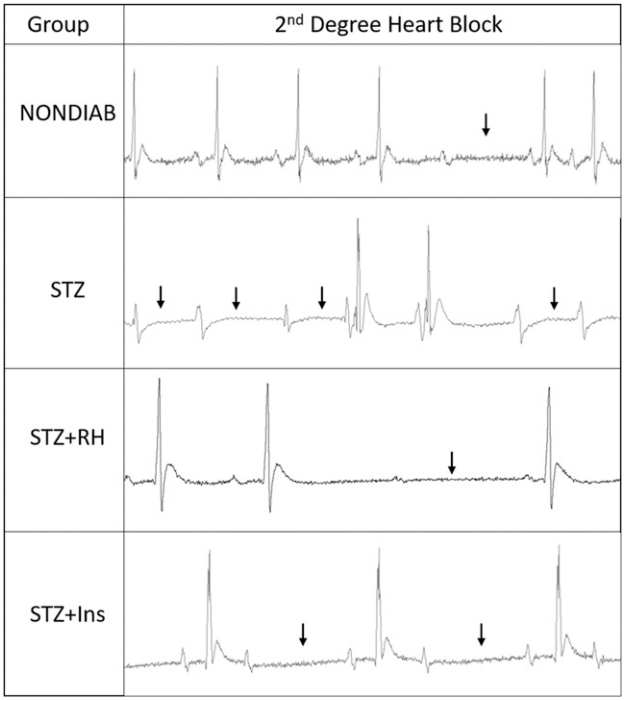

Figure 5—Representative ECG tracings during severe hypoglycemia. Rats in the NONDIAB group experienced some second-degree heart block (dropped QRS complex [arrows]) during severe hypoglycemia. The frequency of second-degree heart block was significantly increased in the STZ and STZ+Ins groups. Recurrent antecedent hypoglycemia markedly decreased the frequency of second-degree heart block in rats in the STZ+RH group.

hypoglycemia-induced increases in epinephrine levels alone are not likely to be the only mediators of severe hypoglycemia–induced cardiac arrhythmias. Sympathetic and parasympathetic innervation of the heart may also contribute to severe hypoglycemia–induced fatal cardiac arrhythmias. However, further studies are needed to address each of these mechanisms.

The role of hypokalemia in increasing the risk of severe hypoglycemia–induced mortality and the potential for potassium supplementation to reduce this mortality in both nondiabetic and diabetic rats have been previously reported (3,14). In a clinical study, Robinson et al. (15) demonstrated that potassium supplementation reduces QT dispersion during moderate hypoglycemia in healthy patients. However, in this study potassium levels fell to a similar level during hypoglycemia in all groups, indicating that hypokalemia is not likely to account for the observed differences in cardiac arrhythmias and mortality.

Prolongation of the QT interval is thought to be proarrhythmic (16). Interestingly, all three streptozotocin-treated groups had increased QTc at baseline compared with the nondiabetic controls. Insulin treatment for 2 weeks (STZ+Ins) and 3 days of insulin-induced recurrent hypoglycemia (STZ+RH) had no effect on baseline QTc intervals on the day of the clamp. Because the STZ+RH group had markedly reduced arrhythmias compared to the other groups despite QTc prolongation, it is suggested that QTc prolongation is a marker of severe hypoglycemia and is associated with cardiac arrhythmias, but it may not be sufficient to cause fatal arrhythmias during severe hypoglycemia.

Hypoglycemia is a known activator of the sympathetic nervous system, which might be expected to increase heart rate. In these studies, however, all groups demonstrated a decreased heart rate during hypoglycemia compared to baseline, suggesting that vagal tone is increased. Sinus bradycardia during hypoglycemia has been noted clinically

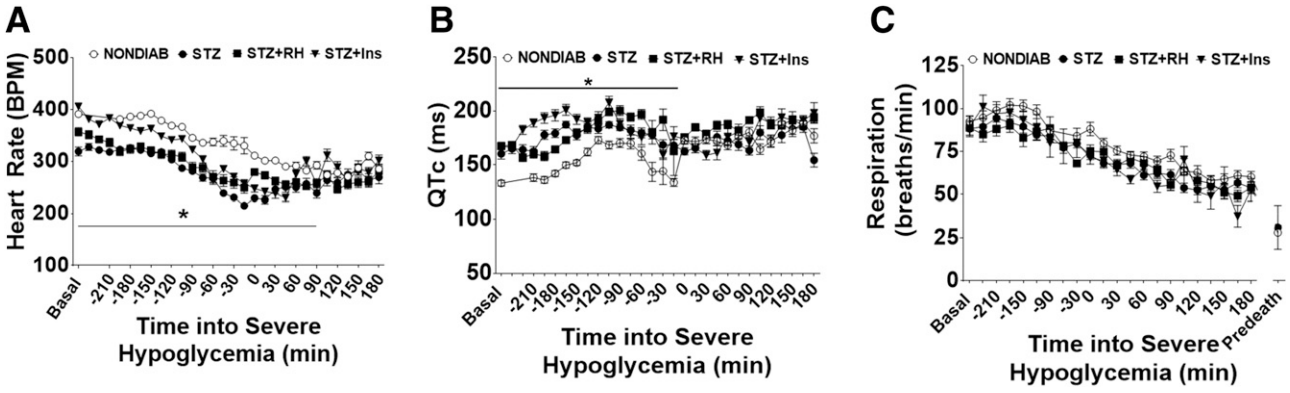

Figure 6—ECG analyses. *A*: Heart rate was decreased at baseline and during the majority of the clamp in the STZ (black circles) and STZ+RH (black squares) groups compared with the NONDIAB group (white circles). Basal heart rate was restored to normal in the STZ+Ins group (black triangles). As blood glucose was decreasing (time −150 to 0 min), heart rate decreased in all groups compared with their respective baseline values. During severe hypoglycemia, heart rate continued to decrease in the rats in the NONDIAB group but increased in those in the STZ and STZ+RH groups, with more variability in the STZ+Ins group. *$P < 0.05$, NONDIAB vs. STZ and STZ+RH. *B*: Baseline QTc was higher in all STZ groups than in the NONDIAB group. During severe hypoglycemia, QTc slightly increased from basal in all groups, but QTc length was similar during severe hypoglycemia in all groups. *$P < 0.05$, NONDIAB vs. STZ, STZ+RH, and STZ+Ins. *C*: Respiration tended to decrease during severe hypoglycemia in all groups, but no differences were found between the NONDIAB, STZ, STZ+Ins, and STZ+RH groups. Respiratory arrest occurred after a fatal arrhythmia and immediately before death. Data are means ± SEMs (n = 12–32 rats/group). Time 0 = start of severe hypoglycemia.

(17,18). Therefore, the current findings indicate *1*) an important role for the parasympathetic nervous system that is dependent on the depth and duration of hypoglycemia, and *2*) the utility of this model to understand better the pathophysiological response to hypoglycemia. Because bradyarrhythmias preceded sudden death during severe hypoglycemia in our rat model, future studies should address to what extent increased vagal tone potentially drives severe hypoglycemia–induced cardiac arrhythmias.

Evidence indicates that both parasympathetic and sympathetic control of the heart are diminished in diabetes. Previous studies showed that rats injected with streptozotocin had a lower heart rate 10–14 days after injection, and insulin treatment in rats injected with streptozotocin slightly increased heart rate, returning it to normal (19). Similarly, in the current study, rats in our STZ and STZ+RH groups had lower basal heart rates than rats in the NODIAB group; these lower rates were restored to normal with insulin treatment in streptozotocin-injected rats (STZ+Ins group). Thus, streptozotocin-induced insulin deficiency leads to altered autonomic control of the heart and may explain the decreased heart rate in streptozotocin-treated rats in our study.

In our rat model, the level of hypoglycemia necessary to observe cardiac arrhythmias was <15 mg/dL glucose. Although profoundly low, such glucose levels have been associated clinically with sudden death (6,7). Our rat model is therefore useful to study the mechanisms linking hypoglycemia to sudden death and, importantly, how we can prevent these potentially life-threatening arrhythmias.

In summary, severe hypoglycemia–induced fatal cardiac arrhythmias are *1*) increased by type 1 diabetes and, conversely, *2*) reduced by antecedent recurrent hypoglycemia. Because people with insulin-treated diabetes often experience hypoglycemia, understanding the mechanisms of how recurrent hypoglycemia reduces severe hypoglycemia–induced cardiac arrhythmias and mortality is important and could lead to better treatment strategies to reduce overall mortality in people at risk for severe hypoglycemia.

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Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. C.M.R. designed and conducted the experiments and wrote the manuscript. J.V. and J.B. conducted the experiments and wrote the manuscript. M.L. designed and conducted the experiments. A.S., A.J., and D.D.-I. conducted the experiments. S.J.F. designed the experiments and edited the manuscript. S.J.F. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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