Severe Hypoglycemia-Induced Fatal Cardiac Arrhythmias are Augmented by Diabetes and Attenuated by Recurrent Hypoglycemia

Running title: Severe Hypoglycemia-Induced Cardiac Arrhythmias

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Abstract

We previously demonstrated that insulin-mediated severe hypoglycemia induces lethal cardiac arrhythmias. However, whether chronic diabetes and insulin deficiency exacerbates, and recurrent antecedent hypoglycemia ameliorates, susceptibility to arrhythmias remains unknown. Thus, adult Sprague Dawley rats were randomized into four groups: 1) non-diabetic (NONDIAB), 2) streptozotocin-induced insulin-deficient (STZ), 3) STZ with antecedent recurrent hypoglycemia (STZ+RH) (3 days, ~40-45 mg/dl, 90 minutes), and 4) insulin-treated STZ (STZ+INS). Following treatment protocols, all rats underwent hyperinsulinemic (0.2 $U \cdot kg^{-1} \cdot min^{-1}$), severe hypoglycemic (10-15 mg/dl) clamps for 3 hours with continuous electrocardiogram recordings. During matched nadirs of severe hypoglycemia, STZ+RH required a 1.7-fold higher glucose infusion rate compared to STZ, consistent with the blunted epinephrine response. 2nd degree heart block was increased 12- and 6.8-fold in STZ and STZ+Ins, respectively, compared to NONDIAB, yet decreased 5.4-fold in STZ+RH compared to STZ. Incidence of 3rd degree heart block in STZ+RH was 5.6%, 7.8-fold less than STZ (44%). Mortality due to severe hypoglycemia in STZ+RH was 5%, 6.2-fold less than STZ (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.

Introduction

People with Type 1 diabetes experience an average of two episodes of symptomatic hypoglycemia per week and at least one episode of severe, temporarily disabling hypoglycemia per 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 that experience repeated episodes of hypoglycemia. This increased risk is thought to be due to the recurrent hypoglycemia induced blunted counterregulatory response and hypoglycemia unawareness (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 or not recurrent hypoglycemia is also beneficial during severe hypoglycemia to reduce fatal cardiac arrhythmias is unknown. We therefore wished to test, in 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 contribution of insulin therapy per se, versus insulin-induced

hypoglycemia, in mediating susceptibility to arrhythmias. Additionally, since 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 as compared to nondiabetic rats.

Research Design and Methods

Animals. Adult, male Sprague-Dawley rats (250-300g; Charles River Laboratories) were housed individually in temperature- and light-controlled environments and fed ad libitum with 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 and jugular vein and electrocardiogram (ECG) lead placement as previously described (3) (Figure 1A,B).

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

Insulin treatment. Two days after STZ injection, one group of rats were implanted with insulin pellets (LinPlant, ~2U/day, Lin Shin, Toronto, ON, Canada) subcutaneously (STZ+Ins; 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 tail vein ~2 times daily.

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

Hyperinsulinemic-severe hypoglycemic clamp. All four groups of overnight fasted, awake, unrestrained rats were subjected to hyperinsulinemic (0.2 $U \cdot kg^{-1} \cdot min^{-1}$, Humulin R) severe hypoglycemic (10-15 mg/dL) clamps with continuous electrocardiograms for 3 hours as previously described (3).

Arterial blood samples were obtained throughout the clamp for blood glucose, electrolytes, and gases (pHOx Plus C ABG machine; Nova Biomedical, Waltham, MA). Epinephrine was measured by ELISA (Abnova, Taiwan). Insulin was measured by ELISA (Crystal Chem, Downers Grove, IL). Respiration was determined by counting visible breaths.

Electrocardiograms were recorded and arrhythmias assessed using PowerLab 26T (LabChart, ADInstruments, Colorado Springs, CO) as previously described (3).

Statistical analyses.

All data are represented as mean ± standard error of the mean (SEM). Analysis of variance 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 Freeman-Halton extension was used to determine significance for mortality and incidence of arrhythmias. Significance was determined at $p<0.05$.

Results

For two weeks following initial randomization, rats had an average glucose of 107±1, 528±7, 523±9, and 308±13 mg/dl for the NONDIAB, STZ, STZ+RH, and STZ+Ins groups, respectively (Figure 2A). Body weight increased in the NONDIAB rats during the experiment, but did not change in the insulin-deficient (STZ, STZ+RH) or insulin-treated (STZ+Ins) groups (Figure 2B). For the STZ+RH treated rats, who underwent 90 minute periods of recurrent hypoglycemia on days 13, 14, and 15, glucose levels were 44±2, 43±4, and 43±2 mg/dl, respectively (Figure 2C). During these three days prior to the severe hypoglycemic clamp, saline injected rats (STZ) had glucose values of 528±17, 517±8, and 522±19 mg/dl, respectively; NONDIAB rats had glucose values of 108±7, 109±3, and 106±1 mg/dl, respectively; and the chronically insulin treated STZ+Ins group had glucose levels of 398±47, 233±48, and 335±40 mg/dl, respectively, clamp (Figure 2C).

Severe Hypoglycemic Clamp

Glucose levels during the 3 hour severe hypoglycemic clamp were evenly matched among the four groups (NONDIAB 12±0.2; STZ 13±0.3; STZ+RH 13±0.5; STZ+INS 13±0.5 mg/dl; Figure 3A). 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 (Figure 3B). Epinephrine levels were similar among the groups during the basal period (prior to insulin infusion). During severe hypoglycemia, epinephrine increased in all groups; however, this response was blunted in the STZ+RH group (NONDIAB 3124±81; STZ 3508±387; STZ+RH 1856±348; STZ+Ins 2695±336 pg/ml; $p<0.03$; Figure 3C).

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

Severe hypoglycemia-induced cardiac arrhythmias were consistently increased in STZ and STZ+Ins rats whereas recurrent hypoglycemia reduced these arrhythmias. First degree heart block was increased in STZ (1.6±0.8/min) and STZ+Ins (0.6±0.2/min) compared to NONDIAB (0.009±0.007/min; $p<0.05$; Figure 4B). Second degree heart block was similarly increased in STZ (18±2/min) and STZ+Ins (10±4/min) versus NONDIAB (1.5±0.7/min; $p<0.05$; Figure 4C). Antecedent recurrent hypoglycemia virtually eliminated 1st degree heart block (0.004±0.003/min; $p<0.007$) and reduced 2nd degree heart block by 82% (3±0.8/min; $p<0.001$). As shown in Figure 5, STZ and STZ+Ins rats had an increased frequency of second degree heart block compared to both the NONDIAB and STZ+RH rats. The presence of third degree heart block was 20%, 44%, and 42% in NONDIAB, STZ, and STZ+Ins rats, respectively, but was reduced to just 5.6% in STZ+RH rats ($p<0.04$; Fisher exact test; Figure 4D). Nonsustained

ventricular tachycardia (Vtach, defined as 4 or more premature ventricular contractions in a row) was present in 50% of the STZ+Ins rats ($p<0.05$, Fisher exact test), while the NONDIAB (10.5%), STZ (32%), and STZ+RH (12.5%; $p<0.05$ vs STZ+Ins) rats had a similar incidence of nonsustained Vtach (Figure 4E). Premature ventricular contractions (PVCs) were similar among the NONDIAB, STZ, and STZ+RH rats while the STZ+Ins group had an increased amount of PVCs ($p<0.05$, ANOVA, Figure 4F).

Sensitivity, specificity, and positive predictive value to predict mortality were 100%, 90%, and 71%, respectively, for 3rd degree heart block, and 64%, 58%, and 26%, respectively, for 2nd degree heart block. First degree heart block and non-sustained ventricular tachycardia were highly specific, but not sensitive predictors of mortality.

Heart rate was variable throughout the clamp (Figure 6A). At baseline, STZ (301±8) and STZ+RH (357±8 beats per minute (BPM); $p<0.01$) groups had lower heart rates compared to NONDIAB (367±4 BPM). Twelve days of insulin treatment in STZ rats increased baseline heart rate (STZ+Ins 406±6 BPM) to levels greater than the NONDIAB group. As blood glucose levels declined with insulin infusion, heart rate decreased in all groups (NONDIAB 294±2; STZ 246±8; STZ+RH 265±6; STZ+Ins 273±6 BPM).

The QT interval (QTc) was increased at baseline in all STZ groups (STZ 160±5; STZ+RH 167±3 ms; STZ+Ins 167±3 ms) compared to NONDIAB (123±5 ms; $p<0.05$; Figure 6B). 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 rats, respectively.

Respiration (Figure 6C), 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; STZ 3.3±0.1; 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 (1st, 2nd, and 3rd degree) which were increased in insulin-deficient (STZ) rats compared to NONDIAB rats. Interestingly, insulin treatment of STZ 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 previously findings (3). As indicated, 2nd and 3rd degree heart block were highly sensitive and specific to predict mortality. Additionally, detailed temporal analysis revealed that respiratory arrest consistently followed fatal cardiac arrhythmias, thus revealing that respiratory arrest is a consequence of fatal arrhythmias, not a cause. These data indicate that 1) insulin-deficiency and hyperglycemia increase severe

hypoglycemia induced cardiac arrhythmias, 2) insulin treatment in STZ rats 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 STZ and STZ+Ins did not reach statistical significance. This study may not have been powered sufficiently to detect a mortality difference with STZ compared to NONDIAB. Previously, in a higher-powered study, severe hypoglycemia-induced mortality was increased in diabetic rats (n=95) and reduced in non-diabetic rats that underwent recurrent hypoglycemia preconditioning (n=27) (3, 14).

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 has shown that non-selective beta adrenergic receptor blockade prevents mortality due to severe hypoglycemia (3). As previous noted in our lab and others, three days of recurrent moderate hypoglycemia leads to a blunted epinephrine response during hypoglycemia on the subsequent day (see Figure 3) (4, 9-11). 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 compared to STZ. It should be noted epinephrine levels were similar in the NONDIAB, STZ, and STZ+Ins groups while arrhythmias were significantly greater in the STZ and STZ+Ins groups. Thus, hypoglycemia induced increases in epinephrine levels alone are unlikely the only mediators of severe hypoglycemia-induced cardiac arrhythmias. Sympathetic and parasympathetic innervation of the heart may also

be contributing 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 non-diabetic and diabetic rats have been previously reported (3, 15). In clinical studies, Robinson et al. (16) demonstrated that potassium supplementation reduces QT dispersion during moderate hypoglycemia in healthy patients. However, in this study potassium levels fell similarly in all groups, indicating that hypokalemia is unlikely to account for the observed differences in cardiac arrhythmias and mortality.

Prolongation of the QT interval is thought to be proarrhythmic (17). Interestingly, all three STZ groups had increased baseline QTc compared to non-diabetic controls. Insulin treatment for two weeks (STZ+Ins) as well as 3 days of insulin-induced recurrent hypoglycemia (STZ+RH) had no effect on baseline QTc intervals on the day of the clamp. Since STZ+RH had markedly reduced arrhythmias despite QTc prolongation, it is suggested that QTc prolongation is a marker of severe hypoglycemia and associated with cardiac arrhythmias, but 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 suggesting that vagal tone is increased. Sinus bradycardia during hypoglycemia has been noted clinically (18, 19). Therefore, the current findings indicate 1) an important role of the parasympathetic nervous system that is dependent on depth and duration of hypoglycemia, and 2) the utility of this model to better understand the pathophysiological response to hypoglycemia. Since increased frequency of 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.

There is evidence that both parasympathetic and sympathetic control of the heart are diminished in diabetes. It has been shown previously that streptozotocin injected rats had a decreased heart rate 10-14 days after injection, and insulin treatment in streptozotocin injected rats slightly increased heart rate back to normal (20). Similarly, in the current study, our STZ and STZ+RH rats had lower basal heart rates compared to nondiabetic rats which was restored back to normal with insulin treatment in STZ rats (STZ+Ins). Thus, STZ-induced insulin-deficiency leads to altered autonomic control of the heart and may explain the decreased heart rate in STZ treated rats in our study.

In our rat model, the level of hypoglycemia necessary to observe cardiac arrhythmias was <15 mg/dl. Although profoundly low, such glucose levels have been associated with sudden death clinically (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 2) conversely, reduced by antecedent recurrent hypoglycemia. Since 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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CMR designed and conducted the experiments and wrote the manuscript. JVW conducted the experiments and wrote the manuscript. JB conducted the experiments and wrote the manuscript. ML designed and conducted the experiments. AS conducted the experiments. DDI conducted the experiments. SJF designed the experiments, edited the manuscript and is the guarantor of this manuscript and takes full responsibility for its content. CMR, JVW, JB, ML, AS, DDI, and SJF report no conflicts of interest.

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Figure Legends

Figure 1. Experimental protocol. A) Rats were divided into 4 groups: 1) Non-diabetic (n = 28), 2) STZ insulin-deficient (n = 32), 3) STZ + Recurrent hypoglycemia (n = 20), and 4) STZ + insulin treatment (n=12). B) All rats underwent surgery for catheter and ECG lead placement (day 0). Two days later, rats were injected with either streptozotocin (STZ) or Na citrate buffer. On day 4, one group of rats were treated with subcutaneous insulin pellets. On day 8, vascular lines were externalized, cleared, and replaced under the skin. On day 13-15, one group of rats underwent recurrent hypoglycemia (DIAB+RH) while the other groups of rats were given saline. On day 16, all rats underwent hyperinsulinemic/severe hypoglycemic (10-15 mg/dl) clamps.

Figure 2. Glucose and body weight. A) Prior to STZ injection (Basal) all rats had normal glucose. After STZ injection (day 3), glucose levels were increased and remained elevated for the duration of the study in both the STZ (closed circle) and STZ+RH (closed square) groups whereas insulin-treatment in STZ rats (closed triangle) had lower glucose levels. Buffer injected NONDIAB rats (open circle) had normal glucose for the duration of the study. *$p<0.001$ for NONDIAB vs STZ, STZ+RH, and STZ+Ins; $^{\#}p<0.001$ for STZ+Ins versus STZ and STZ+RH. B) Body weight increased from basal to the end of the study for NONDIAB. Body weight did not change in the STZ, STZ+RH, and STZ+Ins groups throughout the experiment. *$p<0.05$ for NONDIAB vs STZ, STZ+RH, and STZ+Ins. C) NONDIAB, STZ, and STZ+Ins rats 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. After insulin injection, glucose levels steadily declined and rats were hypoglycemic (~40-45 mg/dl) for 90 minutes before recovering with IP glucose injection and free access to food. Shown are the mean values of glucose on days 13, 14, and 15 for each group. Data are mean ± SEM.

Figure 3. Glucose parameters during the hyperinsulinemic-severe hypoglycemic clamp. A) Glucose levels during the clamp were evenly matched among the NONDIAB (open circle), STZ (closed circle), STZ+RH (closed square), and STZ+Ins (closed triangle) groups. It took 3 ½ hours on average to reach a glucose of 15 mg/dl. Time 0 is the start of severe hypoglycemia (<15 mg/dl). Rats were clamped at 10-15 mg/dl for 3 hours. B) Mean glucose infusion rates during severe hypoglycemia were lower in the STZ rats compared to NONDIAB, but STZ+RH required a significantly higher glucose infusion rate than the STZ and STZ+Ins rats 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 (1856 ± 348 pg/ml) compared to NONDIAB (3124±81), STZ (3508±387), and STZ+Ins (2695±336 pg/ml) groups. *$p < 0.05$. Data are mean ± SEM.

Figure 4. Mortality and cardiac arrhythmias during severe hypoglycemia. A) Mortality due to severe hypoglycemia was 31% in the STZ group (black bar), 33% in STZ+Ins group (horizontal slash bar), and 14% in the NONDIAB group (white bar), p=NS. Recurrent hypoglycemia reduced mortality to just 5% in the STZ+RH rats (diagonal slash bar; $^{\#}p<0.035$ vs STZ). B) 1st degree heart block was nearly absent in NONDIAB (0.009 ± 0.007/min) and STZ+RH (0.004 ± 0.003/min) rats whereas STZ (1.6 ± 0.8/min) and STZ+Ins (0.6 ± 0.19/min) rats had an increased amount of 1st degree heart block. C) 2nd degree heart block was significantly increased in the STZ (18 ± 2/min) and STZ+Ins (10 ± 4/min) rats compared to the

NONDIAB rats (1.5 ± 0.7/min), and recurrent hypoglycemia significantly reduced 2nd degree heart block (STZ+RH: 3 ± 0.8/min). D) Incidence of 3rd degree heart block was 44% in STZ, 42% in STZ+Ins, and 20% in NONDIAB, p=NS. Recurrent hypoglycemia decreased 3rd degree heart block to 5.6% in STZ+RH ($^{\#}p<0.04$ vs STZ and STZ+Ins). E) Incidence of non-sustained ventricular tachycardia (V-Tach) was similar in NONDAIB (10%), STZ (32%), and STZ+RH (11%), but increased in STZ+Ins (50%). F) Premature ventricular contractions (PVCs) were increased in the STZ+Ins group (1.8 ± 0.9/min; $p<0.05$) compared to NONDIAB (0.33 ± 0.2/min), STZ (0.56 ± 0.1), and STZ+RH (0.17 ± 0.1/min). $^{*}p<0.05$ vs NONDIAB, $^{\#}p<0.05$ vs STZ, ANOVA. Data are mean ± SEM. N = 12-32/group.

Figure 5. Representative ECG tracings during severe hypoglycemia. NONDIAB experienced some 2nd degree heart block (dropped QRS complex denoted by arrow) during severe hypoglycemia. The frequency of 2nd degree heart block was significantly increased in the STZ and STZ+Ins groups. Recurrent antecedent hypoglycemia markedly decreased the frequency of 2nd degree heart block in STZ+RH rats.

Figure 6. ECG analyses. A) Heart rate was decreased at baseline and for the majority of the clamp in STZ (closed circle) and STZ+RH (closed square) groups compared to NONDIAB (open circle). STZ+Ins (closed triangle) restored basal heart rate back to normal. As blood glucose was decreasing (time -150 to 0 minutes), heart rate decreased in all groups compared to their respective baseline. During severe hypoglycemia, heart rate continued to decrease in the NONDIAB rats, but increased in the STZ and STZ+RH rats with more variability in the STZ+Ins rats. $^{*}p<0.05$ for NONDIAB vs STZ and STZ+RH. B) Baseline QTc was higher in all STZ groups compared to NONDIAB. QTc slightly increased during severe hypoglycemia from basal in all groups, but QTc length was similar during severe hypoglycemia in all groups. $^{*}p<0.05$ for NONDIAB vs STZ, STZ+RH, and STZ+Ins. C) Respiration tended to decrease during severe hypoglycemia in all groups, but there were no differences between the NONDIAB, STZ, STZ+Ins, and STZ+RH rats. Following a fatal arrhythmia and immediately prior to death, respiratory arrest occurred. Data are mean ± SEM, N = 12-28/group. Time 0 = start of severe hypoglycemia.

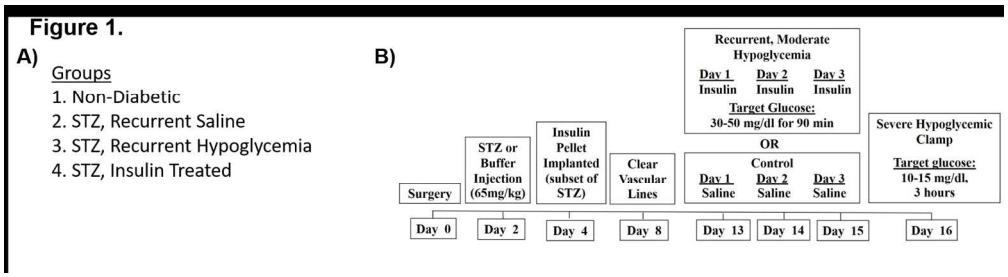

Figure 1. Experimental protocol. A) Rats were divided into 4 groups: 1) Non-diabetic (n = 28), 2) STZ insulin-deficient (n = 32), 3) STZ + Recurrent hypoglycemia (n = 20), and 4) STZ + insulin treatment (n=12). B) All rats underwent surgery for catheter and ECG lead placement (day 0). Two days later, rats were injected with either streptozotocin (STZ) or Na citrate buffer. On day 4, one group of rats were treated with subcutaneous insulin pellets. On day 8, vascular lines were externalized, cleared, and replaced under the skin. On day 13-15, one group of rats underwent recurrent hypoglycemia (DIAB+RH) while the other groups of rats were given saline. On day 16, all rats underwent hyperinsulinemic/severe hypoglycemic (10-15 mg/dl) clamps.

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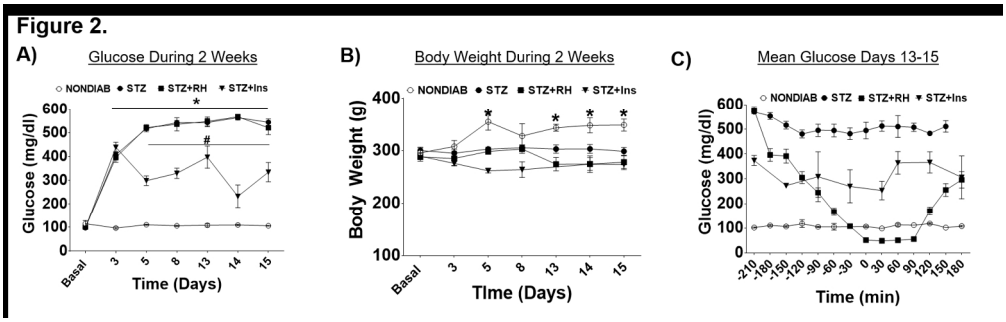

Figure 2. Glucose and body weight. A) Prior to STZ injection (Basal) all rats had normal glucose. After STZ injection (day 3), glucose levels were increased and remained elevated for the duration of the study in both the STZ (closed circle) and STZ+RH (closed square) groups whereas insulin-treatment in STZ rats (closed triangle) had lower glucose levels. Buffer injected NONDIAB rats (open circle) had normal glucose for the duration of the study. *p<0.001 for NONDIAB vs STZ, STZ+RH, and STZ+Ins; #p<0.001 for STZ+Ins versus STZ and STZ+RH. B) Body weight increased from basal to the end of the study for NONDIAB. Body weight did not change in the STZ, STZ+RH, and STZ+Ins groups throughout the experiment. *p<0.05 for NONDIAB vs STZ, STZ+RH, and STZ+Ins. C) NONDIAB, STZ, and STZ+Ins rats 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. After insulin injection, glucose levels steadily declined and rats were hypoglycemic (~40-45 mg/dl) for 90 minutes before recovering with IP glucose injection and free access to food. Shown are the mean values of glucose on days 13, 14, and 15 for each group. Data are mean ± SEM.

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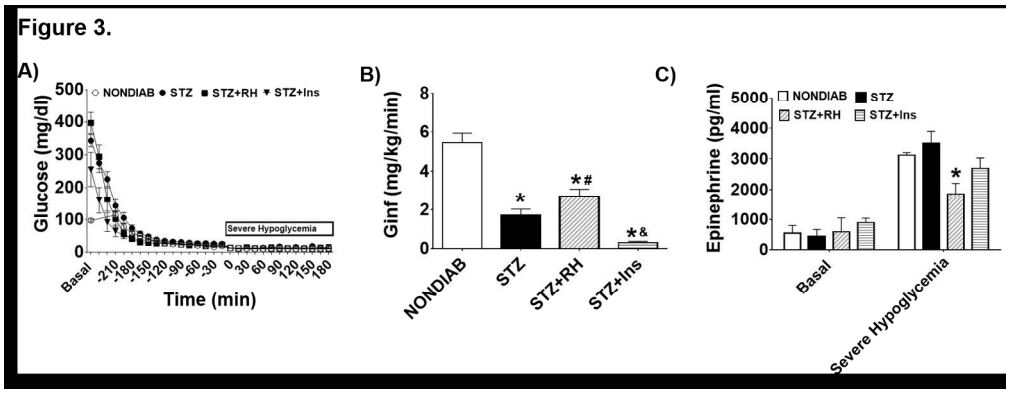

Figure 3. Glucose parameters during the hyperinsulinemic-severe hypoglycemic clamp. A) Glucose levels during the clamp were evenly matched among the NONDIAB (open circle), STZ (closed circle), STZ+RH (closed square), and STZ+Ins (closed triangle) groups. It took 3 ½ hours on average to reach a glucose of 15 mg/dl. Time 0 is the start of severe hypoglycemia (<15 mg/dl). Rats were clamped at 10-15 mg/dl for 3 hours. B) Mean glucose infusion rates during severe hypoglycemia were lower in the STZ rats compared to NONDIAB, but STZ+RH required a significantly higher glucose infusion rate than the STZ and STZ+Ins rats 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 (1856 ± 348 pg/ml) compared to NONDIAB (3124±81), STZ (3508±387), and STZ+Ins (2695±336 pg/ml) groups. *p < 0.05. Data are mean ± SEM.

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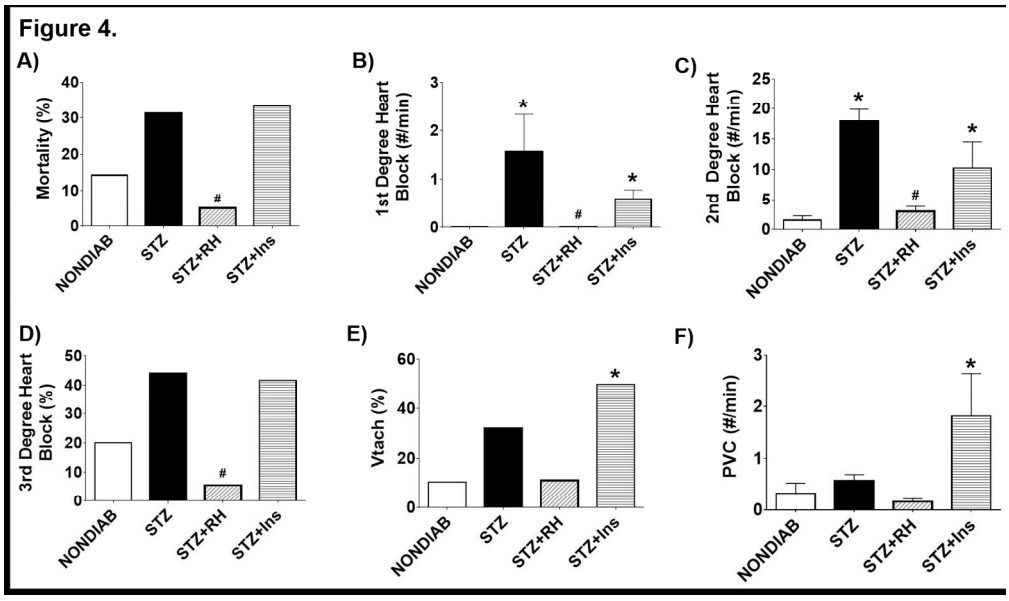

Figure 4. Mortality and cardiac arrhythmias during severe hypoglycemia. A) Mortality due to severe hypoglycemia was 31% in the STZ group (black bar), 33% in STZ+Ins group (horizontal slash bar), and 14% in the NONDIAB group (white bar), p=NS. Recurrent hypoglycemia reduced mortality to just 5% in the STZ+RH rats (diagonal slash bar; #p<0.035 vs STZ). B) 1st degree heart block was nearly absent in NONDIAB (0.009 ± 0.007/min) and STZ+RH (0.004 ± 0.003/min) rats whereas STZ (1.6 ± 0.8/min) and STZ+Ins (0.6 ± 0.19/min) rats had an increased amount of 1st degree heart block. C) 2nd degree heart block was significantly increased in the STZ (18 ± 2/min) and STZ+Ins (10 ± 4/min) rats compared to the NONDIAB rats (1.5 ± 0.7/min), and recurrent hypoglycemia significantly reduced 2nd degree heart block (STZ+RH: 3 ± 0.8/min). D) Incidence of 3rd degree heart block was 44% in STZ, 42% in STZ+Ins, and 20% in NONDIAB, p=NS. Recurrent hypoglycemia decreased 3rd degree heart block to 5.6% in STZ+RH (#p<0.04 vs STZ and STZ+Ins). E) Incidence of non-sustained ventricular tachycardia (V-Tach) was similar in NONDAIB (10%), STZ (32%), and STZ+RH (11%), but increased in STZ+Ins (50%). F) Premature ventricular contractions (PVCs) were increased in the STZ+Ins group (1.8 ± 0.9/min; p<0.05) compared to NONDIAB (0.33 ± 0.2/min), STZ (0.56 ± 0.1), and STZ+RH (0.17 ± 0.1/min). *p<0.05 vs NONDIAB, #p<0.05 vs STZ, ANOVA. Data are mean ± SEM. N = 12-32/group.

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Figure 5.

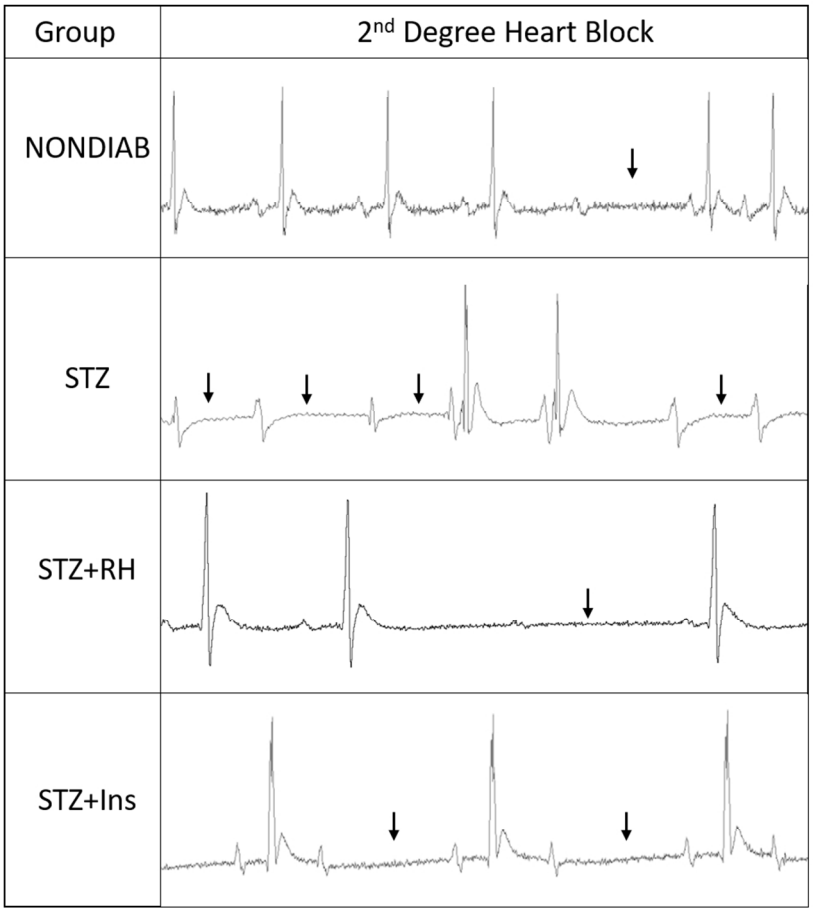

Figure 5. Representative ECG tracings during severe hypoglycemia. NONDIAB experienced some 2nd degree heart block (dropped QRS complex denoted by arrow) during severe hypoglycemia. The frequency of 2nd degree heart block was significantly increased in the STZ and STZ+Ins groups. Recurrent antecedent hypoglycemia markedly decreased the frequency of 2nd degree heart block in STZ+RH rats.

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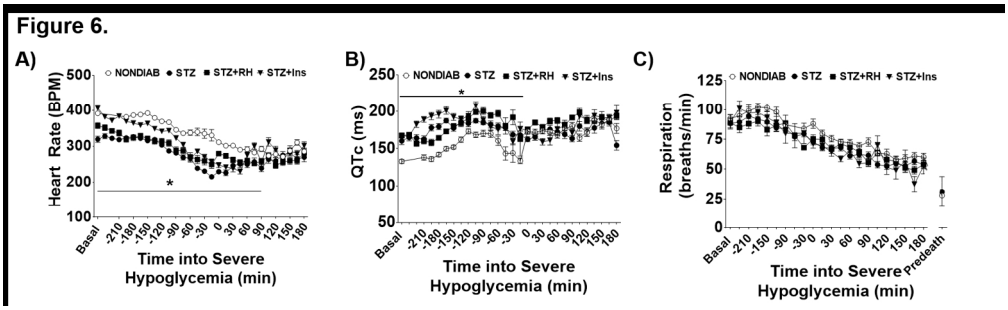

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