

# Severe Hypoglycemia–Induced Lethal Cardiac Arrhythmias Are Mediated by Sympathoadrenal Activation

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For people with insulin-treated diabetes, severe hypoglycemia can be lethal, though potential mechanisms involved are poorly understood. To investigate how severe hypoglycemia can be fatal, hyperinsulinemic, severe hypoglycemic (10–15 mg/dL) clamps were performed in Sprague-Dawley rats with simultaneous electrocardiogram monitoring. With goals of reducing hypoglycemia-induced mortality, the hypotheses tested were that: 1) antecedent glycemic control impacts mortality associated with severe hypoglycemia; 2) with limitation of hypokalemia, potassium supplementation could limit hypoglycemia-associated deaths; 3) with prevention of central neuroglycopenia, brain glucose infusion could prevent hypoglycemia-associated arrhythmias and deaths; and 4) with limitation of sympathoadrenal activation, adrenergic blockers could prevent hypoglycemia-induced arrhythmic deaths. Severe hypoglycemia-induced mortality was noted to be worsened by diabetes, but recurrent antecedent hypoglycemia markedly improved the ability to survive an episode of severe hypoglycemia. Potassium supplementation tended to reduce mortality. Severe hypoglycemia caused numerous cardiac arrhythmias including premature ventricular contractions, tachycardia, and high-degree heart block. Intracerebroventricular glucose infusion reduced severe hypoglycemia-induced arrhythmias and overall mortality.  $\beta$ -Adrenergic blockade markedly reduced cardiac arrhythmias and completely abrogated deaths due to severe hypoglycemia. Under conditions studied, sudden deaths caused by insulin-induced severe hypoglycemia were mediated by lethal cardiac arrhythmias triggered by brain neuroglycopenia and the marked sympathoadrenal response. *Diabetes* 62:3570–3581, 2013

**T**he rate of sudden death in young people with type 1 diabetes is 10-fold higher than in age-matched control subjects (1). Sudden nocturnal deaths account for up to 27% of all unexplained deaths in people with type 1 diabetes (2,3). The aptly named “dead in bed syndrome” identifies otherwise healthy young individuals with type 1 diabetes who were found dead in bed with no clear cause of death (1–9). It has been speculated that their deaths were due to excess insulin administration that resulted in severe hypoglycemia (3,5–11). However, the mechanisms by which severe hypoglycemia can be lethal is unclear.

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During hypoglycemia, lack of glucose supply to neurons can lead to confusion, brain damage (12), seizures (13,14), and even death. Indeed, 6–10% of deaths in young people with type 1 diabetes are directly attributable to hypoglycemia (15–17). It has been hypothesized that abrupt cardiac arrhythmias contribute to severe hypoglycemia-induced sudden death. Clinical studies using electrocardiograms (ECGs) have found that cardiac arrhythmias associated with corrected QT (QTc) prolongation occur during moderate hypoglycemia (18–21). QTc prolongation represents dispersion of ventricle depolarization and can lead to increased risk of fatal cardiac arrhythmias (22–24). In the setting of insulin-induced severe hypoglycemia, it remains unknown whether fatal cardiac arrhythmias are mediated by brain neuroglycopenia per se or systemic factors acting directly on the heart. Systemically, both the insulin-induced decrement in potassium levels and the counterregulatory-induced increase in catecholamine levels have been speculated to contribute to arrhythmias (25–27). It has been shown previously that the administration of potassium or  $\beta$ -blockers can reduce mild cardiac arrhythmias during moderate hypoglycemia (~45 mg/dL) (26). However, it remains to be determined whether the prevention of hypokalemia or blocking the actions of the catecholamines protects against lethal arrhythmias and cardiorespiratory arrest during severe hypoglycemia.

In order to determine preventable causes of death during hypoglycemia, severe hypoglycemic clamps were performed in Sprague-Dawley rats with simultaneous arterial blood sampling and ECG monitoring. It was hypothesized that brain neuroglycopenia and the marked sympathoadrenal response during severe hypoglycemia trigger fatal cardiac arrhythmias.

## RESEARCH DESIGN AND METHODS

Nine-week-old male Sprague-Dawley rats (Charles River Laboratories) were housed individually in a temperature- and light-controlled environment and fed ad libitum with standard rat chow diet and water. All studies were done in accordance with and approved by the Animal Studies Committee at Washington University School of Medicine.

**Implantation of vascular catheters and ECG leads.** Vascular catheters were implanted as previously described (28). One ECG wire lead was placed in the right supraclavicular fossa and another was placed exterior to the lower left rib cage and sutured to underlying muscle. Reference lead was placed subcutaneously.

**Hyperinsulinemic/severe hypoglycemic clamp.** Four to ten days postsurgery, overnight fasted, precannulated, awake, unrestrained rats were subjected to hyperinsulinemic (0.2 units  $\cdot$  kg<sup>-1</sup>  $\cdot$  min<sup>-1</sup>) severe hypoglycemic (10–15mg/dL) clamps as previously described (28–30). Hypoglycemia was maintained for a predetermined duration (60 min for studies 1 and 2, 90 min for study 3, and 180 min for studies 4 and 5).

Insulin was measured by ELISA (Crystal Chem, Downers Grove, IL). Glucagon was measured by radioimmunoassay (Millipore, Billerica, MA). Epinephrine and norepinephrine levels were determined by a single isotope derivative (radioenzymatic) method as previously described (28). Blood pressure (BP) (noninvasive BP monitor; Columbus Instruments, Columbus, OH) was measured by tail cuff in select studies.

**Arterial blood gases.** Blood samples were taken throughout the clamp for analysis of electrolytes and blood gases (pHOx Plus C arterial blood gas machine; Nova Biomedical, Waltham, MA). Respiration was calculated by visibly counting breaths.

**Seizures.** Seizure-like behavior was noted during the hypoglycemic clamps as previously described (28,29). A subgroup of four rats had electroencephalogram (EEG) recording (XLTEK Video EEG System, Oakville, Ontario) to analyze brain wave activity during hypoglycemia.

**Study 1: pooled mortality analysis in control, diabetic, and recurrently hypoglycemic rat experiments.** Control ( $n = 123$ ), diabetic ( $n = 95$ ), and recurrent hypoglycemic ( $n = 27$ ) rats that underwent 1 h of severe hypoglycemia (28–30) were analyzed for mortality.

**Diabetes.** Diabetes was induced by injection of streptozotocin (STZ) (65 mg/kg i.p.; Sigma, St. Louis, MO) 2 weeks prior to the clamp as previously described (29). Control rats received injections of 0.1 mmol/L sodium citrate (Fisher Scientific) buffer.

**Recurrent hypoglycemia.** Moderate hypoglycemia (25–40 mg/dL) was induced for 3 h/3 days in nondiabetic rats as previously described (28). Control rats received saline injections. Severe hypoglycemic clamps were performed on the fourth day.

**Study 2: potassium supplementation.** Potassium (KCl, 0.375 mEq/mL; Hospira, Lake Forest, IL) was infused with saline at 4  $\mu$ L/min. Nondiabetic rats received potassium ( $n = 12$ ) or saline ( $n = 15$ ), and diabetic rats received potassium ( $n = 28$ ) or saline ( $n = 45$ ) throughout a 1-h hyperinsulinemic/severe hypoglycemic clamp as described above.

**Study 3: cardiac arrhythmias.** Nondiabetic rats ( $n = 6$ ) underwent severe hypoglycemic clamps for 90 min as described above, with continuous ECG recording (ADI Powerlab 26T; ADInstruments, Colorado Springs, CO). ECG analyses included heart rate, QTc length, and other arrhythmias (ADI LabChart Pro 7). QTc was calculated using Bazett formula (31).

**Study 4: intracerebroventricular glucose infusion.** Intracerebroventricular (ICV) cannulas (internal cannula 31 g, outer guide cannula 24 g, 9.5 mm depth; Plastics One, Roanoke, VA) were placed on the skull (–2.8 mm posterior to bregma) using stereotaxic equipment under isoflourane. Rats recovered 1 week before vascular and lead implantations. D-glucose (2 mmol/L i.c.v.) ( $n = 9$ ) (Sigma) or D-mannitol (2 mmol/L i.c.v.) ( $n = 9$ ) (Sigma) was infused (0.1  $\mu$ L/min) throughout a 3-h hyperinsulinemic/severe hypoglycemic clamp as described above. Correct cannula placement was confirmed by postmortem staining with Evans blue dye.

**Study 5: adrenergic blockade.**  $\alpha$ -Blockers (prazosin [ $\alpha_1$ ], 1 mg/mL, 1  $\mu$ L/min; Sigma) and  $\beta$ -blockers (propranolol [ $\beta_1$  and  $\beta_2$ ], 1 mg/mL, 2.5  $\mu$ L/min; Sigma) were infused intravenously concurrently ( $n = 13$ ) or individually ( $n = 5$ –6) or with saline (control,  $n = 12$ ) starting 30 min prior to insulin infusion and continued throughout a 3-h hypoglycemic clamp as described above.

**Statistical analyses.** All data are represented as means  $\pm$  SEM. ANOVA (one- and two-way), repeated-measures ANOVA, and Student *t* test were used to determine significance. Holm-Sidak was used for post hoc analyses. Fisher exact test with the Freeman-Halton extension was used for mortality and arrhythmia incidence. Significance was determined at  $P < 0.05$ .

## RESULTS

**Study 1: pooled mortality analysis in control, diabetic, and recurrently hypoglycemic rat experiments.** For an understanding of how diabetes and recurrent hypoglycemia might impact survival during severe hypoglycemia, pooled data were analyzed from 1) nondiabetic, 2) uncontrolled STZ diabetic, and 3) recurrent moderate hypoglycemic prior to severe hypoglycemic rats. Basal glucose levels for nondiabetic, diabetic, and recurrently hypoglycemic rats were  $88 \pm 5$ ,  $348 \pm 37$ , and  $74 \pm 2$  mg/dL, respectively. During the 1 h of hypoglycemia, glucose levels were similar for control ( $12 \pm 0.2$  mg/dL), diabetic ( $12 \pm 0.2$  mg/dL), and recurrent hypoglycemia ( $12 \pm 0.4$  mg/dL) rats. As expected, the recurrently hypoglycemic rats had a blunted epinephrine response to hypoglycemia ( $2,001 \pm 241$  vs.  $3,487 \pm 474$  pg/mL in controls) (28). During severe hypoglycemia, all rats lost their righting reflex, went into a coma, and experienced brief seizure-like activity. Mortality due to severe hypoglycemia was 21% in nondiabetic rats and increased to 36% in diabetic rats ( $P < 0.05$ ) (Fig. 1A). Interestingly, rats that had been previously treated with recurrent moderate hypoglycemia

had markedly less mortality (4%) during severe hypoglycemia ( $P < 0.05$ ) (Fig. 1A). Differences in death rates were hypothesized to be due to hypokalemia, neuroglycopenia, or the sympathoadrenal response, each of which may have increased susceptibility to cardiac arrhythmias.

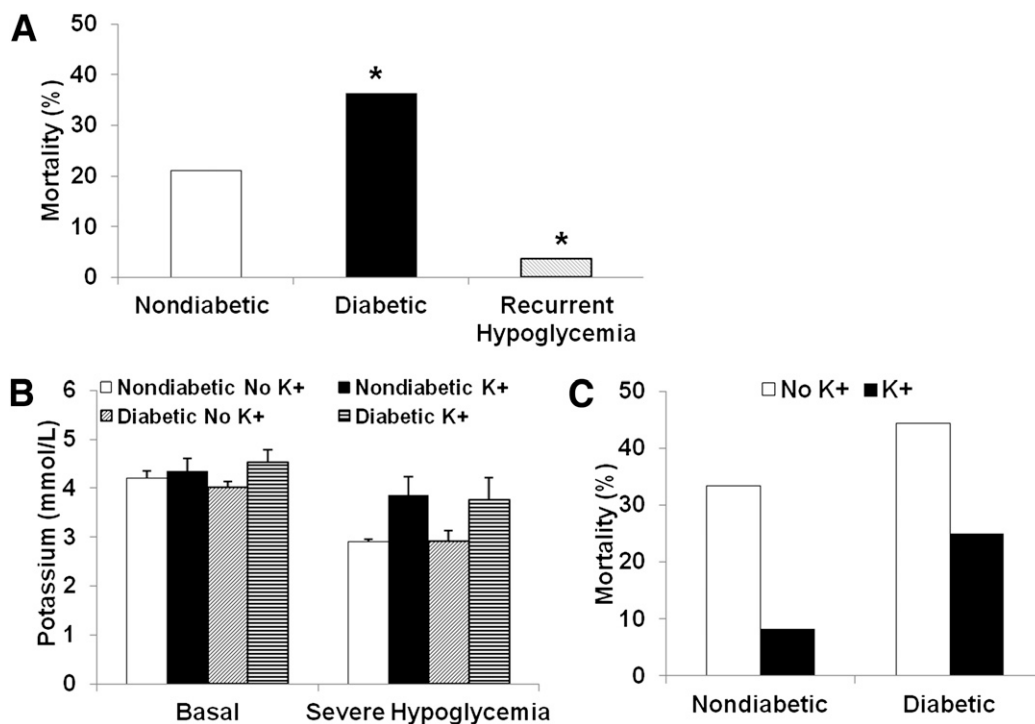
**Study 2: potassium supplementation.** In order to determine if the cause of death due to severe hypoglycemia resulted from hypokalemia, potassium was supplemented in nondiabetic and diabetic rats during a 1-h severe hypoglycemic clamp. Control rats had basal glucose levels of  $127 \pm 7$  mg/dL and  $116 \pm 12$  mg/dL with or without potassium supplementation, respectively. Basal glucose levels for diabetic rats were  $479 \pm 77$  mg/dL and  $394 \pm 54$  mg/dL, respectively. Mean glucose levels during severe hypoglycemia were not different among the nondiabetic rats that did ( $12.6 \pm 1.18$  mg/dL) or did not ( $12.5 \pm 0.76$  mg/dL) receive potassium or the diabetic rats that did ( $12.1 \pm 0.84$  mg/dL) or did not ( $13.4 \pm 0.39$  mg/dL) receive potassium. Potassium levels decreased during severe hypoglycemia in both nondiabetic rats ( $4.2 \pm 0.15$  to  $2.9 \pm 0.06$  mmol/L) and diabetic rats ( $4.0 \pm 0.11$  to  $2.9 \pm 0.23$  mmol/L) that did not receive potassium (Fig. 1B). From basal to severe hypoglycemia, potassium supplementation maintained plasma potassium levels in nondiabetic ( $4.4 \pm 0.26$  to  $3.9 \pm 0.38$  mmol/L) and diabetic ( $4.5 \pm 0.25$  to  $3.8 \pm 0.47$  mmol/L) rats (Fig. 1B). Mortality associated with severe hypoglycemia trended to be reduced with potassium supplementation compared with rats that did not receive potassium in both nondiabetic (from 33 to 8%) and diabetic (from 44 to 25%) rats ( $P < 0.08$ ) (Fig. 1C).

**Study 3: cardiac arrhythmias.** Severe hypoglycemia-induced death seemed to be related to the proarrhythmic condition of hypokalemia; thus, cardiac arrhythmias were hypothesized to mediate severe hypoglycemia-induced mortality. Nondiabetic rats ( $n = 6$ ) were subjected to severe hypoglycemic clamps with continuous ECG recording. Glucose was clamped between 10–15 mg/dL for 90 min during which all rats died (Fig. 2A and B).

During insulin infusion, heart rate remained normal until it increased at the onset of severe hypoglycemia (Fig. 2C). Severe hypoglycemia increased the QTc interval ( $172 \pm 8$  ms) compared with basal ( $122 \pm 2$  ms), which represents a 40% increased ventricular depolarization and repolarization phase ( $P < 0.001$ ) (Fig. 2D).

Epinephrine and norepinephrine increased during severe hypoglycemia compared with the basal period ( $P < 0.001$ ) (Fig. 2E and F). Plasma epinephrine peaked shortly after the onset of severe hypoglycemia and then decreased as severe hypoglycemia continued, possibly indicating adrenal exhaustion. Plasma norepinephrine continued to increase throughout severe hypoglycemia.

Cardiac arrhythmias did not occur during moderate hypoglycemia. After the onset of severe hypoglycemia, premature ventricular contractions (PVCs) and narrow complex second-degree heart block (Mobitz II) were noted as initial arrhythmias (Fig. 3). Premature atrial contractions (PACs) and first-degree heart block were also observed as early arrhythmias (Fig. 3). Occasionally, brief runs of nonsustained ventricular tachycardia occurred, but these rhythms did not precede death. With longer duration of severe hypoglycemia, a sequential pattern and progression of arrhythmias that preceded death were noted. Frequent and higher-grade second-degree heart block (4:1) was noted followed by third-degree heart block (complete atrioventricular



**FIG. 1.** Severe hypoglycemia-induced mortality in diabetic, nondiabetic, and recurrently hypoglycemic rats and the effects of potassium supplementation (studies 1 and 2). **A:** Analysis of mortality associated with severe hypoglycemia revealed 21% mortality in nondiabetic control rats ( $n = 123$ ). Mortality was increased in diabetic rats to 36% ( $n = 95$ ) and decreased to 4% ( $n = 27$ ) in rats that underwent recurrent hypoglycemia prior to severe hypoglycemia ( $*P < 0.05$ , Fisher exact test, Freeman-Halton extension). **B:** Potassium levels in rats that had no potassium supplementation versus rats with potassium supplementation both in the basal state and during severe hypoglycemia. Rats without supplementation had potassium levels significantly decrease during severe hypoglycemia, while rats with supplementation maintained normal potassium during severe hypoglycemia. Data expressed as means  $\pm$  SEM. **C:** Potassium supplementation in nondiabetic and diabetic rats trended to reduce severe hypoglycemia-induced mortality from 33 to 8% ( $n = 12-15$ ) and 44 to 25% ( $n = 28-45$ ), respectively, but this did not reach significance.

dissociation), characterized by a terminal bradycardic ventricular rhythm (Fig. 3).

Oxygen and carbon dioxide levels remained normal throughout the experiment until just before death, when oxygen levels decreased and carbon dioxide levels increased (Supplementary Fig. 1A). As previously noted, potassium levels decreased during severe hypoglycemia compared with basal ( $P < 0.05$ ) (Supplementary Fig. 1B).

BP increased from  $105 \pm 14/60 \pm 2$  mmHg during the basal period to  $177 \pm 16/93 \pm 9$  mmHg during severe hypoglycemia and remained elevated until immediately prior to death, when it suddenly decreased (Supplementary Fig. 1C).

Seizure-like activity was noted in all rats. A subset of rats had simultaneous EEG recordings during the clamp. During severe hypoglycemia, EEG tracings showed characteristic high-amplitude slow waves. EEG isoelectricity coincided with a nonresponsive comatose state (Supplementary Fig. 1D). Witnessed seizures coincided with high-frequency, high-amplitude spikes of increased electrical activity on the EEG. There was no correlation found between seizures and mortality.

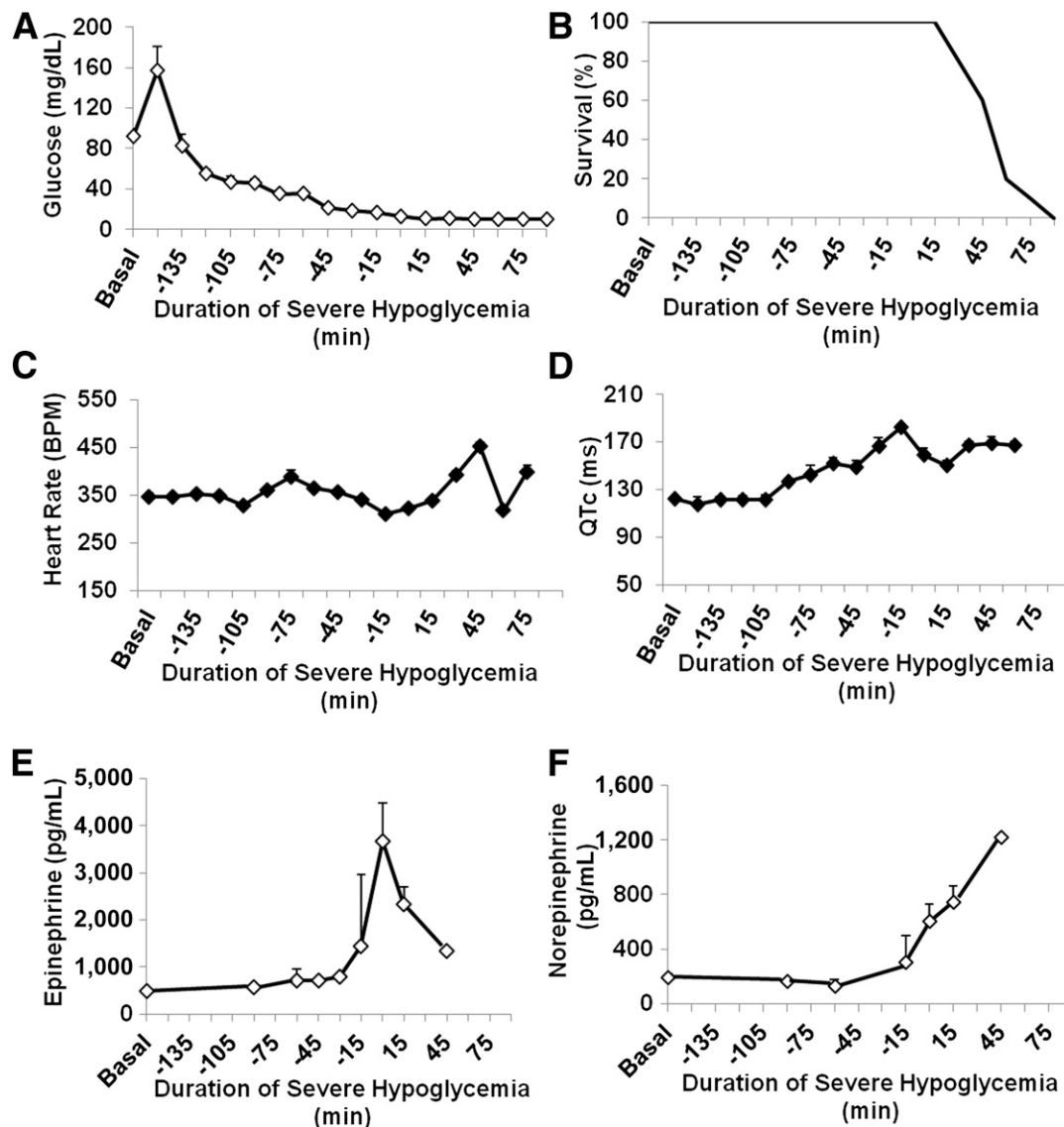
**Study 4: ICV glucose infusion.** For an understanding of the extent to which neuroglycopenia mediates sudden death, glucose was infused into the third ventricle of the brain during severe hypoglycemia. Compared with controls, ICV glucose infusion reduced mortality due to severe hypoglycemia from 86 to 33% ( $P < 0.05$ ) (Fig. 4A). Systemic blood glucose was similar during severe hypoglycemia in ICV mannitol (MAN) ( $n = 9$ )-infused and ICV glucose ( $n = 9$ )-infused rats ( $12 \pm 0.1$  vs.  $12 \pm 0.2$  mg/dL, respectively) (Fig. 4B). Despite matched glucose levels, the ICV glucose group

required a higher peripheral glucose infusion rate ( $5.4 \pm 0.6$  mg/kg/min;  $P < 0.005$ ) during severe hypoglycemia compared with MAN ( $4.1 \pm 0.41$  mg/kg/min) (Fig. 4C). Insulin levels were comparable between MAN ( $7.5 \pm 0.78$  ng/mL) and glucose ( $8.8 \pm 0.49$  ng/mL) groups during severe hypoglycemia.

During severe hypoglycemia, heart rate was lower in the glucose compared with MAN group ( $P < 0.05$ ) (Fig. 4D). Mean heart rate increased in the MAN group during severe hypoglycemia compared with basal ( $414 \pm 9$  vs.  $373 \pm 4$  bpm;  $P < 0.01$ ), while ICV glucose infusion prevented this rise in heart rate (basal:  $357 \pm 4$  bpm; hypoglycemia:  $357 \pm 8$  bpm). QTc increased during severe hypoglycemia compared with basal in both MAN and glucose groups ( $P = \text{NS}$ ) (Fig. 4E).

Cardiac arrhythmias were absent in 25% of the ICV glucose-infused rats. Frequencies of PVCs and second-degree heart block were nonsignificantly reduced in the glucose compared with the MAN group ( $P < 0.07$ ) (Supplementary Fig. 2A and B). Incidence of third-degree heart block was decreased in the glucose group compared with the MAN group ( $P < 0.05$ ) (Supplementary Fig. 2C) and preceded death in both groups, suggesting that third-degree heart block was the terminal fatal arrhythmia associated with severe hypoglycemia. Other life-threatening arrhythmias such as ventricular tachycardia were witnessed in 33% of the MAN and 0% of the glucose group (Supplementary Fig. 2D).

The norepinephrine response was blunted during severe hypoglycemia in the glucose ( $464 \pm 48$  pg/mL) compared with the MAN group ( $1,441 \pm 646$  pg/mL;  $P < 0.05$ ) (Fig. 4F). During severe hypoglycemia, epinephrine increased to



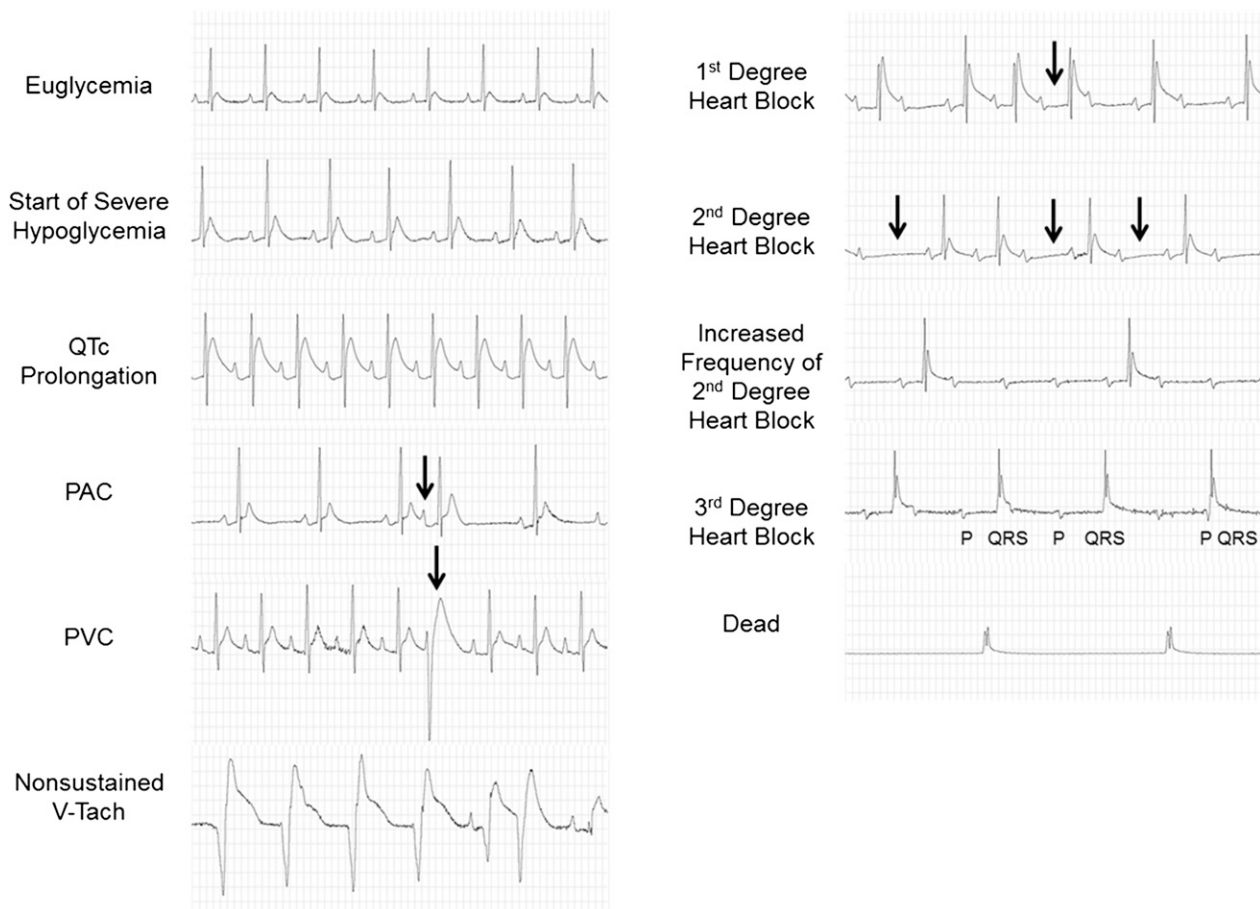
**FIG. 2.** Severe hypoglycemic clamp in control experiments (study 3). **A:** Glucose levels of control, nondiabetic rats during a severe hypoglycemic clamp. With insulin infusion and careful glucose infusion, plasma glucose levels are decreased over a 2.5-h period to 15 mg/dL (time 0). Severe hypoglycemia (10–15 mg/dL) was maintained for 1.5 h. **B:** Mortality associated with severe hypoglycemia started as early as 25 min after the onset of severe hypoglycemia. All rats had died by 90 min of severe hypoglycemia. **C:** Heart rate during the clamp remained normal until just before severe hypoglycemia when glucose levels reach ~35 mg/dL, at which time heart rate increases. At the onset of severe hypoglycemia, heart rate decreases. Heart rate increases again immediately prior to death. The slower heart rates that occur toward the end of the clamp can be attributed to multiple cardiac arrhythmias associated with bradycardia. **D:** QTc prolongation starts to occur during insulin infusion when glucose levels are moderately hypoglycemic (~35 mg/dL) and persists throughout the period of severe hypoglycemia. Mean QTc during severe hypoglycemia increased to  $158 \pm 5$  ms compared with  $122 \pm 2$  ms in the basal period ( $P < 0.01$ ). **E:** Epinephrine levels increased from  $495 \pm 60$  pg/mL in the basal period and peak at  $3,034 \pm 383$  pg/mL at the onset of severe hypoglycemia but declined as severe hypoglycemia persisted ( $P < 0.001$ ). **F:** Norepinephrine levels increased from  $197 \pm 20$  pg/mL in the basal period, started to increase at the onset of severe hypoglycemia, and continued to rise throughout severe hypoglycemia, peaking at  $748 \pm 116$  pg/mL ( $P < 0.001$ ).  $n = 6$ ; data expressed as means  $\pm$  SEM.

a similar extent in both the MAN ( $1,926 \pm 320$  pg/mL) and glucose ( $2,155 \pm 448$  pg/mL) groups. From basal to severe hypoglycemia, plasma potassium levels fell in MAN ( $4.7 \pm 0.3$  to  $3.6 \pm 0.4$  mmol/L) and glucose ( $4.4 \pm 0.3$  to  $3.1 \pm 0.5$  mmol/L) rats.

**Study 5: adrenergic blockade.** For testing of the hypothesis that the sympathoadrenal response increases risk of fatal cardiac arrhythmias during severe hypoglycemia, rats had adrenergic receptor blockers infused during a hyperinsulinemic/severe hypoglycemic clamp. Severe hypoglycemia-induced mortality was 33% in control rats ( $n = 12$ ), while combined  $\alpha/\beta$ -blocker infusion ( $n = 13$ ) and  $\beta$ -blocker infusion alone ( $n = 5$ )

completely prevented death ( $P < 0.029$ ) (Fig. 5A).  $\alpha$ -Blocker infusion alone had no effect on mortality (50%;  $n = 6$ ).

Glucose levels during severe hypoglycemia were equally matched in control,  $\alpha/\beta$ -,  $\alpha$ -, and  $\beta$ -blocker-infused rats ( $12 \pm 0.2$ ,  $12 \pm 0.3$ ,  $12 \pm 0.5$ , and  $12 \pm 0.5$  mg/dL, respectively) (Fig. 5B). Consistent with a blunted counterregulatory response, glucose infusion rate was increased during severe hypoglycemia in  $\alpha/\beta$ -blocker-infused rats ( $P < 0.01$ ) (Fig. 5C) compared with controls, with no difference in  $\alpha$ - and  $\beta$ -blocker alone-infused rats. Insulin levels were not different between the groups (Supplementary Fig. 3A).



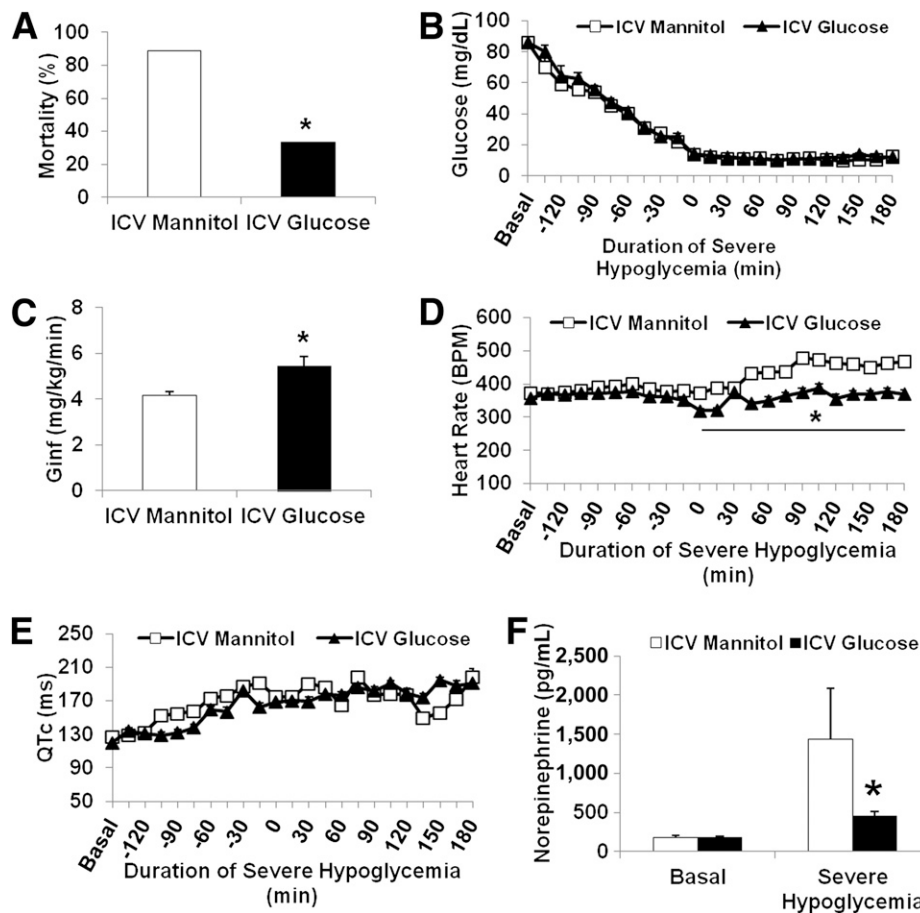
**FIG. 3.** ECG changes in response to severe hypoglycemia (study 3). ECG recordings from one representative control rat from basal (euglycemia) through severe hypoglycemia-induced death. The order of the graphs is a typical representation of what occurs in all rats during severe hypoglycemia and just prior to death. At the top of the left column, the ECG shows a rat in normal sinus rhythm during euglycemic conditions. During moderate hypoglycemia, cardiac arrhythmias are rarely witnessed. At the start of severe hypoglycemia, heart rate tends to return to basal levels followed by QTc prolongation and the initiation of several types of cardiac arrhythmias. Cardiac arrhythmias that occur during severe hypoglycemia are PACs (noted by arrow), PVCs (noted by arrow), nonsustained ventricular tachycardia (V-Tach), first-degree heart block (PR prolongation noted by arrow), and second-degree heart block (absent QRS complex noted by arrow). With severe hypoglycemia of increased frequency high-grade, second-degree heart block was noted followed by third-degree heart block. At the time of cardiorespiratory arrest, a lower-voltage ECG with a markedly decreased heart rate was noted.

During severe hypoglycemia, heart rate was lower in  $\alpha/\beta$ -blocker-infused rats ( $225 \pm 5$  bpm) and  $\beta$ -blocker-infused rats ( $198 \pm 4$  bpm) than in controls ( $331 \pm 5$  bpm) and  $\alpha$ -blocker-infused ( $339 \pm 9$  bpm) rats ( $P < 0.05$ ) (Fig. 5D). Sinus tachycardia was experienced in all rats that died. More than 30 min of tachycardia resulted in 100% mortality (Supplementary Fig. 3B).  $\beta$ -Blocker and combined  $\alpha/\beta$ -blocker infusion completely abrogated hypoglycemia-induced tachycardia and mortality. Sinus tachycardia is therefore a predictor of mortality in these experiments, and prevention of tachycardia was associated with a mortality benefit.

QTc prolongation increased during severe hypoglycemia in control ( $163 \pm 2$  ms),  $\alpha$ -blocker-infused ( $210 \pm 5$  ms), and  $\beta$ -blocker-infused ( $164 \pm 2$  ms) rats ( $P < 0.001$ ), but QTc prolongation was blunted with  $\alpha/\beta$ -blocker ( $134 \pm 3$  ms;  $P < 0.001$ ) infusion (Fig. 5E). QTc prolongation was associated with increased risk of mortality in control and  $\alpha$ -blocker rats (Supplementary Fig. 3C). Interestingly, the  $\beta$ -blocker-infused rats had QTc prolongation similar to that in control rats (Fig. 5E), yet all survived, indicating that QTc prolongation may not be a direct mediator of severe hypoglycemia-induced lethality.

Arrhythmia incidence was lower with  $\alpha/\beta$ - and  $\beta$ -blocker infusion compared with controls. PVCs and second-degree heart block occurred in only 25 and 8% of the  $\alpha/\beta$ -blocker rats, respectively ( $P < 0.001$ ). Frequency of PVCs was reduced in  $\alpha/\beta$ - and  $\beta$ -blocker-infused rats compared with controls ( $P < 0.001$ ) (Fig. 6A).  $\alpha$ -Blocker-infused rats had nonsignificantly lower frequency of PVCs. Frequency of second-degree heart block was significantly reduced in combined  $\alpha/\beta$ -blocker-infused rats ( $P < 0.001$ ) and trended to be reduced in  $\alpha$ - and  $\beta$ -blocker-infused rats compared with controls (Fig. 6B). Third-degree heart block was prevented in  $\alpha/\beta$ -blocker-infused rats ( $P < 0.024$ ) and  $\beta$ -blocker-infused rats ( $P = \text{NS}$ ), with no difference in  $\alpha$ -blocker-infused rats compared with controls (Fig. 6C).

Epinephrine and norepinephrine significantly increased during severe hypoglycemia compared with basal in all groups (Fig. 6D and E); however, the actions of epinephrine and norepinephrine were blocked with the adrenergic blockers. Consistent with decreased epinephrine clearance by  $\beta$ -blockers, epinephrine levels were significantly higher in  $\beta$ -blocker-infused rats compared with controls ( $P < 0.004$ ) and  $\alpha/\beta$ -blocker rats ( $P < 0.011$ ) (Fig. 6D).



**FIG. 4.** Severe hypoglycemic clamp with ICV glucose infusion (study 4). **A:** Mortality due to severe hypoglycemia was significantly reduced in ICV glucose-infused compared with ICV MAN-infused (control) (33 vs. 89%;  $*P < 0.05$ , Fisher exact test, Freeman-Halton extension) rats. **B:** Blood glucose levels were evenly matched between the ICV MAN-infused and ICV glucose-infused rats. Once blood glucose levels reached 15 mg/dL, severe hypoglycemia (starting at time 0) was maintained for a duration of 3 h. **C:** Mean glucose infusion (Ginf) rates during severe hypoglycemia were significantly elevated in ICV glucose-infused ( $5.4 \pm 0.6$  mg/kg/min) compared with ICV MAN-infused ( $4.2 \pm 0.41$  mg/kg/min) rats ( $*P < 0.01$ , *t* test). **D:** Heart rate remained normal in both groups until 45 min into severe hypoglycemia, when ICV MAN-infused rats had elevated heart rate. ICV glucose-infused rats had lower heart rates throughout severe hypoglycemia compared with controls. Mean heart during severe hypoglycemia was  $414 \pm 9$  bpm and  $357 \pm 8$  bpm in ICV MAN-infused and glucose-infused rats, respectively ( $*P < 0.05$ , *t* test). **E:** QTc length increased after the start of insulin infusion during moderate hypoglycemia. During severe hypoglycemia, QTc length was not different between ICV MAN-infused and glucose-infused rats, with mean QTc values of  $180 \pm 2$  and  $187 \pm 3$  ms, respectively. **F:** Norepinephrine levels were increased during severe hypoglycemia in ICV MAN-infused rats compared with the basal state, but this response was blunted in ICV glucose-infused rats ( $*P < 0.05$ , *t* test).  $n = 9$ /group. Data expressed as means  $\pm$  SEM.

Norepinephrine was significantly increased in the  $\alpha$ -blocker group ( $P < 0.001$ ) compared with the control,  $\alpha/\beta$ -, and  $\beta$ -blocker groups (Fig. 6E). Glucagon was significantly higher in  $\alpha$ -blocker-infused rats compared with other groups ( $P < 0.001$  for AUC) (Fig. 6F), consistent with the elevated norepinephrine-mediated, unopposed,  $\beta$ -adrenergic stimulation of pancreatic  $\alpha$ -cells.

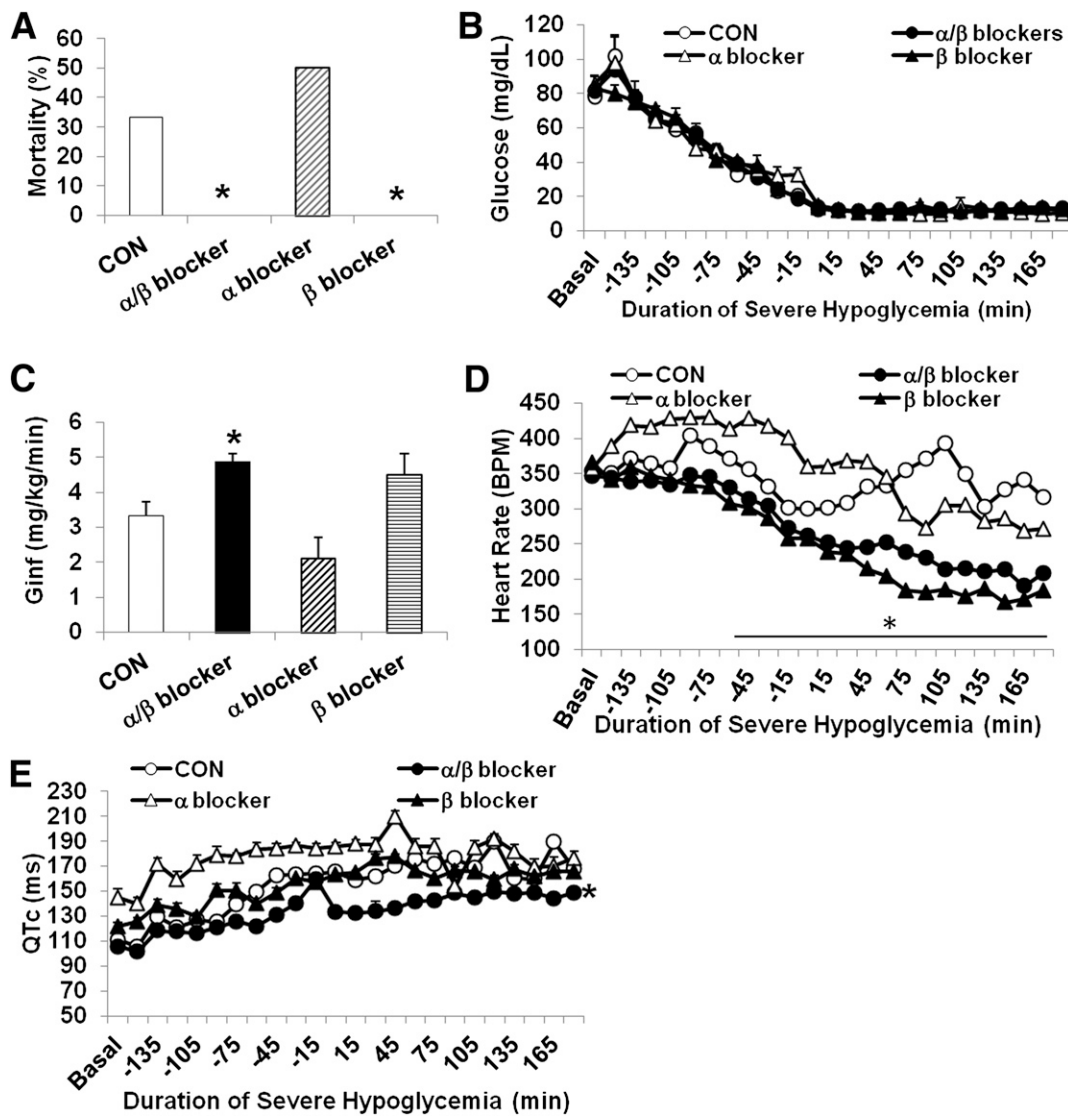
Potassium levels decreased in all groups during severe hypoglycemia ( $P < 0.01$ ) (Table 1). Oxygen and carbon dioxide levels remained normal in all groups until just before death. Compared with controls, respiration rates were lower in the  $\alpha/\beta$ -,  $\alpha$ -, and  $\beta$ -blocker-infused rats during severe hypoglycemia ( $P < 0.001$ ) and dropped precipitously immediately prior to death.

Mean systolic BP during severe hypoglycemia for control,  $\alpha/\beta$ -,  $\alpha$ -, and  $\beta$ -blocker-infused rats was  $133 \pm 14$ ,  $140 \pm 10$ ,  $129 \pm 8$ , and  $112 \pm 4$  mmHg, respectively (Supplementary Fig. 3D). Mean diastolic BP during severe hypoglycemia was  $91 \pm 16$ ,  $90 \pm 9$ ,  $92 \pm 8$ , and  $77 \pm 4$  mmHg, respectively (Supplementary Fig. 3E). Systolic and diastolic BP was significantly lower in  $\beta$ -blocker-infused rats ( $P < 0.001$ ).

Based on the composite observations in the presented experiments, mechanisms of sudden cardiac death due to insulin-induced severe hypoglycemia are proposed (Fig. 7).

## DISCUSSION

Mortality owing to iatrogenic hypoglycemia represents a major concern for insulin-treated diabetic patients and their families. Determining the mechanisms by which hypoglycemia causes sudden death is critically important in order to find treatment strategies that could protect at-risk patients. Based on ECG anomalies reported during moderate hypoglycemia (26), it has been speculated that the "dead in bed syndrome" may be mediated by hypoglycemia-induced fatal arrhythmias. In the current study, it is shown for the first time that fatal cardiac arrhythmias occur during severe hypoglycemia and can be reduced by ICV glucose infusion and prevented by  $\beta$ -adrenergic blockade, indicating that brain neuroglycopenia and the striking sympathoadrenal response mediate fatal cardiac arrhythmias during severe hypoglycemia (Fig. 7A).

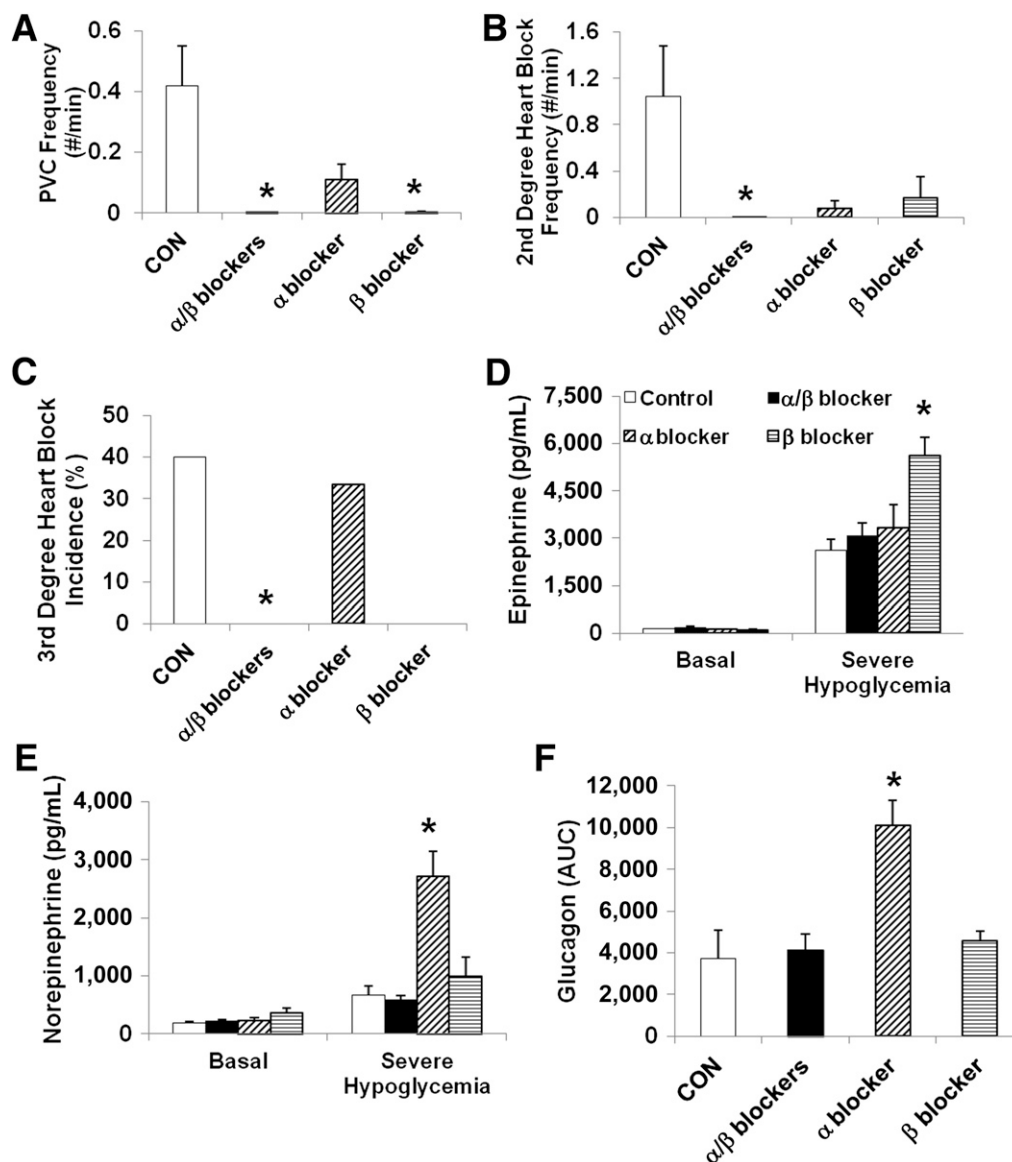


**FIG. 5.** Severe hypoglycemic clamp with adrenergic blockade (study 5). **A:** Control rats had a mortality rate of 33% ( $n = 12$ ) in response to severe hypoglycemia.  $\alpha$ -Blockade had no effect on mortality (50%;  $n = 6$ ). Death was prevented in both  $\alpha/\beta$ -blocker-infused ( $n = 13$ ) and  $\beta$ -blocker-infused ( $n = 5$ ) rats ( $*P < 0.029$ , Fisher exact test, Freeman-Halton extension). **B:** Glucose levels were evenly matched throughout the clamp. Glucose was clamped between 10 and 15 mg/dL for 3 h. **C:** Mean glucose infusion (Ginf) rate during severe hypoglycemia.  $\alpha/\beta$ -Blockade significantly increased glucose infusion rate.  $\alpha$ -Blockade and  $\beta$ -blockade alone did not differ compared with controls, but  $\alpha$ -blocker rats had a lower glucose infusion rate compared with  $\alpha/\beta$ -blocker-infused and  $\beta$ -blocker-infused rats ( $*P < 0.01$ , ANOVA). **D:** Heart rate was consistently lower in  $\alpha/\beta$ -blockade and  $\beta$ -blockade rats throughout the clamp compared with control and  $\alpha$ -blockade rats ( $*P < 0.001$  for individual time points, ANOVA). **E:** QTc length was significantly increased in all groups during severe hypoglycemia ( $P < 0.001$ , ANOVA). Combined  $\alpha/\beta$ -blockade significantly blunted the QTc prolongation compared with controls ( $*P < 0.001$  for mean QTc during severe hypoglycemia, ANOVA). Data expressed as means  $\pm$  SEM. CON, control.

In these experiments, diabetes per se nearly doubled the mortality risk associated with severe hypoglycemia. Uncontrolled diabetes is hypothesized to increase risk of fatal cardiac arrhythmias via altering the myocardium composition (32) and potential cardiac mechanical defects (33). It is unclear whether these previously reported chronic maladaptive changes induced by diabetes were manifest in the current studies because the duration of diabetes in the current study was only of 2–4 weeks' duration. It was speculated that relative whole-body potassium depletion in diabetic rats might predispose to arrhythmic deaths. Although systemic potassium levels in diabetic rats were not different from those in controls, a potential role for an altered cardiac *myo*-cellular potassium gradient in diabetic rats could not be ruled out. Aside from potential chronic

maladaptations associated with insulin deficiency, it may be that the greater absolute decrement in blood glucose levels in diabetic (348 to 12 mg/dL) versus control (88 to 12 mg/dL) rats may have contributed to increased mortality. Finally, although STZ diabetic rats have been shown to be at higher risk for arrhythmias (34), in the absence of ECG data from these preliminary experiments the cause of increased mortality rates in diabetic rats remains unknown.

Recurrent hypoglycemia significantly reduced severe hypoglycemia-induced mortality. These findings are consistent with a preconditioning effect of recurrent hypoglycemia to limit severe hypoglycemia-induced seizures, brain damage, and cognitive dysfunction (28). The blunted catecholamine response in the recurrently hypoglycemic rats would render them more prone to severe hypoglycemia



**FIG. 6.** Severe hypoglycemia-induced arrhythmias and catecholamine levels during adrenergic blockade (study 5). **A:** Compared with that in control rats, PVC frequency during severe hypoglycemia was significantly decreased in  $\alpha/\beta$ - and  $\beta$ -blockade rats ( $*P < 0.001$ , ANOVA), with a trend for decreased frequency in  $\alpha$ -blockade rats. **B:** Second-degree heart block frequency was significantly reduced in  $\alpha/\beta$ -blockade rats ( $*P < 0.01$ , ANOVA), with only one rat experiencing second-degree heart block.  $\alpha$ - and  $\beta$ -blockade alone trended to reduce frequency of second-degree heart block, but this was not significantly different compared with controls. **C:** Incidence of third-degree heart block was 40% in control rats and 33% in  $\alpha$ -blockade rats. No third-degree heart block occurred in  $\alpha/\beta$ - or  $\beta$ -blockade rats ( $*P < 0.02$ , Fisher exact test, Freeman-Halton extension). **D:** Epinephrine levels increased significantly in all groups during severe hypoglycemia. The  $\beta$ -blockade group had significantly higher epinephrine levels compared with all other groups ( $*P < 0.01$ , ANOVA). **E:** Norepinephrine levels increased significantly in all groups during severe hypoglycemia. Norepinephrine levels in the  $\alpha$ -blockade group were significantly higher compared with all three other groups ( $*P < 0.001$ , ANOVA). **F:** Glucagon area under the curve (AUC) was significantly higher with  $\alpha$ -blockade and similar with  $\alpha/\beta$ - and  $\beta$ -blockade compared with controls ( $*P < 0.005$ , two-way ANOVA).  $n = 5$ –13. Data expressed as means  $\pm$  SEM. CON, control.

but, paradoxically, less vulnerable to severe hypoglycemia-induced death. This putative blunted adrenergic mechanism leading to improved survival is supported by both the reduced norepinephrine response in the ICV glucose study, as well as the reduced cardiac stimulation noted in the  $\beta$ -blockade study, with both studies noting a reduction of arrhythmias and death. In the absence of ECG recordings, though, the extent to which cardiac arrhythmias were reduced by antecedent recurrent hypoglycemia remains unknown.

Compared with human ECG recordings, rat ECGs are characterized by shorter interval durations, more rapid

heart rate, absent Q waves, and absent isoelectric ST-segments (35). Supporting our assertion that the reported rat ECG changes truthfully reflect human ECG responses during hypoglycemia are clinical ECG studies that note similar hypoglycemia-induced QTc prolongation (20,21,26,36), sinus bradycardia (18,36), and multifocal ventricular ectopies (18). Furthermore, the reduction in heart rate and BP with  $\beta$ -blockade in rats during hypoglycemia corroborates with responses seen in humans (26). Therefore, the rat ECG changes presented here, that suggest  $\beta$ -blockers have the potential to prevent severe hypoglycemia-induced sudden death, may have clinical applicability.



TABLE 1

Arterial blood gases, electrolytes, and respiratory rates during basal, severe hypoglycemia, and predeath conditions for adrenergic blockade study (study 5)

	Control	$\alpha/\beta$ -Blockers	$\alpha$ -Blocker	$\beta$ -Blocker
Potassium (mmol/L)				
Basal	4.4 $\pm$ 0.14	4.0 $\pm$ 0.11	4.7 $\pm$ 0.1	5.0 $\pm$ 0.14
Severe hypoglycemia	3.12 $\pm$ 0.18*	2.84 $\pm$ 0.08*	3.01 $\pm$ 0.21*	3.74 $\pm$ 0.05*†
Predeath	3.6 $\pm$ 0.48	N/A	4.2 $\pm$ 0.21	N/A
Oxygen saturation (%)				
Basal	96 $\pm$ 1.9	97 $\pm$ 0.28	97 $\pm$ 0.3	97 $\pm$ 0.32
Severe hypoglycemia	97 $\pm$ 0.34	98 $\pm$ 0.15	98 $\pm$ 0.36	98 $\pm$ 0.14
Predeath	26 $\pm$ 12*	N/A	35 $\pm$ 0.28*	N/A
Carbon dioxide (mmHg)				
Basal	32 $\pm$ 1.7	33 $\pm$ 0.69	32 $\pm$ 0.87	34 $\pm$ 0.91
Severe hypoglycemia	32 $\pm$ 1.4	38 $\pm$ 0.97*†	36 $\pm$ 2.2	42 $\pm$ 2.2*†
Predeath	48 $\pm$ 2.5*	N/A	63 $\pm$ 11*	N/A
Respiration (breaths/min)				
Basal	86 $\pm$ 4	88 $\pm$ 4	96 $\pm$ 8	86 $\pm$ 4
Severe hypoglycemia	76 $\pm$ 3	51 $\pm$ 2*†	66 $\pm$ 8*	54 $\pm$ 2*†
Predeath	28 $\pm$ 8*	N/A	8	N/A

Data are means  $\pm$  SEM. Potassium levels decreased in all groups during severe hypoglycemia.  $\beta$ -Blockade resulted in higher potassium levels during severe hypoglycemia compared with controls. Oxygen saturation remained normal in all groups throughout severe hypoglycemia. In control and  $\alpha$ -blocker-infused rats, oxygen decreased and carbon dioxide levels increased just prior to death. Respiration was significantly decreased during severe hypoglycemia in  $\alpha/\beta$ -,  $\alpha$ -, and  $\beta$ -blocker-infused rats compared with basal. Respiration decreased just prior to death in control and  $\alpha$ -blocker-infused rats. Of the  $\alpha$ -blocker-infused rats that died, predeath respiration was only noted for 1 rat; therefore, there are no SEM values and the data are not significant. Note that since  $\alpha/\beta$ - and  $\beta$ -blocker-infused rats did not die, predeath values were not applicable. \* $P < 0.05$  vs. basal, † $P < 0.05$  vs. control.  $n = 5$ –13/group.

A key concept highlighted in the findings is the importance of the duration of severe hypoglycemia, and not hypoglycemic nadir alone, as a critically important component in determining arrhythmias and sudden death. Indeed a consistent, time-dependent sequence of events leading to severe hypoglycemia-induced sudden death was observed (Fig. 7B). The ability to survive an episode of severe hypoglycemia depended on the ability to escape from sinus tachycardia. In rats that died, sinus tachycardia was followed by increased frequency of PVCs and second-degree heart block, which culminated into third-degree heart block associated with bradycardia followed by the rapid sequential onset of reduced cardiac output (noted by hypotension), respiratory depression, hypoxemia, hypercarbia, and acidosis. The sensitivity and specificity of sinus tachycardia (heart rate  $>400$  bpm) as a predictor of mortality was 88 and 74%, respectively, while third-degree heart block was 89 and 95%, respectively. The sensitivity and specificity of QTc prolongation ( $>200$  ms) as a predictor of mortality was 81 and 60%, respectively, while second-degree heart block was 94 and 53%, respectively. Hypoglycemic seizures were also noted to occur in most rats but were not associated with severe hypoglycemia-induced mortality, consistent with other studies demonstrating survival after hypoglycemia-induced seizures (28,29).

Moderate hypoglycemia has been shown to lead to QTc prolongation (ventricular dispersion) and be proarrhythmic (18–21,26). In the present studies, QTc lengthening was associated with increased risk of mortality. With adrenergic blockade during hypoglycemia, blunted QTc prolongation may have contributed to reduced incidence of arrhythmias and prevention of death. However, hypoglycemia-induced QTc prolongation in both the  $\beta$ -blocker and the ICV glucose-infused rats was similar to their respective controls, indicating that QTc prolongation

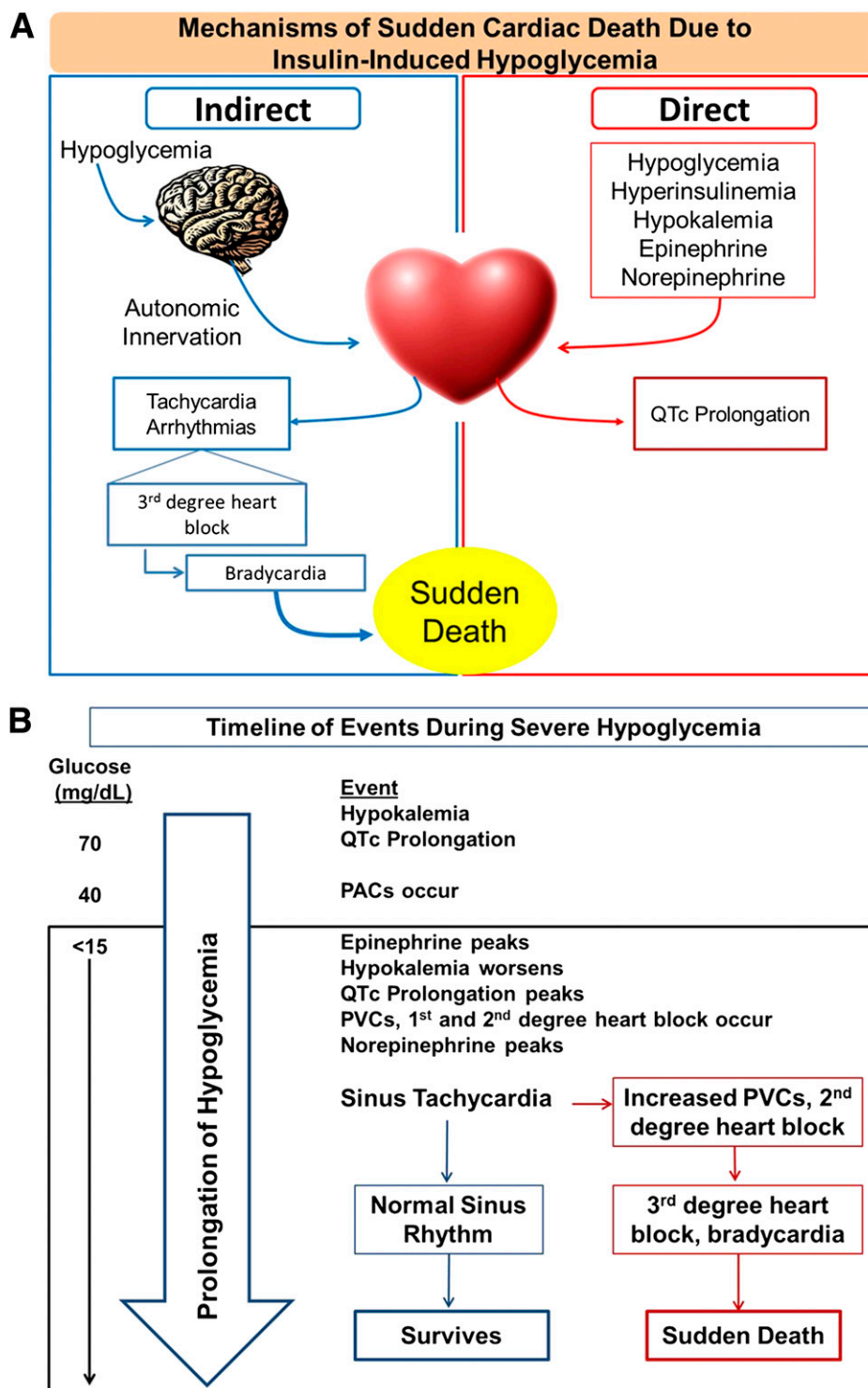
per se does not lead to cardiac arrhythmias and death. Taken together, these studies indicate that QTc prolongation is a predictive marker of ensuing potentially lethal cardiac arrhythmias but alone is not sufficient to cause cardiac arrhythmias.

A role for potassium in potentiating ECG anomalies during moderate hypoglycemia has been noted (25,26). Potassium supplementation in the current studies tended to reduce mortality in nondiabetic and diabetic rats, indicating that hypokalemia increases risk of death due to severe hypoglycemia. However, in the ECG studies, similar degrees of hypokalemia were noted in rats that lived and died, suggesting that in the setting of insulin-induced hypoglycemia, the level of hypokalemia achieved was not sufficient to cause lethal cardiac arrhythmias.

It was questioned whether cardiac arrhythmias could have been secondary to respiratory depression. In these studies, the overall respiratory rate and oxygen and carbon dioxide levels did not change. Thus, changes in respiratory drive were not primary contributors to severe hypoglycemia-induced sudden death. In these studies, respiratory arrest (noted by apnea, hypoxemia, and hypercarbia) only developed after fatal cardiac arrhythmias.

The hypothalamus also plays a critical role in regulating the hypoglycemic counterregulatory response (37) and heart contractility (38). The reduced mortality rate with ICV glucose infusion demonstrated that the brain is important in reducing cardiac arrhythmias during severe hypoglycemia. Of note, the ICV glucose dose infused was not enough to blunt the epinephrine response, consistent with other studies (37). Thus, even in the presence of elevated epinephrine, prevention of brain neuroglycopenia reduces severe hypoglycemia-induced cardiac arrhythmias and mortality.

Although adrenergic receptors located within the brain have been shown to be involved in mediating the



**FIG. 7.** Proposed mechanism of sudden cardiac death due to insulin-induced severe hypoglycemia. **A:** In the setting of insulin-induced hypoglycemia, altered levels of circulating nutrients (glucose), electrolytes (potassium), and hormones (insulin, epinephrine, and norepinephrine) may have a direct arrhythmogenic effect on the heart. Additionally, since hypoglycemia is detected at the level of the brain, indirect (i.e., central nervous system) effects of hypoglycemia also contribute to fatal cardiac arrhythmias via the efferent nervous system and local release of norepinephrine at nerve terminals within the heart. Autonomic innervation of the adrenal gland results in epinephrine release. Both epinephrine and norepinephrine act at the level of the heart to increase risk of fatal cardiac arrhythmias. In the setting of insulin administration, hypokalemia is also thought to contribute to cardiac arrhythmias. Direct and indirect actions on the heart lead to QTc prolongation. Hypoglycemia-induced sudden cardiac death results from enhanced adrenergic signaling at the level of the heart that leads to sinus tachycardia. Tachycardia is followed by third-degree heart block that culminates into a fatal bradycardic rhythm causing cardiorespiratory failure and sudden death. Red, circulating glucose, electrolytes, and hormones; blue, autonomic innervations. **B:** In response to hyperinsulinemic conditions, yet before hypoglycemia starts, hypokalemia and QTc prolongation were noted to occur. During moderate levels of hypoglycemia (~40 mg/dL), PACs occur occasionally. At the start of severe hypoglycemia (15 mg/dL), epinephrine peaks. As severe hypoglycemia prolongs, hypokalemia worsens, QTc prolongation peaks, and cardiac arrhythmias develop (PVCs and first- and second-degree heart blocks). Norepinephrine peaks ~1 h into severe hypoglycemia and is followed by sinus tachycardia. If tachycardia is followed by a return to normal sinus rhythm, the rat survives (blue boxes). If tachycardia is followed by an increase in the frequency of PVCs and second-degree heart block, then third-degree heart block associated with bradycardia will manifest and lead to sudden cardiac death (red boxes).

counterregulatory response (39), the current study cannot distinguish whether the adrenergic blocker effect was mediated via central or peripheral actions. Regardless of their site of action, their effect was profound. It is speculated that the  $\beta$ -adrenergic norepinephrine response may be the lethal arrhythmogenic provocateur based on the following: 1) epinephrine levels peaked early and then declined before the onset of serious arrhythmias, whereas norepinephrine continued to rise during severe hypoglycemia and peaked immediately before the time of arrhythmogenic death; 2) the reduced rates of hypoglycemia-induced arrhythmias and deaths with ICV glucose infusion were associated with a blunted norepinephrine response and not a blunted epinephrine response; and 3)  $\beta$ -blockade markedly reduced arrhythmias and completely prevented death, even in the presence of high epinephrine levels. Taken together, it is proposed that the hypoglycemia-induced norepinephrine response, acting primarily via  $\beta$ -adrenergic receptors, mediates severe hypoglycemia-induced fatal cardiac arrhythmias. Future research is needed to investigate potential life-saving effects of  $\beta$ 1- and/or  $\beta$ 2-receptor blockade in the setting of hypoglycemia.

In conclusion, diabetes worsens, while recurrent antecedent hypoglycemia protects against, severe hypoglycemia-induced mortality. Thus, the odds of surviving an episode of severe hypoglycemia are dependent on antecedent blood glucose control. Deaths due to severe hypoglycemia were mediated by brain neuroglycopenia and the sympathoadrenal response that leads to fatal cardiac arrhythmias causing cardiac failure followed by respiratory arrest. Implications of the current studies could lead to improvement in treatment strategies that aim to reduce the mortality of individuals at risk for insulin-induced hypoglycemia.

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C.M.R. designed and performed experiments, analyzed data, and wrote the manuscript. D.D.-I., Y.S.C., J.V., and K.J. performed experiments and analyzed data. S.J.F. designed experiments, analyzed data, and revised 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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