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Absence of glucose transporter 4 diminishes electrical activity of mouse hearts during hypoxia

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Abstract

Insulin resistance, which characterizes type 2 diabetes, is associated with reduced translocation of glucose transporter 4 (GLUT4) to the plasma membrane following insulin stimulation, and diabetic patients with insulin resistance show a higher incidence of ischaemia, arrhythmias and sudden cardiac death. The aim of this study was to examine whether GLUT4 deficiency leads to more severe alterations in cardiac electrical activity during cardiac stress due to hypoxia. To fulfil this aim, we compared cardiac electrical activity from cardiac-selective GLUT4-ablated (G4H−/−) mouse hearts and corresponding control (CTL) littermates. A custom-made cylindrical 'cage' electrode array measured potentials (V_{es}) from the epicardium of isolated, perfused mouse hearts. The normalized average of the maximal downstroke of V_{es}($|dV_{es}/dt_{min}|_{na}$), which we previously introduced as an index of electrical activity in normal, ischaemic and hypoxic hearts, was used to assess the effects of GLUT4 deficiency on electrical activity. The $d|Ves/dt_{min}|_{na}$ of G4H−/− and CTL hearts decreased by 75 and 47%, respectively ($P < 0.05$),30 min after the onset of hypoxia. Administration of insulin attenuated decreases in values of $|d\,Ves/dt_{min}|_{na}$ in G4H−/− hearts as well as in CTL hearts, during hypoxia. In general, however, G4H−/− hearts showed a severe alteration of the propagation sequence and a prolonged total activation time. Results of this study demonstrate that reduced glucose availability associated with insulin resistance and a reduction in GLUT4-mediated glucose transport impairs electrical activity during hypoxia, and may contribute to cardiac vulnerability to arrhythmias in diabetic patients.

Heart disease is the major cause of diabetes-related morbidity and mortality, accounting for more than 70% of deaths in patients with diabetes (Gu *et al.* 1998; Grundy *et al.* 1999). Diabetic patients also show a higher incidence of arrhythmias and sudden cardiac death

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(Curb *et al.* 1995; Balkau *et al.* 1999). Type 2 diabetes, the most common form, is characterized by an impaired response of cells to insulin (insulin resistance), resulting in increased insulin requirements to maintain glucose homeostasis.

Fatty acids provide ~70% of the energy generation in basal conditions in the heart (Taegtmeyer, 1994; Abel, 2004). However, during anoxic conditions, oxidative metabolism of fatty acids is greatly reduced and glucose becomes the predominant fuel for the heart to maintain ATP production by anaerobic glycolysis (Opie, 1976; Liedtke, 1981; Myears *et al.* 1987). Elevated rates of glycolysis are required during anoxia because of the low efficiency of ATP generation by glycolysis relative to aerobic metabolism (Dietrich & Elzinga, 1992).

Glucose transport is an important regulator of myocardial glucose utilization and mediates the increase in glucose uptake that is stimulated by insulin and increased extracellular concentrations of glucose (Morgan *et al.* 1961; Manchester *et al.* 1994; Clow *et al.* 2004). Glucose enters myocytes via glucose transporters GLUT1 and GLUT4 (Mueckler, 1990). GLUT1 predominantly resides in the plasma membrane and plays a major role in basal glucose uptake in quiescent cells (Kraegen *et al.* 1993; Laybutt *et al.* 1997; Young *et al.* 1997; Abel *et al.* 1999; Davey *et al.* 2007). GLUT4, the most abundant glucose transporter in the heart, cycles to the sarcolemma from an intracellular storage compartment in response to contraction, ischaemia or to hormones such as insulin or catecholamines. Insulin, released from the pancreas in response to high blood glucose levels, stimulates GLUT4 translocation, which contributes to its blood glucose-lowering effect (Slot *et al.* 1991; Rett *et al.* 1996; Egert *et al.* 1997; Fischer *et al.* 1997; Russell *et al.* 1998). The underlying mechanisms by which insulin and ischaemia promote GLUT4 translocation are distinct (Rett *et al.* 1996; Egert *et al.* 1997; Russell *et al.* 1999; Bryant *et al.* 2002) and additive (Russell *et al.* 1998). Insulin resistance, which characterizes type 2 diabetes, is associated with reduced translocation of GLUT4 to the plasma membrane following insulin stimulation (Gibbs *et al.* 1995; Desrois *et al.* 2004; Cook *et al.* 2010).

Abel *et al.* (1999) generated mice with myocardium-restricted deletion of GLUT4 (G4H−/−). Abel and coworkers (Abel *et al.* 1999; Tian & Abel, 2001; Abel, 2005) reported impaired insulin-stimulated glucose uptake in G4H−/− hearts. Those features of G4H−/− hearts reflect characteristics of insulin-resistant diabetic hearts. The deteriorated glucose metabolism of G4H−/− hearts resulted in reduced contractile performance. The hearts maintained normal function in basal conditions, but showed systolic and diastolic contractile dysfunction during ischaemia owing to a marked inability to increase glucose transport (Tian & Abel, 2001). GLUT4 deficiency in the heart has also been linked to hypertrophy, impaired excitation–contraction coupling, and altered Ca^{2+} and pH handling (Huggins *et al.* 2008; Domenighetti *et al.* 2010).

Other studies have linked changes in cardiac cellular electrical activity and ATP derived from glycolysis during anoxia, hypoxia and ischaemia (MacLeod & Daniel, 1965; MacLeod & Prasad, 1969; Prasad & MacLeod, 1969; McDonald *et al.* 1971; McDonald & MacLeod, 1971, 1973; Beller *et al.* 1976; Willerson *et al.* 1977; Winston *et al.* 1990; Nakaya *et al.* 1991; Ikenouchi*et al.* 1993; Jansen *et al.* 2003). Therefore, we hypothesized that the deficiency of GLUT4 would lead to dysfunction of cardiac electrical activity.

To test our hypothesis, we used a previously established index of electrical activity developed in our laboratory (Sohn *et al.* 2007, 2009, 2011) to investigate the effects of GLUT4 and insulin on cardiac electrical activity using G4H−/− and corresponding control (CTL)hearts. We measured mouse 64 extracellular surface electrograms from isolated, perfused mouse hearts with a cylindrical electrode array. Using these electrograms, we determined the normalized average of the magnitude of the maximal downstroke of the surface potentials,($|\mathrm{d}V_{es}/\mathrm{d}t_{min}|_{na}$), total activation time (TAT) and the correlation coefficient of propagation sequences (C_{as}). The results of our study show electrical impairment of G4H −/− hearts during hypoxia and may provide insight into−arrhythmogenesis and other electrical consequences in individuals with diabetes, who universally exhibit decreased myocardial glucose utilization (Boudina & Abel, 2007).

Methods

Experimental procedure

The experimental protocol for these studies was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Utah and conforms to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (2010). Female mice at 10–14 weeks of age with cardiac-selective GLUT4 ablation (G4H−/−) or control littermate mice were studied (see Table 1; Abel *et al.* 1999; Tian & Abel, 2001). Mice were injected with 3 units kg^{-1} of heparin and 50 mg kg^{-1} of sodium pentobarbital in sequence at 7 min intervals, and more sodium pentobarbital (25 mg kg^{-1}) was injected as needed. After a deep plane of anaesthesia had been attained, the heart was rapidly excised and Langendorff perfused with a modified Krebs solution, which was composed of (mM): NaCl, 118; $NaHCO_3$, 22; KCl, 4.1;$CaCl_2$, 2.5; $MgSO_4$, 1.2; EDTA, 0.5; and KH_2PO_4, 1.2. The perfusate was bubbled with a mixture of 95% O_2 and 5% CO_2 for 100% oxygen saturation. For hypoxic solutions, the concentration of $NaHCO_3$ was lowered to adjust the pH to 7.4, the perfusate was bubbled with a mixture of 95% N_2 and 5% CO_2, and the temperature was maintained at 37°C. A cylindrical ‘cage’ electrode array fabricated in our laboratory, as shown in Fig. 1, was described in detail previously (Sohn *et al.* 2007, 2011). This electrode array was slipped over the perfused mouse hearts to measure 64 unipolar epicardial surface potentials referenced to an electrode positioned at the top of the anterior mid-line of the heart. The heart and electrode array were enclosed inside a custom-made Plexiglass chamber, in which the ambient temperature was maintained at 37°C. During hypoxia, a hypoxic solution with 25 mmHg measured P_{O2} and lacking insulin and glucose was continuously supplied through a syringe bathing the surface of the heart to minimize differences that may occur in oxygen penetration in the tissue (Sohn *et al.* 2009).

Protocols involved varying concentrations of glucose (0 and 10 mM) and insulin (0 and 2 units l^{-1}) and P_{O2} within the perfusate. There were six experimental conditions labelled as follows: i2_CTL, i2_G4H−/−i0_CTL, i0_G4H−/−, g0_CTL and g0_G4H−/−, where ‘CTL’ represents control, i2 and i0 represent−2−units l^{-1} and no insulin, respectively, and g0 represents absence of glucose in the perfusate. Studies were conducted in CTL and G4H−/− hearts in the presence and absence of insulin during hypoxia (i2_CTL, i0_CTL, i2_G4H−/− and i0_G4H−/−). Additional studies were conducted in normoxic CTL and G4H−/− the

hearts that were studied in absence of glucose (g0_CTL and g0_G4H−/−). Each experimental procedure consisted of the following four parts: heart cannulation and stabilization (1 h); baseline perfusion (30 min); induction of hypoxia or glucose deprivation (30 min); and reoxygenation or glucose (30 min; see Table 2).

Data acquisition and analysis

Cardiac extracellular potentials from 64 electrodes were simultaneously recorded, amplified and digitized at 4 kHz with 12-bit resolution, and stored on a Macintosh computer. The activation time at each electrode was determined as the time of the minimal slope of the potentials (Punske *et al.* 2003). To quantify changes in action potential propagation sequence over the epicardial surface, we defined the correlation coefficient of action potential propagation sequences (C_{as}) as the Pearson product–moment correlation coefficient of two activation maps from the end of baseline and other times during experimental interventions. Total activation time (TAT) was defined by the time difference between the latest activation time and the earliest activation time recorded from the 64 electrodes; TAT_n denotes TAT normalized to the value at the end of baseline. Values of $\mathrm{d}V_{es}/\mathrm{d}t_{min}$ at each electrode were normalized to the corresponding $|\mathrm{d}V_{es}/\mathrm{d}t_{min}|$ value at the end of baseline recordings and denoted as $|\mathrm{d}V_{es}/\mathrm{d}t_{min}|_n$, and all values were averaged to yield $|\mathrm{d}V_{es}/\mathrm{d}t_{min}|_{na}$. Signals were quality checked and eliminated based on two criteria; if the standard deviation of baseline $|\mathrm{d}V_{es}/\mathrm{d}t_{min}|_n$ values of a channel exceeded 2.2 or if $|\mathrm{d}V_{es}/\mathrm{d}t_{min}|_n$ of the channel exceeded 1.5 during experiments. Heart rates (HR) were normalized to the value at the end of baseline and are indicated as HR_n.

Statistics

Quantitative results are expressed as means ± SEM. A linear mixed model was applied for statistical comparisons of $|\mathrm{d}V_{es}/\mathrm{d}t_{min}|_{na}$, because values of $|\mathrm{d}V_{es}/\mathrm{d}t_{min|n}$ from electrode sites were correlated with each other. Compound symmetry covariance structure and maximal likelihood were used in the application of the linear mixed model (Littell *et al.* 2006). Statistical analysis of other indexes, such as C_{as}, TAT_n and HR_n, was performed by oneway ANOVA. Statistical significance was determined by $P < 0.05$.

Results

Statistical data on our experimental cohorts and baseline values are presented in Table 1. Consistent with earlier reports, the weight of G4H−/− hearts was significantly greater than that of control hearts (Abel *et al.* 1999). Values of $|\mathrm{d}V_{es}/\mathrm{d}t_{min}|$ (in volts per second), TAT (in milliseconds) and HR (in beats per minute) showed no statistically significant differences during baseline conditions for all experiments (Table 1).

Representative potentials at the time of activation for each set of experimental conditions are shown in Fig. 2. Signal morphology typically consisted of an upstroke followed by downstroke. The G4H−/− hearts showed noticeable changes in signal morphology during hypoxia compared with control hearts. In control hearts, the amplitudes of the 64 extracellular potentials decreased during hypoxia, but each potential still showed a clear downstroke with detectable time of activation. In G4H−/− hearts, however, there were not

only larger decreases in the amplitudes but also profound alterations in the signal morphology during hypoxia and the absence of insulin-accentuated decreases in $|dV_{es}/dt_{min}$ and alterations in the signal morphology. For the example in Fig. 2A, the value of $|dV_{es}/dt_{min}$ for i2_CTL decreased by 24% during hypoxia but recovered close to the baseline values during reoxygenation. In the absence of insulin, control hearts (i0_CTL) showed a larger decrease in $|dV_{es}/dt_{min}|$ during hypoxia, and the values did not return to baseline during reoxygenation (Fig. 2B). In i2_G4H/morphologies hearts during hypoxia, electrogram changed from the standard upstroke followed by a downstroke to having multiple peaks, but in general the altered morphology of these potentials recovered to prehypoxia morphologies during reoxygenation (Fig. 2C). The $|dV_{es}/dt_{min}$ decreased by 56% during hypoxia and did not return to the prehypoxic level during reoxygenation (Fig. 2C). For G4H−/− hearts perfused without/insulin (i0_G4H−/−) during hypoxia, all signals showed profound decreases in amplitude and $|dV_{es}/dt_{min}$. A majority of electrodes showed no clear activation times, with some electrodes having multiple downstrokes and altered morphology. This altered morphology and the values of $|dV_{es}/dt_{min}$ did not recover during reoxygenation.

Mean values and standard error of $|dV_{es}/dt_{min|na}$ from multiple hearts as a function of time during hypoxia are shown in Fig. 3. Initially, values of $|dV_{es}/dt_{min\ na}$ decreased for all four cohorts at the onset of hypoxia. |After several minutes of hypoxia, $|dV_{es}/dt_{min}|_{na}$ values for the G4−/− groups were lower than the CTL groups. In the absence of insulin, values of $|dV_{es}/dt_{min\ |na}$ were significantly lower for both CTL and G4H−/− groups (open circles and open triangles). The $dV_{es}/dt_{min|na}$ for i2_CTL, i0CTL, i2_G4H−/− and i0_G4H−/− decreased to 0.75, 0.53, 0.38and 0.25 at the end of hypoxia, and recovered to 0.82, 0.73,0.58 and 0.37 at the end of reoxygenation, respectively.

Typical examples of activation time maps for each heart type before, during and after hypoxia are shown in Fig. 4. Typically, the base of the right ventricle was the last part to activate and exhibited the slowest surface propagation speed. To quantify alterations in activation sequence, C_{as} was computed by comparing maps generated during hypoxia and reoxygenation with baseline maps. The maps of propagation sequence for i2_CTL and i0_CTL hearts remained qualitatively consistent throughout the experimental protocol, with small differences in C_{as} (Fig. 4A and B). For G4H−/−hearts, propagation sequences changed markedly by−the−end of hypoxia, without recovery of the baseline sequence during reoxygenation. Values of TAT are listed for each map, indicating the increased propagation time associated with hypoxia.

Significant differences in $|dV_{es}/dt_{min}|_{na}$ values between G4H−/− and corresponding control hearts (i2_G4H−/−*versus* i2_CTL and i0_G4H−/− *versus* i0_CTL, $P < 0.05$) at the end of hypoxia and reoxygenation are indicated in Fig. 5. Insulin availability created significant differences in values of $|dV_{es}/dt_{min}|_{na}$ measured at the end of hypoxia for each heart type (i2_G4H−/− *versus* i0_G4H−/− and i2_CTL *versus* i0_CTL, $P < 0.05$; Fig. 5A). The decrease of C_{as} was closely related to the decrease of $|dV_{es}/dt_{min\ |na}$, and there were significant differences in C_{as} values between G4H−/− and corresponding CTL hearts at the ends of hypoxia and reoxygenation (Fig. 5B). The increase of TAT_n was also related to the decrease of $|dV_{es}/dt_{min|na}$ during hypoxia. Values of TAT_n usually recovered to baseline by

the end of reoxygenation, except in the case of i0_G4H−/− (Fig. 5C). The HR_n appeared to be independent of heart type and insulin availability, decreasing by ~54% after the onset of hypoxia and recovering to the baseline levels at the onset of reoxygenation (Fig. 5D).

The G4H−/− hearts have previously been shown to have higher basal glycogen levels compared with CTL hearts, as a compensatory effect (Tian & Abel, 2001). To investigate the influence of the higher glycogen levels in G4H−/− hearts, perfusate with no glucose was administered for 30 min (Table 2; g0_G4H−/− and g0_CTL). The CTL hearts showed significantly lower at $|dV_{es}/dt_{min}|_n$ and C_{as} values than G4H−/− hearts the end of glucose suspension and glucose resupply (Fig. 6). The CTL hearts also showed much larger increases in TAT_n than G4H−/− hearts. Values of HR_n for both G4H−/− and CTL hearts decreased by ~20% in the absence of glucose and recovered after glucose was resupplied, showing no significant differences.

Discussion

The present study reveals a prominent role for GLUT4 in the maintenance of proper electrical responses during hypoxic stress. Previous studies have shown mechanical consequences of impaired glucose transport during stress (Tian & Abel, 2001; Huggins *et al.* 2008; Domenighetti *et al.* 2010). Our study shows that the electrical activity of the heart also depends greatly on the ability to transport glucose during hypoxia. We tested the effects of the absence of GLUT4 on electrical activity during hypoxia using control and G4H−/− mouse hearts. This study demonstrated greater alterations in the electrical activity of G4H−/− hearts *versus* control hearts during hypoxia. Both $|dV_{es}/dt_{min}|_{na}$ and C_{as} of G4H−/− hearts were significantly lower than for CTL hearts at the end of hypoxia and even at the end of reoxygenation.

The fast inward sodium current (I_{Na}) generates the upstroke of the action potential, and our previous study suggested that decreases in $|dV_{es}/dt_{min}$ n are related to decreases in I_{Na} (Sohn *et al.* 2011). |A reduction in intracellular pH, as occurs during hypoxia, leads to sodium channel inactivation (Nonner *et al.* 1980) and was reflected in the reduced values of $|dV_{es}/dt_{min}|_{na}$ measured during hypoxia in both G4H−/− and CTL hearts. For G4H−/− hearts, where impaired glucose transport reduces available energy for ionic homeostasis via Na^+–K^+-ATPase, Na^+–Ca^{2+} exchanger and Na^+–H^+ exchanger (Barth & Tomaselli, 2009), reduced extrusion of H^+ from the cells may exacerbate Na^+ accumulation and inactivation, as revealed in the lower measured values of $|dV_{es}/dt_{min|na}$.

Changes in| C_{as} represented alterations in activation sequence. In i0_G4H−/− hearts, $|dV_{es}/dt_{min}|_n$ decreased by 75%, C_{as} reduced to 0.07, TAT_n increased threefold,| and the morphology of the potential signals frequently showed multiple downstrokes during hypoxia. These suggested decreases in I_{Na} and regional inactivation, which lead to slow conduction, conduction block and activation sequence change.

Given that insulin stimulates GLUT4 translocation, we expected to observe improved electrical activity in CTL hearts as measured by $|dV_{es}/dt_{min}|_n$ at the end of hypoxia in the presence of insulin.| We also observed that the electrical activity of G4H−/− hearts was

improved by insulin. The $|dV_{es}/dt_{min}|_n$ of G4H−/− hearts was significantly improved at the end of hypoxiaes and reoxygenation by administration of insulin. Without insulin, we observed more frequent atrioventricular conduction blocks, premature ventricular activations and signs of local conduction block. It has previously been demonstrated that there are no changes in the rate of glucose uptake in G4H−/−hearts after insulin administration (Tian & Abel, 2001), which suggests that the improved electrical activity in G4H−/− hearts in the presence of insulin is not due to increased glucose transport. Insulin stimulates not only glucose transport but also glycogen synthesis in CTL hearts. While G4H−/− hearts might not increase glucose transport in response to insulin, the increase in glycogen content could contribute to the improved electrical activity. In addition, insulin also has direct effects on the cardiac electrophysiology, such as L-type Ca^{2+} current (Aulbach *et al.* 1999) and the Na^{+}–Ca^{2+} exchange (Villa-Abrille *et al.* 2008). The enhanced electrical activity of G4H −/− hearts with insulin may therefore result from the direct effect of insulin on the electrical activity rather than improved energy metabolism.

Abel and co-workers (Abel *et al.* 1999; Tian & Abel, 2001) reported a threefold increase in GLUT1 expression and 54% higher glycogen content in G4H−/− hearts relative to CTL in basal conditions. The increased GLUT1 expression yields a threefold increase in the rate of glucose transport. The effect of increased glycogen in G4H−/− hearts may be responsible for significantly higher values of $|dV_{es}/dt_{min}|_{na}$ and C_{as} when exogenous glucose was not supplied (Fig. 6). Tian & Abel (2001) reported that 20 h of fasting removed the compensatory effects of increased glycogen levels in G4H−/− hearts. We also fasted both G4H−/− and CTL mice For examine 20 h to the effects of depleted glycogen on the electrical activity (data not shown). We found that the majority of the fasted G4H−/− hearts stopped beating and could not provide viable data during hypoxia. This demonstrated the crucial role of GLUT4 during hypoxia. Taken together, these results support the hypothesis that reduced glucose availability may contribute to cardiac vulnerability to arrhythmias in diabetic patients with insulin resistance, many of whom develop reduced GLUT4-mediated glucose transport. In addition to the increased glycogen levels and hypertrophy reported in G4H−/− hearts, partial and total atrioventricular block were also−frequently observed during hypoxia in these studies. These observations provide a phenotype that shares many similarities to Wolff–Parkinson–White syndrome with glycogen storage disease (Light, 2006).

Heart rates did not show a strong dependence on available energy or metabolism like the other parameters, $|dV_{es}/dt_{min}|_n$, C_{as} and TAT_n. Heart rate decreased by ~45% quickly at the onset of hypoxia and recovered immediately following reoxygenation regardless of genotype or insulin administration (Fig. 6). Likewise, when glucose supply was arrested without hypoxia (Fig. 6), there were significant differences in $|dV_{es}/dt_{min}|_n$ and C_{as} values between G4H−/− and CTL, However HR_n values of G4H−/−and CTL hearts were virtually identical.

The epicardial surface potentials were measured using a custom-made cylindrical electrode cage. This method of measurement does not damage or restrict the heart. In addition, this method eliminates the need for voltage-sensitive dyes and electromechanical uncouplers or other methods to reduce the contraction of the heart as required for optical mapping techniques. Di-4-ANEPPS, a commonly used dye in optical mapping studies, has been

shown to alter cardiac electrophysiology, including prolongation of the PQ interval and QRS duration, and reduction of conduction velocity (Nygren *et al.* 2003; Larsen *et al.* 2010, 2012). Likewise, excitation–contraction uncouplers, such as 2,3-butanedione monoxime, cytochalasin D and diacetyl monoxime, frequently used for optical mapping have been shown to have effects on action potential duration, conduction velocity, electrical restitution curve, calcium transient and ventricular fibrillation activation patterns (Qin *et al.* 2003; Baker *et al.* 2004; Cheng *et al.* 2004; Kettlewell *et al.* 2004). Additional evidence has shown that uncouplers affect metabolism, where the administration of 2,3-butanedione monoxime before or after ischaemia increased myocardial ATP and reduced myocardial injury (Habazettl *et al.* 1996; Moriguchi *et al.* 2010). Hence, the methods used in this study offer the advantage of allowing the heart to contract freely and do not alter characteristics of energy metabolism or electrophysiology. However, the cylindrical shape of the electrode array has limitations in that it does not fit the exact shape of the heart, allowing variable contact for the electrodes, and the fixed size of the cage limits the heart size used for optimal contact with the electrodes. The i0_CTL and i0_G4H−/− mice were slightly older than the i2_CTL and i2_G4H−/− mice. However, we confirmed that the parameters−we−used ($|\mathrm{d}V_{es}/\mathrm{d}t_{min}|_{na}$ C_{as}, TAT_n and HR_n) did not show any clear dependence on the age within the animals used. Our study investigated only global hypoxia. Although partial and total atrioventricular block occurred frequently in this study, ventricular tachycardia and ventricular fibrillation were very rare. Regional anoxic/ischaemic conditions may generate more frequent ventricular tachycardia and ventricular fibrillation by creating electrophysiological heterogeneity in larger hearts.

The experimental design for these studies, while maintaining simplicity, yielded a fundamental relationship between glucose transport and electrical activity, thus warranting further studies that can begin to address the more complex nature of the diabetic condition. A limitation to this work was the choice to provide only glucose as the energy substrate to reduce contributions from allosteric regulation. Glucose becomes the predominant fuel for the heart during hypoxia/ischaemia due to anaerobic ATP production by glycolysis and was therefore the primary target in these studies. The availability of fatty acids may modulate the electrical response through additional energy reserve. Pyruvate has been shown to protect the myocardium from ischaemia–reperfusion injury by providing an energy reserve and suppressing oxidative stress (Crestanello *et al.* 1995, 1998; Mallet, 2000; Arya *et al.* 2006). Studies have also shown that exogenous lactate during ischaemia may have adverse effects, such as increased intracellular lactate concentration and decreased glycolysis (Saman & Opie, 1984; Cross *et al.* 1995). Other studies showed a protective effect of externally supplied lactate against ischaemic injury (Doenst *et al.* 1996; Tokuno *et al.* 1999). Future investigations are required to determine whether substrate competition has a significant impact on the electrical activity.

The use of hypoxia to stress the heart reduces the complexities of electrical responses by eliminating the accumulation of potassium and metabolic wastes in the extracellular space that are associated with ischaemia. Our results show that the evaluation of ischaemia and the direct measurement of ionic currents during ischaemia are now warranted.

In conclusion, this study demonstrated the vital role of GLUT4 in maintaining cardiac electrical activity during hypoxia, and illustrates the direct link between metabolism and electrical function. Given the reduction in GLUT4-mediated glucose uptake in the hearts of patients with diabetes, these findings may provide additional insight into arrhythmic complications in this high-risk population, particularly in the context of ischaemia.

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New Findings

What is the central question of this study?

The aim of this study was to examine quantitatively whether glucose transporter 4 deficiency leads to more severe alterations in cardiac electrical activity during cardiac stress.

What is the main finding and what is its importance?

When compared with hearts from corresponding control littermates, the measured epicardial potentials from the surface of cardiac-selective glucose transporter 4-ablated mouse hearts during hypoxia showed the following differences: (i) significant decreases in the maximal downstroke of the potentials; (ii) increased activation time; and (iii) greater alterations in the activation sequence.

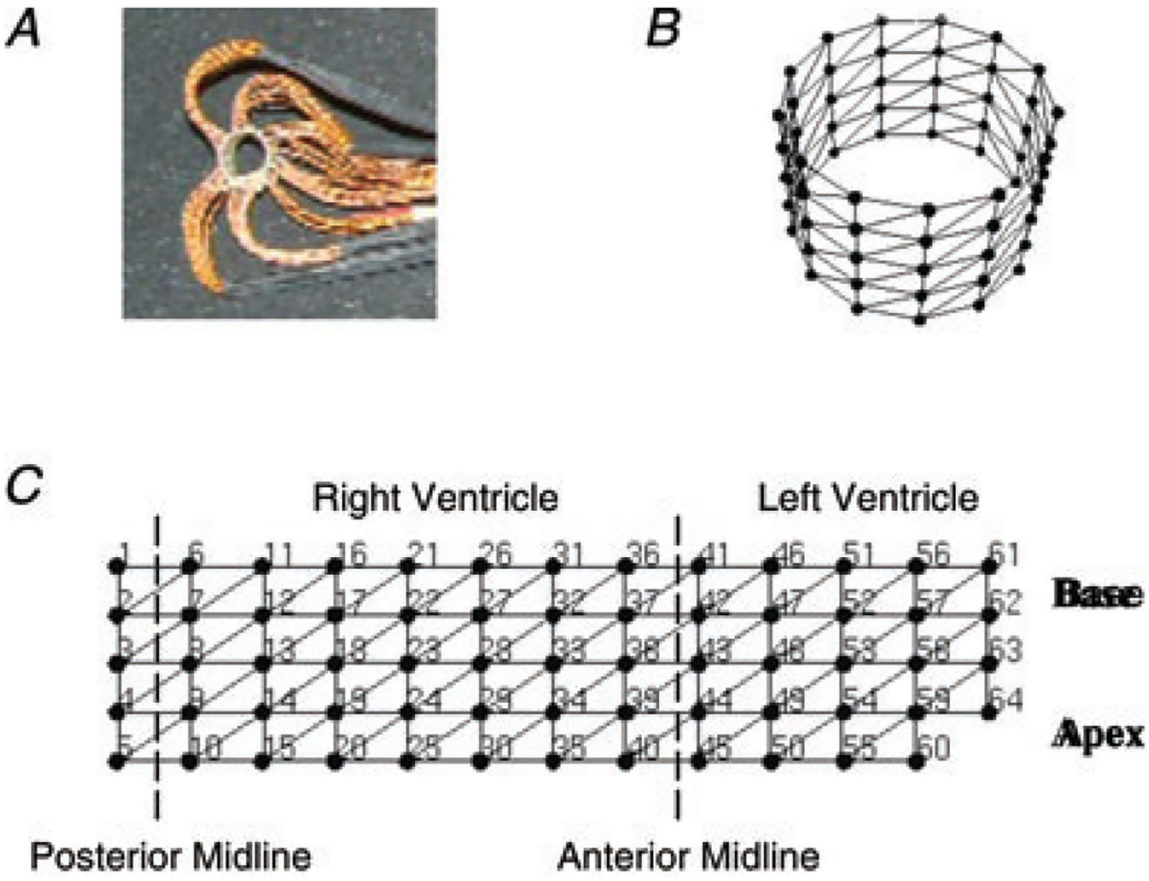

Figure 1. Cylindrical 64-electrode array

A, photograph of the electrode array. *B*, the triangulated mesh geometry of the electrode array in three dimensions. The electrode array formed a cylinder 8.03 mm in diameter and 5.16 mm in length. *C*, two-dimensional triangulated mesh of the electrode array with electrode numbers. Five electrodes were arranged in each column with 1.29 mm interelectrode distance, and 13 columns were arranged circumferentially with 1.94 mm interelectrode spacing.

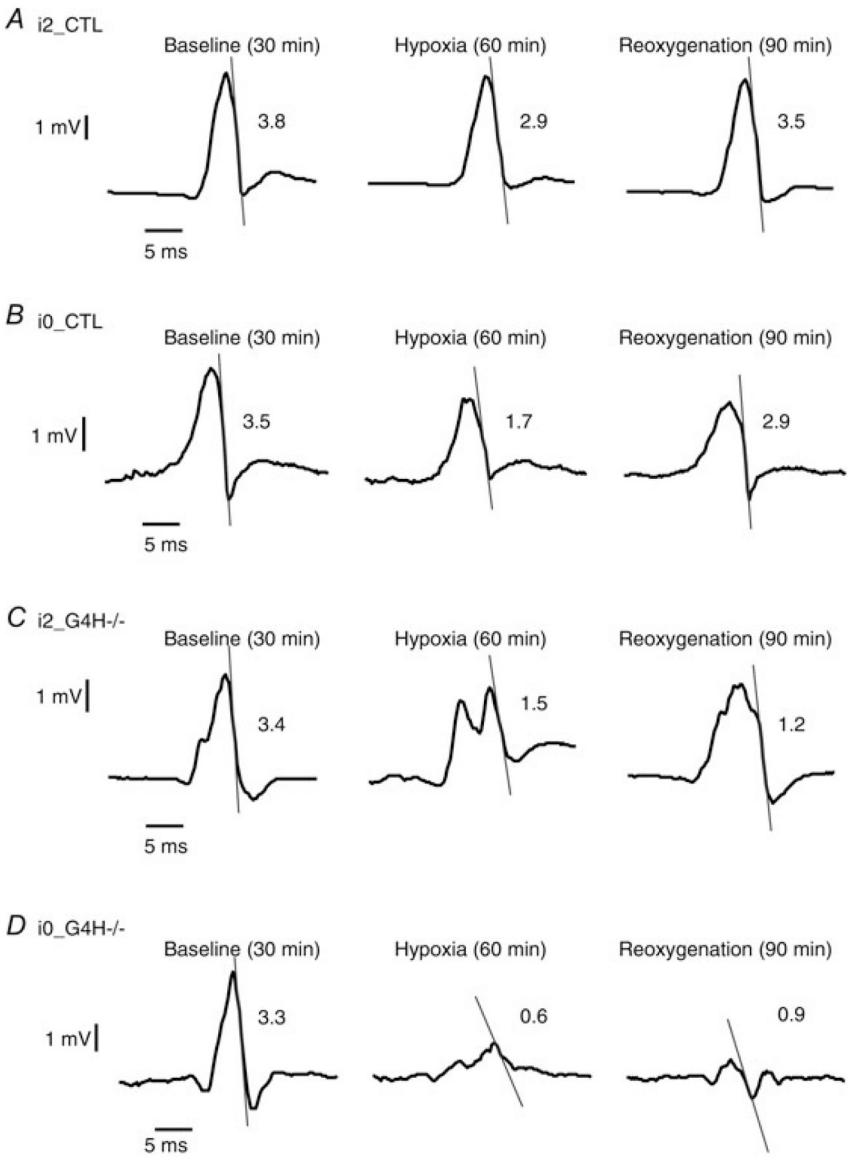

Figure 2.

Typical examples of recorded potentials with $|\mathrm{d}V_{es}/\mathrm{d}t_{min}$ (magnitude of the maximum downstroke of the surface potentials) marked by tangential lines and| values indicated (in volts per second) during baseline conditions, during hypoxia and during reoxygenation for each cohort

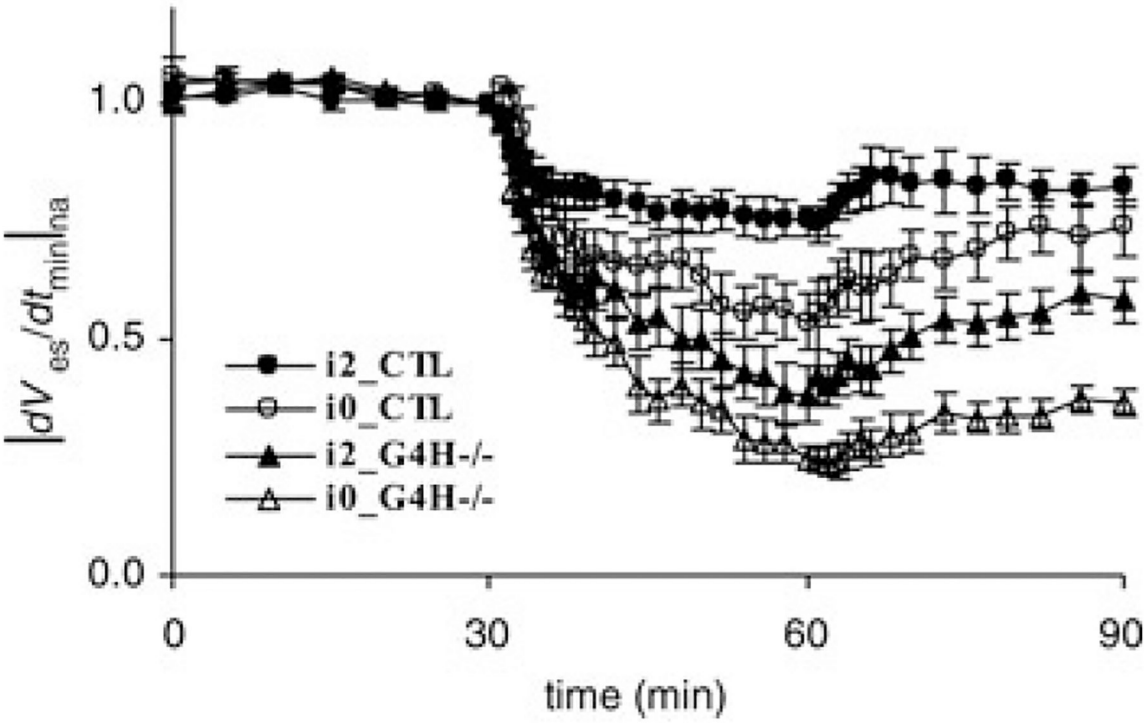

Figure 3.

Mean values and standard error of $|\mathrm{d}V_{es}/\mathrm{d}t_{min}$ na(normalized average of the magnitude of the maximum | downstroke of the surface potentials) as a function of time during hypoxia for each cohort

Times are as follows: 0–30 min, baseline; 30–60 min, hypoxia; and 60–90 min, reoxygenation.

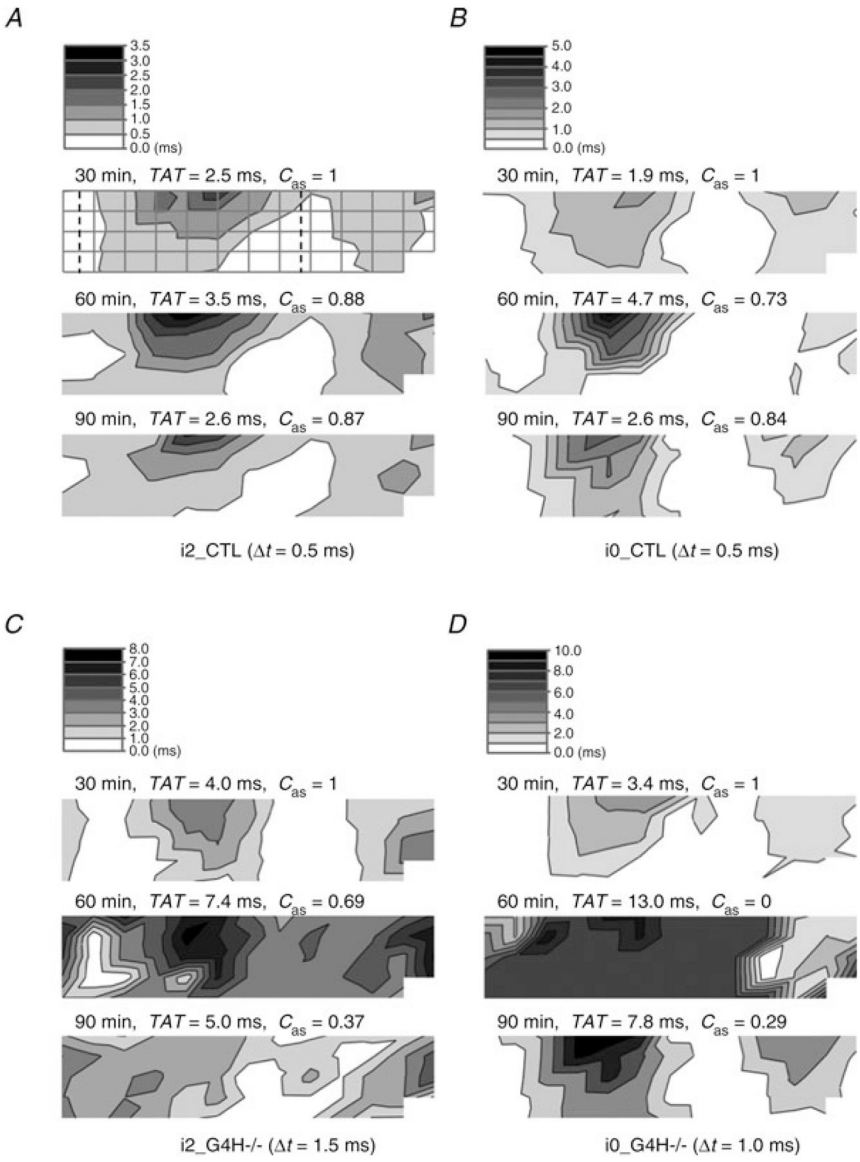

Figure 4. Activation time maps for selected hearts during hypoxia

For each set of experimental conditions, activation time maps are displayed at the end of baseline (30 min), at the end of hypoxia (60 min) and at the end of reoxygenation (90 min). The total activation time (TAT) and correlation coefficient of action potential propagation sequences (C_{as}) are also listed above the corresponding activation time map. The electrode positions as shown in Fig. 1C are overlaid on the first map in *A*. White indicates early activation and black indicates late activation.

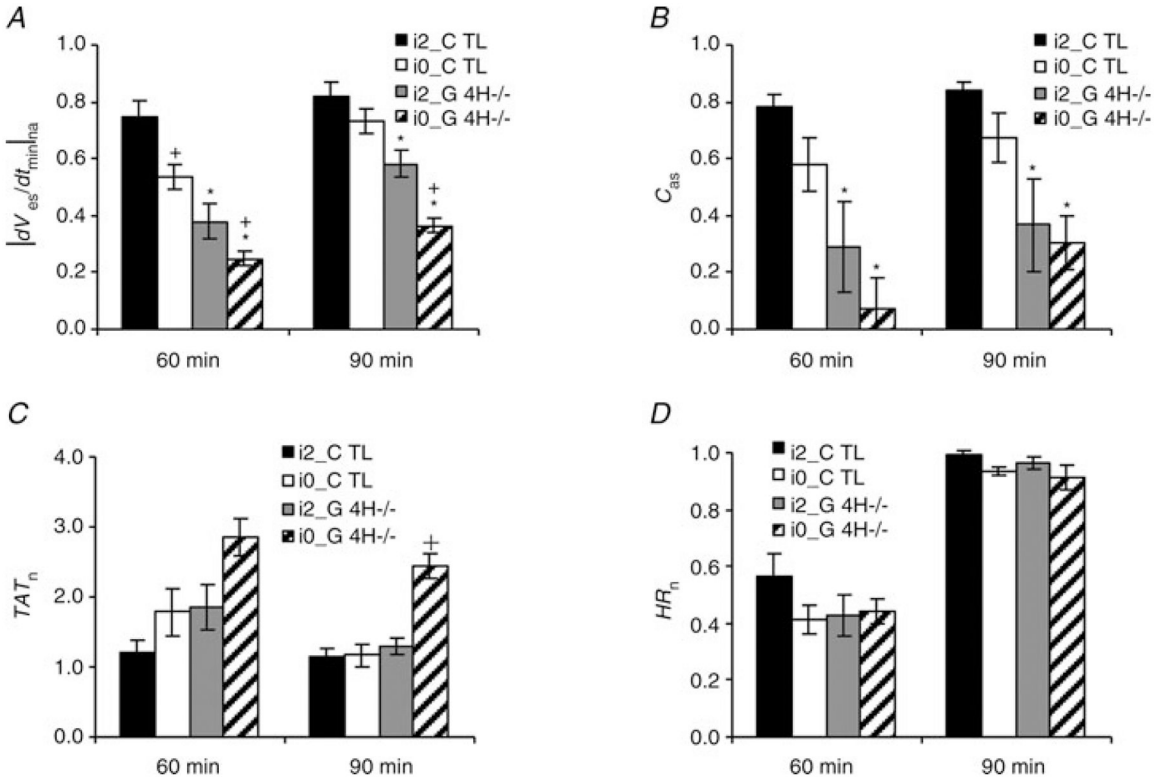

Figure 5.

Values of $|\mathrm{d}V_{es}/\mathrm{d}t_{min}|_{na}$ (*A*), C_{as} (*B*), TAT_n (*C*) and normalized heart rate (HR_n) (*D*) at the end of hypoxia (60 min) and at the end of reoxygenation (90 min)

**P* < 0.05 for G4H−/− *versus* CTL. +*P* < 0.05 for with *versus* without insulin.

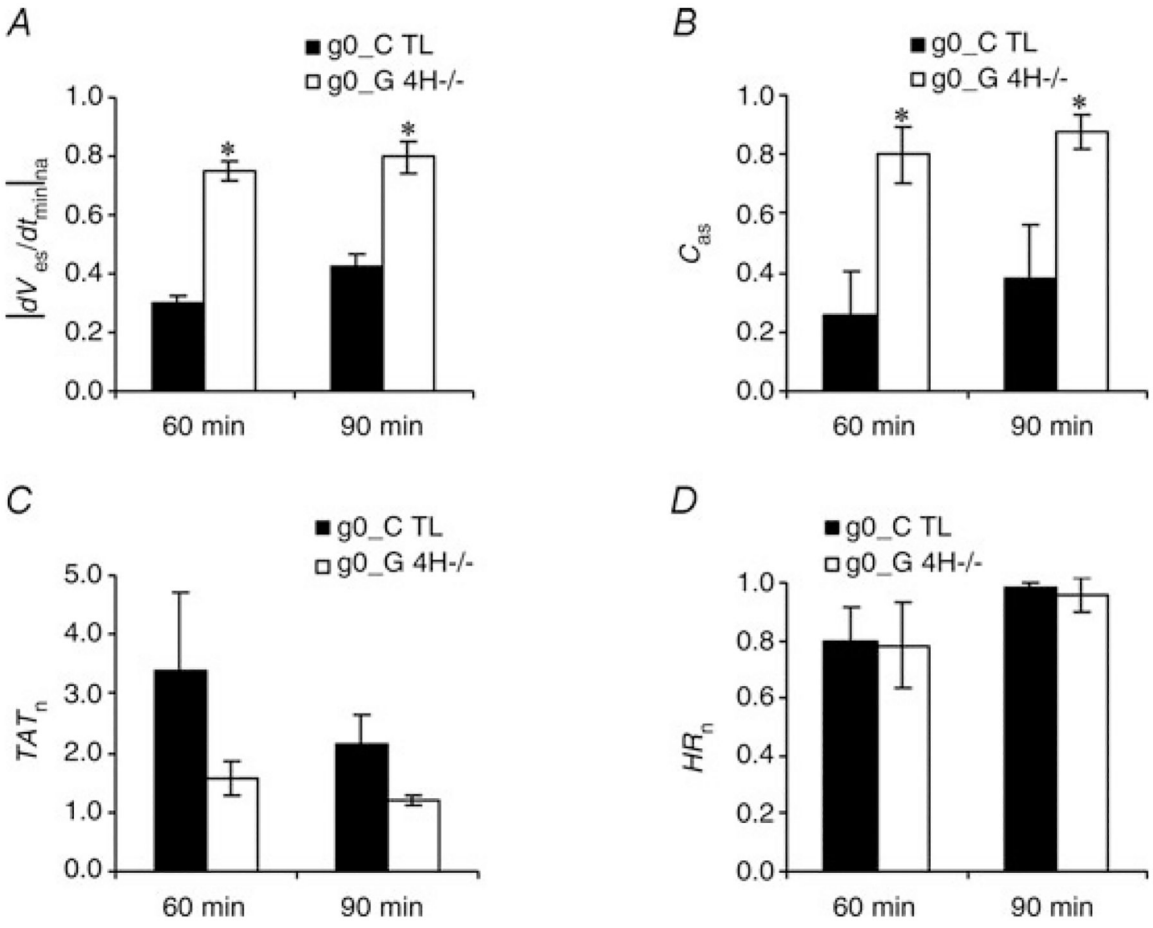

Figure 6.

Values of $|\mathrm{d}V_{es}/\mathrm{d}t_{min}|_{na}$ (*A*), C_{as} (*B*), TAT_n (*C*) and HR_n (*D*) at the end of glucose withdrawal (60 min) and at the end of glucose resupply (90 min)

$^{*}P < 0.05$ *versus* corresponding CTL.

Table 1.

General characteristics of the animals studied and values for $|\mathrm{d}V_{es}/\mathrm{d}t_{min}$ (magnitude of the maximum downstroke of the surface potentials, in volts per second), total activation time (in milliseconds) and heart rate (in beats per minute) for each cohort during baseline conditions prior to intervention

Parameter	i2_CTL	i0_CTL	i2_G4H−/−	i0_G4H−/−	g0_CTL	g0_G4H−/−
No. of mice	7	10	6	9	4	4
Age (weeks)	10.8 ± 0.7	12.8 ± 0.3†	11.6 ± 0.6	13.2 ± 0.4†	11.5 ± 0.3	10.9 ± 0.1
Body weight (g)	19.3 ± 0.7	21.9 ± 0.7†	20.2 ± 1.0	21.3 ± 0.7	20.8 ± 1.1	20.4 ± 1.3
Heart weight (mg)	126 ± 6	132 ± 8	160 ± 13*	161 ± 9*	117 ± 5	182 ± 12*
$\lvert \mathrm{d}V_{es}/\mathrm{d}t_{min}\rvert$ (V s^{-1})	4.0 ± 0.4	3.9 ± 0.2	4.8 ± 1.0	4.1 ± 0.3	3.7 ± 0.3	4.0 ± 0.5
Total activation time (ms)	3.1 ± 0.4	2.7 ± 0.5	3.4 ± 0.2	4.1 ± 0.7	3.2 ± 0.4	4.2 ± 0.4
Heart rate (beats min^{-1})	374 ± 12	361 ± 10	363 ± 9	368 ± 11	367 ± 13	343 ± 13

*$P < 0.05$ *versus* control (CTL).

†$P < 0.05$ *versus* with insulin.

Table 2.

Experimental protocols

		0–30 min	30–60 min	60–90 min
P_{O2}	i2_CTL	>550 mmHg	~19 mmHg	>550 mmHg
	i0_CTL	>550 mmHg	~19 mmHg	>550 mmHg
	i2_G4H−/−	>550 mmHg	~19 mmHg	>550 mmHg
	i0_G4H−/−	>550 mmHg	~19 mmHg	>550 mmHg
	g0_CTL	>550 mmHg	>550 mmHg	>550 mmHg
	g0_G4H−/−	>550 mmHg	>550 mmHg	>550 mmHg
Insulin	i2_CTL	2 units l^{-1}	2 units l^{-1}	2 units l^{-1}
	i2_G4H−/−	2 units l^{-1}	2 units l^{-1}	2 units l^{-1}
	i0_CTL	No insulin	No insulin	No insulin
	i0_G4H−/−	No insulin	No insulin	No insulin
	g0_CTL	No insulin	No insulin	No insulin
	g0_G4H−/−	No insulin	No insulin	No insulin
Glucose	i2_CTL	10 mM	10 mM	10 mM
	i0_CTL	10 mM	10 mM	10 mM
	i2_G4H−/−	10 mM	10 mM	10 mM
	i0_G4H−/−	10 mM	10 mM	10 mM
	g0_CTL	10 mM	No glucose	10 mM
	g0_G4H−/−	10 mM	No glucose	10 mM

The G4H−/− and control (CTL) hearts in the presence and absence of glucose and insulin were tested during 30 min of hypoxia ($P_{O2} \approx 19$ mmHg). Additional protocols looked separately at the effects of insulin and glucose on electrical activity.

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