Improvement of the metabolic status recovers cardiac potassium channel synthesis in experimental diabetes

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Abstract

Aims: The fast transient outward current, \( I_{\text{to,fast}} \), is the most extensively studied cardiac \( K^+ \) current in diabetic animals. Two hypotheses have been proposed to explain how type-1 diabetes reduces this current in cardiac muscle. The first one is a deficiency in channel expression due to a defect in the trophic effect of insulin. The second one proposes flawed glucose metabolism as the cause of the reduced \( I_{\text{to,fast}} \). Moreover, little information exists about the effects and possible mechanisms of diabetes on the other repolarizing currents of the human heart: \( I_{\text{to,slow}} \), \( I_{\text{Kr}} \), \( I_{\text{Ks}} \), \( I_{\text{Kur}} \), \( I_{\text{Kslow}} \) and \( I_{\text{K1}} \).

Methods: We recorded cardiac action potentials and \( K^+ \) currents in ventricular cells isolated from control and streptozotocin- or alloxan-induced diabetic mice and rabbits. Channel protein expression was determined by immunofluorescence.

Results: Diabetes reduces the amplitude of \( I_{\text{to,fast}} \), \( I_{\text{to,slow}} \) and \( I_{\text{Kslow}} \) in ventricular myocytes from mouse and rabbit, with no effect on \( I_{\text{ss}} \), \( I_{\text{Kr}} \) or \( I_{\text{K1}} \). The absence of changes in the biophysical properties of the currents and the immunofluorescence experiments confirmed the reduction in channel protein synthesis. Six-hour incubation of myocytes with insulin or pyruvate recovered current amplitudes and fluorescent staining. The activation of AMP-K reduced the same \( K^+ \) currents in healthy myocytes and prevented the pyruvate-induced current recovery.

Conclusion: Diabetes reduces \( K^+ \) current densities in ventricular myocytes due to a defect in channel protein synthesis. Activation of AMP-K secondary to deterioration in the metabolic status of the cells is responsible for \( K^+ \) channel reductions.

Keywords: AMPK, heart, insulin, metabolism, pyruvate, repolarization.

Diabetic cardiomyopathy is commonly associated with impaired contractility and electrocardiographic abnormalities. Most of the electric disorders are related to cardiac repolarization, such as prolonged QTc interval and increased QT dispersion, even in patients who have suffered diabetes for brief period (reviewed in Casis & Echevarria 2004). These alterations can be the cause of the higher incidence of complex arrhythmias and sudden death that have been observed in patients with diabetes mellitus (Grossmann et al. 1997, Robillon et al. 1999, Shiono et al. 2001, Tu et al. 2010).
The electrocardiographic alterations are due to a diabetes-induced prolongation of cardiac action potential duration (APD) secondary to impairments in potassium repolarizing currents (Magyar et al. 1992, Casis et al. 2000, Zhang et al. 2006). Experimental studies show that different repolarizing currents are reduced depending on the animal species used for the induction of type-1 diabetes. In the human heart, ventricle, the main ventricular repolarizing currents are the transient outward, the slow transient outward, the rapid delayed rectifier, the slow delayed rectifier and the sustained K+ currents (I_{to,fast}, I_{to,slow}, I_{Kr}, I_{ks} and I_{ks} respectively), whereas in the atria, they are the I_{to,fast}, the ultrarapid (I_{Kur}) and the I_{Kslow} currents (Beuckelmann et al. 1993, Li et al. 1996, Spector et al. 1996, Pond et al. 2000, Van Wagoner & Nerbonne 2000).

I_{to,fast} has been the most extensively studied K+ current in diabetic animals. Two hypotheses have been proposed to explain how type-1 diabetes alters changes in this potassium current in cardiac muscle. The first hypothesis involves insulin deficiency, as it affects gene expression of a large number of proteins (O’Brien & Granner 1996) including potassium channels. In support of this hypothesis, incubation of diabetic cardiac myocytes with insulin for 6 h restores I_{to,fast} current to control values, an effect that is blocked by inhibitors of protein synthesis (Shimoni et al. 1999). The second hypothesis postulates that the cause of the reduced cardiac I_{to,fast} in diabetes mellitus is the flawed glucose metabolism. This second hypothesis is supported by the fact that in diabetic cardiomyocytes, 6-h incubation with metabolism improvers such as L-carnitine or glutathione recovers the potassium currents (Xu et al. 1996b, 2002). Both trophic and metabolic mechanisms may require an increase in the expression of I_{to,fast} channel forming proteins, as current amplitude is the only biophysical parameter affected, and current recovery is complete after a delay of several hours.

Moreover, there is no information about the possible mechanisms involved in the consequences of diabetesthe on the other repolarizing currents. Here, we have used the streptozotocin-induced model of diabetes mellitus in mouse ventricular myocytes to explore the effects of diabetic cardiomyopathy on K+ repolarizing currents, including I_{to,fast}, I_{to,slow}, I_{Kslow} and I_{ks} K+ repolarizing currents. In addition, we explored the effects of diabetes on I_{to,slow}, I_{Ks} and I_{K1} were explored in the alloxan-induced model of diabetes in rabbit ventricular myocytes. We also explored the possible mechanism of these effects.

**Materials and methods**

The investigation fulfils the Spanish (RD 1201/2005) and European (D2003/65/CE and R2007/526/CE) Guidelines for the Care of Animals Used in Experimental and other Research Purposes, it also conforms to the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health and has been approved by the Ethics Committee for Animal Care of the Universidad del País Vasco and the Universidad de Colima.

Animals were obtained from the Animal Facilities of the Universidad de Colima and the Universidad del País Vasco and maintained in standard conditions. Swiss mice weighing 30–45 g and New Zealand White rabbits weighing 1.5–1.8 kg were randomly divided into a control and an experimental group. The murine experimental group received an ip injection of 100 mg kg\(^{-1}\) of streptozotocin. The rabbit experimental group received an injection of 200 mg kg\(^{-1}\) of alloxan in the ear vein. The experiments were carried out 10 weeks after diabetes induction.

Blood samples were taken just before diabetes induction and immediately before heart extraction. Samples were obtained always early in the morning. As animals also feed early in the morning, these are non-fasting blood samples. Blood glucose levels were measured with the One Touch Basic system (LifeScan; Johnson and Johnson, S.A., New Brunswick, NJ, USA) or the Acutrend Confort Glucometer (Roche Diagnostics, Basel, Switzerland). Glucose values averaged 123 ± 22 mg dL\(^{-1}\) in control (n = 18) and 404 ± 16 mg dL\(^{-1}\) in diabetic (n = 25) mice (P < 0.001), and 106 ± 3.9 mg dL\(^{-1}\) in control (n = 18) and 362 ± 39 mg dL\(^{-1}\) in diabetic (n = 22) rabbits (P < 0.001).

**Cell isolation**

Animals were anaesthetized with chloral hydrate (3 mL kg\(^{-1}\), ip) and killed by cervical dislocation. Once removed, the hearts were retrogradely perfused through the aorta on a Langendorff apparatus. The Tyrode perfusing solution contained NaCl, 118; KCl, 5.4; NaHCO\(_3\), 24; MgCl\(_2\), 1.02; CaCl\(_2\), 1.8; NaH\(_2\)PO\(_4\), 0.42; dextrose, 12; and taurine, 20 (mM). Solution was kept at 37 °C and bubbled with 95% O\(_2\) and 5% CO\(_2\) to ensure pH 7.4. After 5 min, hearts were prefunded with a calcium-free Tyrode for 10 min and then with collagenase type I (0.5 mg mL\(^{-1}\)) and protease type XIV (0.03 mg mL\(^{-1}\)) dissolved in the same nominally Ca\(^{2+}\)-free solution. Finally, the hearts were washed with KB solution (Isenberg & Klockner 1982) of the following composition (in mM): taurine, 10; glutamic acid, 70; creatine, 0.5; succinic acid, 5; dextrose, 10; KH\(_2\)PO\(_4\), 10; KCl, 20; HEPES-K\(^+\), 10; and EGTA-K\(^+\), 0.2. KOH was used to adjust the pH to 7.4. Single cells were obtained by mechanical agitation. Initially, myocytes were isolated from three different regions: the right
ventricle, the epicardium of the left ventricle and the endocardium of the left ventricle. As the results were not significantly different from region to region, we show only the results obtained only in myocytes isolated from the free wall of the left ventricle. The well-known difference in the amplitude of $I_{\text{to,fast}}$ between the three regions made impossible to pool the data from the three regions.

**Patch-clamp**

Action potentials and K$^+$ currents were recorded using the whole-cell configuration of the patch-clamp technique (Hamill et al. 1981). Borosilicate microelectrodes (Sutter Instruments, Novato, CA, USA) were filled with the internal solution (in mM): 1-aspartic acid (potassium salt), 80; KH$_2$PO$_4$, 10; MgSO$_4$, 1; KCl, 50; HEPES-K$^+$, 5; ATP-Na$_2$, 5; EGTA-K$^+$, 10; GTP-Na, 0.2; adjusted to pH 7.2 with KOH and had a tip resistance of 1–2 MΩ.

Capacitive transients elicited by voltage steps from $-50$ to $-60$ mV were integrated to measure cell membrane capacitances. To minimize voltage errors, series resistances were compensated 80%. The ‘Clampex’ program of the ‘pClamp’ software (Molecular Devices, Sunnyvale, CA, USA) controlled the current-and voltage-clamp experimental protocols. A normal external solution (in mM): NaCl, 136; KCl, 5.4; MgCl$_2$, 1; HEPES-Na, 10; CaCl$_2$, 1.8; dextrose, 11; pH adjusted to 7.4 with NaOH was used to elicit cell action potentials. K$^+$ currents were recorded with a bathing solution containing (in mM) choline chloride, 136; MgCl$_2$, 1; HEPES-K$^+$, 4; HEPES, 6; N-Methyl-D-glucamine, 6; CaCl$_2$, 0.1; CoCl$_2$, 0.5; dextrose, 11; and adjusted to pH 7.4 with KOH.

Both current-clamp and voltage-clamp recordings were taken with an Axopatch 200A or 200B patch-clamp amplifiers (Molecular Devices). Potassium currents are rapidly activated. Therefore, experiments were performed at room temperature (20–22 °C) to isolate peak current from capacitive current. Voltage-gated outward K$^+$ currents were routinely evoked during depolarizing voltage steps from a holding potential of $-80$ mV; voltage steps were presented in 10 mV increments at 30-s intervals. Precise voltage protocols are described in detail in the Results section. Experimental protocols were controlled, and cell signals were digitized with a Digidata 1440 analogue/digital interface and pClamp 10.1 (Molecular Devices).

The different K$^+$ current components in mice were measured using the method described by Xu et al. (1999). The current inactivation during 5 s depolarizing steps between −40 and +60 mV from a holding potential of $-80$ mV were fitted using the following expression: $y(t) = A_1 \cdot \exp(-t/\tau_1) + A_2 \cdot \exp(-t/\tau_2) + A_3 \cdot \exp(-t/\tau_3) + A_4$. $\tau_1$, $\tau_2$ and $\tau_3$ are the time constants of the K$^+$ currents inactivation. $A_1$, $A_2$, and $A_3$ and $A_4$ are the amplitudes of the three inactivating current components and the amplitude of the steady state, non-inactivating component of the total current. The quality of fits was assessed by correlation coefficients (R) determination; R values for the fits reported here were $>0.98$. In rabbit myocytes, the maximum current amplitude was measured directly for each current type.

**Immunofluorescence**

Freshly isolated ventricular cardiomyocytes were placed on tissue culture plates containing poly-L-lysine coated round coverslips. There, cells were incubated for 6 h at room temperature with vehicle, insulin or pyruvate. Cells in coverslips were then fixed in methanol during 10 min and washed with phosphate buffer before incubation for 1 h at room temperature in blocking buffer (0.22% gelatine, 1% BSA, 0.1% Triton X-100 and 1% normal donkey serum in phosphate buffer). Primary anti-Kv1.4, Kv1.5, Kv2.1, Kv4.2 and Kv4.3 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and secondary donkey anti-goat Alexa 488 (Invitrogen, Grand Island, NY, USA) antibodies diluted in blocking buffer were applied for 60 and 45 min respectively. Coverslips were mounted in Mowiol reagent (Calbiochem) and analysed in a Zeiss Axio Observer Z1 fluorescence microscope (Zeiss, Jena, Germany).

**Statistical analyses**

All averaged and normalized data are presented as means ± SD. The statistical significance of observed differences between groups was evaluated using a two-tailed Student’s t-test when comparing two groups and ANOVA followed by Bonferroni’s test when more groups were compared. $P$ values are presented in the text; statistical significance was set at $P < 0.05$.

**Results**

**Effect of diabetes on cardiac action potentials**

In mouse ventricular myocytes, diabetes has no effect either on resting membrane potential or on action potential amplitude. The APD at 30, 50 and 90% of repolarization shows a statistically significant increase in diabetic myocytes with respect to control cells (Table 1).

The variability in APD during a train of stimuli renders the patch-clamp technique inaccurate to measure action potentials in isolated rabbit myocytes.
Effects of diabetes on cardiac $K^+$ currents

In isolated mouse ventricular myocytes, outward $K^+$ currents were recorded applying 5 s depolarizing voltage pulses to potentials between −40 and +60 mV from a −80 mV holding potential, and current recording decays were fitted to a three exponential equation. All the time constants were voltage independent (not shown) and, in accordance with the work by Xu et al. (1999), the fast inactivating component corresponded to $I_{to,fast}$ (τ = 46.5 ± 9.6 ms), the medium inactivating component corresponded to $I_{to,slow}$ (τ = 329.6 ± 96.5 ms) and the slow inactivating one corresponded to $I_{k,slow}$ (τ = 1433.2 ± 51 ms). $I_{to}$ was related with the non-inactivating current component.

The amplitude of the total $K^+$ current is reduced in myocytes isolated from murine diabetic hearts compared with control cells (Fig. 1a). The amplitude of each current component obtained from the fitting was normalized to cell capacitance to compare current densities from cells of different sizes. Current density–voltage relationships for the four distinct outward $K^+$ currents in control and diabetic cells are shown in Figure 1b. Diabetic status had no appreciable effect on $I_{to}$ but induced a significant reduction in $I_{to,fast}$, $I_{to,slow}$ and $I_{k,slow}$ current amplitudes with no effects on the inactivation time constants.

The decrease in the current densities could be caused by a shift in the voltage dependence of inactivation to a more negative membrane potential. The voltage dependence of inactivation of $I_{to,slow}$ was studied using a two-pulse protocol, starting from a 1-s prepulse ranging from −120 to 0 mV, followed by a 500-ms pulse to +50 mV (Fig. 4a). Peak current amplitudes at +50 mV were plotted against the prepulse voltage and fitted to the previously described Boltzman equation. $V_{half}$ values were similar in control and diabetic myocytes (−45.5 ± 0.8 vs. −44.9 ± 0.6 mV respectively, ns).

The time dependence of recovery from inactivation was tested by applying a 500-ms conditioning prepulse to +50 mV to inactivate $K^+$ channels, followed by a test pulse to +50 mV (Fig. 2b) at progressively increased intervals. There was no variation between the time dependence of recovery in the two cell types in any of the repolarizing currents studied (Table 2).

In isolated myocytes from rabbit hearts, outward $K^+$ currents were recorded separately. $I_{to,slow}$ was recorded by applying 500-ms depolarizing voltage pulses to potentials between −30 and +60 mV from a holding potential of −80 mV.

The amplitude of the $I_{to,slow}$ current was reduced in myocytes isolated from rabbit diabetic hearts compared with control cells (Fig. 3a). Current density–voltage relationships in control and diabetic cells are shown in Figure 3b. As in murine ventricular myocytes, diabetic status induced a significant reduction in $I_{to,slow}$ current density with no effects on its inactivation time constant in ventricular myocytes from rabbits.

In this case, the decrease in the current density could be caused by a shift in the voltage dependence of inactivation to a more negative membrane potential. The voltage dependence of inactivation of $I_{to,slow}$ was studied using a two-pulse protocol, starting from a 1-s prepulse ranging from −120 to 0 mV, followed by a 500-ms pulse to +50 mV (Fig. 4a). Peak current amplitudes at +50 mV were plotted against the prepulse voltage and fitted to the previously described Boltzman equation. $V_{half}$ values were similar in control and diabetic myocytes (−45.5 ± 0.8 vs. −44.9 ± 0.6 mV respectively, ns).

The time dependence of recovery from inactivation was tested by applying a 500-ms conditioning prepulse to +50 mV to inactivate $I_{to,slow}$ followed by a progressively increased interval by a test pulse to +50 mV (Fig. 4b). There was no difference between the time constant of recovery in the control and the diabetic cells (2.2 vs. 2.3 s in control and diabetic cells respectively).

The rapid delayed rectifier current, $I_{Kr}$, was recorded in rabbit ventricular myocytes by applying 3-s depolarizing voltage pulses to potentials between −30 and +50 mV from a holding potential of −80 mV, followed by a 1.5-s pulse to −40 mV. The current amplitude

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**Table 1** Effects of diabetes on resting membrane potential (RMP), action potential amplitude (APA) and action potential duration (APD) at 30, 50 and 90% of repolarization. Action potentials were recorded in myocytes isolated from the right ventricle of diabetic and control animals.

<table>
<thead>
<tr>
<th></th>
<th>RMP (mV)</th>
<th>APA (mV)</th>
<th>APD30 (ms)</th>
<th>APD50 (ms)</th>
<th>APD90 (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 19)</td>
<td>−83.6 ± 4.2</td>
<td>125.5 ± 11</td>
<td>0.9 ± 0.4</td>
<td>1.88 ± 1</td>
<td>11.2 ± 5.1</td>
</tr>
<tr>
<td>Diabetic (n = 26)</td>
<td>−85.4 ± 4.7</td>
<td>115.5 ± 11</td>
<td>1.6 ± 0.7*</td>
<td>3.5 ± 1.7*</td>
<td>21.4 ± 12*</td>
</tr>
</tbody>
</table>

* $P < 0.05.$
was also normalized to cell capacitance to compare current densities from cells of different sizes. Current density–voltage relationships in control and diabetic cells are shown in Figure 5. Diabetes affected neither stimulated nor I_{Kr} tail current density.

Last, the inward rectifier I_{K1} current was recorded by applying 500-ms hyperpolarizing voltage pulses to potentials between −120 and −30 mV from a holding potential of −40 mV. The I_{K1} was not affected by diabetic status, because current density was similar in control and diabetic cells (Fig. 6).

**Metabolic basis for K⁺ current reduction**

A number of previous works demonstrated that I_{to,fast} current density was recovered in diabetic myocytes after incubation with insulin. In this study, recovery was confirmed, as I_{to,fast} amplitude increased to control values after 6-h incubation with insulin. In addition, I_{to,slow} and I_{K,slow} current densities were also recovered up to control values by insulin (Fig. 7a).

The insulin-mediated upregulation of K⁺ currents can be caused either by the trophic effect of the hormone on protein synthesis or by an improvement of the metabolic status of the cells. To test this second possibility, diabetic myocytes were incubated with pyruvate, and K⁺ currents were recorded. Six-hour incubation with pyruvate resulted in the recovery of I_{to,fast}, I_{to,slow} and I_{K,slow} current densities up to control values (Fig. 7B).

**K⁺ channel expression**

One likely explanation for the decrease in K⁺ current densities with no changes in their biophysical properties...
is a decrease in K$^+$ channel expression. Thus, consistent with the results from electrophysiological experiments, immunofluorescence experiments showed a reduction in Kv4.2, Kv4.3, Kv1.4 and Kv1.5 protein levels in diabetic mouse ventricular myocytes compared with healthy myocytes, whereas Kv2.1 protein expression was not modified. Incubation with either insulin or pyruvate recovered Kv4.2, Kv4.3, Kv1.4 and Kv1.5 channels expression, with no effect on Kv2.1 expression (Fig. 8).

Figure 2 Biophysical properties of K$^+$ outward currents in mice. (a) Current recordings derived from an inactivation protocol in a control ventricular myocytes (left panel). The mean ± SD amplitude at +50 mV of each current component (circles $I_{\text{to,fast}}$, triangles $I_{\text{to,slow}}$, squares $I_{K,\text{slow}}$) was graphed with respect to the prepulse potential, and the points were fitted to a Boltzmann equation (right panel). Filled symbols are control cells, and open symbols are cells from diabetic mice. The inactivation $V_{\text{half}}$ values are shown in Table 2. (b) Current traces derived from an inactivation recovery protocol recorded in a control myocytes (upper left panel). The proportion between the amplitude at the test pulse ($I_t$) and at the conditioning pulse ($I_c$) was plotted with respect to interval time, and the points were fitted to the equation: $I_t/I_c = A[1 + \exp(-t/\tau)]$. The first part of the x-axis is magnified to visualize the absence of effect of diabetes on the recovery from inactivation. The results for each current component in every experimental group are shown in Table 2.
Intracellular mechanism of reduced K\(^+\) channel expression

The AMP-dependent protein kinase, AMP-K, is a cellular energy sensor, which couples metabolic status to protein synthesis. In the next group of experiments, we studied whether or not AMP-K is involved in K\(^+\) channels forming proteins synthesis. We first tested the effects of the adenosine analogue AICAR, a commonly used activator of AMP-K. Incubation of healthy mouse myocytes with AICAR 500 \(\mu\)M reduced the amplitude of \(I_{\text{to,fast}}, I_{\text{to,slow}}\) and \(I_{\text{K,slow}}\) in a similar manner as diabetes did. However, similar to diabetic status, AICAR did not affect \(I_{ss}\) current density (Fig. 9). In addition, when administered to diabetic mouse myocytes together with the AMP-K activator AICAR, puruvic acid lost its ability to restore current densities.

Discussion

The outlook for patients suffering diabetes mellitus has improved considerably, thanks to progress made...
in recent years in the treatment of the disease. However, while life expectancy has been lengthened, different long-term complications have developed. One of the complications of chronic diabetes is diabetic cardiomyopathy, an entity responsible for the appearance of mechanical and electrical abnormalities (Regan 1983, Fein & Sonnenblick 1985). The electrocardiogram of diabetic patients shows clear differences with respect to healthy people. The most frequent alterations are those related to cardiac repolarization, due to the increase in APD induced by diabetes in atrial and ventricular myocytes (Fein et al. 1983, Jourdon & Feuvray 1993, Casis et al. 2000). As expected, in our model of diabetic mice, the APD was also prolonged in ventricular myocytes isolated from the right ventricle.

**Figure 5** Effects of diabetes on the rapid delayed rectifier, $I_{Kr}$, in rabbit cardiac myocytes. (a) $I_{Kr}$ currents traces recorded in a control cell and a cell from a diabetic rabbit during 5000-ms depolarizing voltage steps to potentials between $-40$ and $+50$ mV from a holding potential of $-80$ mV, followed by a $-40$ mV step. (b) Activated $I_{Kr}$ current density–voltage (pA/pF) relationships in control cells (filled symbols; $n = 14$) are compared with cells from diabetic rabbits (open symbols; $n = 28$). (c) Tail $I_{Kr}$ current density at $-40$ mV plotted as a function of the voltage of the prepulse. Mean ± SD.

**Figure 6** Effects of diabetes on the inward rectifier, $I_{K1}$, in rabbit cardiac myocytes. (a) Typical $I_{K1}$ currents traces recorded in a control and a diabetic myocytes during 500-ms hyperpolarizing voltage steps to potentials between $-120$ and $-30$ mV from a holding potential of $-40$ mV. (b) Peak $I_{K1}$ current density–voltage (pA/pF) relationships in control cells (filled symbols; $n = 9$) are compared with cells from diabetic rabbits (open symbols; $n = 7$). Mean ± SD.
Several studies demonstrated that in diabetic myocytes, the main cause for the APD prolongation is the reduction in the K$^+$ repolarizing currents (Magyar et al. 1992, Jourdon & Feuvray 1993, Shimoni et al. 1994). As already mentioned, the main repolarizing currents in the human heart are $I_{\text{to, fast}}$, $I_{\text{to, slow}}$, $I_{Kr}$, $I_{Ks}$, $I_{Kur}$ (or $I_{ss}$) and $I_{Kslow}$ (Beuckelmann et al. 1993, Li et al. 1996, 1996, Spector et al. 1996, Pond et al. 2000, Van Wagoner & Nerbonne 2000). Employing diverse animal models, previous works described that $I_{Ks}$ was reduced in hearts from diabetic dogs and rabbits (Lengyel et al. 2007, 2008), and the transient outward K$^+$ current was reduced in both dogs and rats (Casis et al. 2000, Lengyel et al. 2007). However, there are controversial results regarding the effects of diabetes on $I_{Kr}$, since it has been described to be reduced in diabetic rabbits (Zhang et al. 2006) or unchanged in diabetic rabbits and dogs (Lengyel et al. 2007, 2008). In addition, nothing is known about the effects of diabetes on the $I_{Kslow}$ and $I_{Kur}$. Different animal models are necessary to study these currents.

Here, we employed mouse adult ventricular myocytes, in which four different K$^+$ repolarizing currents have been described: $I_{\text{to, fast}}$, $I_{\text{to, slow}}$, $I_{Kr}$ and $I_{ss}$ (Xu et al. 1999). We also used rabbit ventricular myocytes, in which the main repolarizing currents are $I_{\text{to, slow}}$, $I_{Kr}$ and $I_{K1}$ (Veldkamp et al. 1993, Howarth et al. 1996). Respectively, the molecular correlate of

**Figure 7** Effect of insulin and pyruvate on ventricular repolarizing currents in murine diabetic cardiomyocytes. (a) Outward K$^+$ current density–voltage (Mean ± SD) relationships in control cells (filled circles; $n = 17$) compared with cells from diabetic mice (open circles; $n = 19$) and cells from diabetic mice incubated 6 h with insulin (open triangles, $n = 15$). (b) Outward K$^+$ current density–voltage (Mean ± SD) relationships in control cells (filled circles; $n = 17$) compared with cells from diabetic mice (open circles; $n = 19$) and cells from diabetic mice incubated 6 h with pyruvate (open triangles, $n = 17$).

* $P < 0.05$ with respect to control and diabetic respectively.
Previously, we demonstrated that, despite of its non-homogeneous distribution, $I_{\text{to,fast}}$ is homogeneously reduced by diabetes regardless of its regional localization (Casis et al. 2000). Because the other currents are equally distributed and $I_{\text{to,fast}}$ is greater in the right than the left ventricle, in this work we showed the results found in myocytes isolated only from the right ventricle. We found similar results in the endo- and epicardium of the left ventricle.

The reduction in all these repolarizing currents induced by diabetes may be attributed either to impaired channel protein expression (Howarth et al. 2009) or to altered channel biophysical properties or both. Yet, the absence of differences in inactivation half voltages, in the inactivation kinetics or in recovery from inactivation kinetics suggests an impairment of channels protein expression. This was confirmed by the clear reduction in the immunofluorescence of Kv4.2, Kv4.3, Kv1.4 and Kv1.5 channel proteins seen in diabetic compared with healthy myocytes. Consistent with the lack of effects on $I_{\text{so}}$, immunofluorescence staining of Kv2.1 channels was also unaffected by diabetes.

Clearly, insulin has a role in regulating the expression of different potassium channels in diabetic cardiac myocytes; in vitro incubation of myocytes isolated from diabetic hearts with insulin restores the potassium current amplitudes (Magyar et al. 1995, Xu et al. 1996a,b, 2002, Shimoni et al. 1998). It was proposed that this restoration was due to the effects of insulin on cellular metabolism, because compounds such as dichloroacetate (DCA) or L-carnitine, which improve glucose utilization, also restored $I_{\text{so}}$ current density (Xu et al. 1996a,b). There is more evidence for a metabolic basis of $I_{\text{to,fast}}$ current density decrease in the early stages of diabetes. Thus, $I_{\text{to,fast}}$ is inhibited by diverse intracellular changes occurring during diabetes, such as oxidative stress or protons and amphiphilic fatty acid accumulation (Xu et al. 1996a, 2002, Xu & Rozanski 1998, Rozanski & Xu 2002). There is also evidence for the involvement of protein synthesis in $K^+$ current restoration, because insulin requires at least 5–6 h to restore current amplitude, and the use of inhibitors of protein synthesis blocks the current recovery (Shimoni et al. 1999).

In preliminary experiments, the current recovery was tested after 3, 4, 5 and 6 h of incubation. In agreement with previous works (Rozanski et al. 1998, Shimoni et al. 1998), these experiments showed that current restoration was complete at 5–6 h of insulin or pyruvate incubation. We used this time point to start the current recordings. Insulin and pyruvate needed several hours to exert their effect and both substances restored current densities and channels protein expression. These results point to the requirement...
of protein synthesis to restore the K\textsuperscript{+} currents to control values. The channel protein synthesis is improved by insulin, which improves the metabolic state and stimulates protein synthesis, and, on the other hand, by pyruvate, which only improves the cellular metabolic state. Thus, one conclusion of this work is that diabetes reduces K\textsuperscript{+} current densities in ventricular myocytes due to a defect in channel protein synthesis secondary to a deterioration of the metabolic status of the cells.

To explore the link between the energy supply in cardiomyocytes and ventricular K\textsuperscript{+} channel expression, we tested the involvement of AMP-K. The AMP-activated protein kinase is a cellular energy sensor in heart (Heidrich et al. 2010), which switches on catabolic pathways to generate ATP and switches off energy consuming anabolic pathways including protein synthesis (Hardie 2012). Thus, AMP-K activation in healthy myocytes from rodents reduces the I\textsubscript{to,fast}, I\textsubscript{to,slow} and I\textsubscript{K,slow} densities, whereas I\textsubscript{ss} density was unaffected, in a similar manner than the diabetic status. Moreover, incubation with pyruvate was unable to restore K\textsuperscript{+} current densities in diabetic myocytes when AMP-K was previously activated. Thus, the second conclusion of this work is that AMP-K is the intracellular link between metabolic status and K\textsuperscript{+} channel proteins synthesis in the heart.

In summary, diabetes reduces K\textsuperscript{+} current densities in ventricular myocytes due to a defect in channel protein synthesis induced by the activation of AMP-K secondary to a deterioration of the metabolic status of the cells.

Conflict of interest
The authors have no conflict of interest.

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