Short-term Diabetes Alters K+ Currents in Rat Ventricular Myocytes

Y. Shimoni, L. Firek, D. Severson, W. Giles

Abstract  The electrophysiological properties of single ventricular myocytes from control rats and from rats made diabetic by streptozotocin (STZ) injection (100 mg/kg body weight) have been investigated using whole-cell voltage-clamp measurements. Our major goal was to define the effects of diabetes on rate-dependent changes in action potential duration and the underlying outward K+ currents. As early as 4 to 6 days after STZ treatment, significant elevation of plasma glucose levels occurs, and the action potential duration increases. In both control and diabetic rats, when the stimulation rate is increased, the action potential is prolonged, but this shortening is considerably more pronounced in myocytes from diabetic rats. In ventricular myocytes from diabetic rats, the Ca2+-independent transient outward K+ current (I1) is reduced in amplitude, and its reactivation kinetics are slowed. These changes result in a smaller I1 at physiological heart rates. The steady-state outward K+ current (Ih) also exhibits rate-dependent attenuation, and this phenomenon is more pronounced in cells from diabetic rats. These STZ-induced changes in I1 and Ih also develop when a lower dose (55 mg/kg) of STZ is used and measurements are made after 7 weeks of treatment. These electrophysiological effects are not related to the hypothyroid conditions that accompany the diabetic state, since they cannot be reversed by replacement of the hormone l-triiodothyronine to physiological levels. Direct effects of STZ could be ruled out, since preceding the STZ injection with a bolus injection of 3-O-methylglucose, which prevents development of hyperglycemia, prevents the electrophysiological changes. These results provide new insights into the ionic mechanism(s) involved in cardiovascular complications of diabetes, and they have implications for the metabolic regulation of K+ channel kinetics. (Circ Res. 1994;74:620-628.)

Key Words  diabetes  K+ currents  rat ventricles  cardiac action potential

The cardiovascular complications of diabetes mellitus are well known.1,2 In addition to coronary vessel disease, there are significant derangements in the myocardium itself, including alterations in both mechanical and electrical activity.3,4 Changes in both the action potential configuration5-6 and the ECG of diabetic patients have been described and may be responsible for the increased propensity for cardiac arrhythmias.7 Recent findings have provided important biochemical/electrophysiological evidence concerning the cellular mechanism(s) for the functional changes that occur in the heart in the diabetic state. Changes in the ionic currents that generate the action potentials8,9 have been described, and the amounts of GTP-binding proteins that mediate hormonal and neurotransmitter transduction mechanisms10 are known to be altered significantly. In addition to well-known changes in membrane phospholipid composition,11 ion pump and exchange systems,12 and overall cellular metabolic activity,13,14 there are alterations in the levels of various hormones that, in turn, may also affect cardiac function. For example, a hypothyroid state develops in diabetic animals and patients.1,5

In most previous studies, an animal model of chemically induced diabetes has been used, and the effects of diabetes have been investigated after a period of no less than 4 weeks after, for example, streptozotocin (STZ) injection. However, in this model, blood glucose levels are known to be substantially elevated within only 24 hours. Changes in thyroid hormone levels, which can have dramatic effects on electrical and mechanical activity of the heart,16,17 also occur within only a few days.1,5

The initial goal of the present study was to determine whether cardiac action potentials, which are known to be modified under long-term diabetic conditions,5,16 are also altered by short-term diabetic conditions and to evaluate to what extent changes in the repolarizing K+ currents are responsible for these electrophysiological changes. A well-established animal (rat) model of diabetes mellitus induced by STZ was used.1 The modifications of action potentials and K+ currents were studied as a function of the rate of stimulation, since rate changes, which are an inherent feature of cardiac activity, can significantly change the duration of the action potential and alter its refractory period. We have also investigated whether the observed changes in electrophysiological parameters arise from the hypothyroid state, which itself can alter the electrical activity of the heart.2,16,17

We have consistently observed substantial changes in action potential configuration and K+ currents within 4 to 6 days after induction of diabetic conditions by STZ injection. Our results demonstrate a significant reduction in a Ca2+-independent transient outward K+ current (I1) and provide information concerning the underlying biophysical mechanism(s) for this decrease. Thus, the frequency-dependent decrease in I1 may explain some of the previously described changes in the ECG of
diabetic patients and may also contribute to some of the arrhythmogenic mechanisms associated with diabetes.

Materials and Methods

STZ-Induced Diabetes Model

Male rats were made diabetic by a single intravenous injection of STZ (100 mg/kg body weight) and were killed after 4 to 6 days. This acute and severe model of diabetes has been used extensively for metabolic studies with cardiac myocytes. Normal rats of comparable age and weight were used as controls. Direct measurements confirmed that the STZ-treated rats were hyperglycemic; their plasma glucose levels were 26.3±2.15 mmol/L (mean±SD, n=15) as compared with 7.0±0.66 mmol/L (n=11) in control rats. These findings are consistent with previous studies using this model of STZ-induced insulin deficiency. Plasma measurements of L-triiodothyronine (T3) levels confirmed that the diabetic animals were hypothyroid. The normal value was 0.78±0.17 mmol/L (mean±SD, n=18), whereas 4 to 6 days after STZ injection, the mean value was 0.50±0.16 mmol/L (n=23), again consistent with previous results. In a second series of experiments, thyroid hormone replacement was started 1 day after STZ injection. This was done by scubcutaneous T3 injections of 0.3 µg/100 g body weight for 3 to 4 days before the animals were killed. This dose of T3 is considered to be "physiological," and it proved sufficient to restore plasma T3 levels to normal, with the mean value being 1.06±0.65 mmol/L (n=8). The mean glucose level in the T3-treated group was 25.1±2.6 mmol/L (n=8), which is indistinguishable from the level in the STZ-induced diabetic rat population.

Cell Isolation

Single myocytes from the right ventricles of rat hearts were prepared by enzymatic dispersion. Rats were anesthetized with ether, and after cervical dislocation, their hearts were quickly removed and mounted on a Langendorff apparatus, with aortic cannulation enabling retrograde perfusion at 37°C and at 70 cm H2O pressure. Hearts were first perfused for 5 minutes with a Tyrode's solution containing (mmol/L) CaCl2 1, NaCl 121, KCl 5, MgSO4 1, NaH2PO4 5, sodium acetate 2.8, NaHCO3 24, and glucose 5, bubbled with 95% O2:5% CO2. This was followed by 5 minutes of perfusion with a Tyrode's solution containing low CaCl2 (5 µmol/L). Finally, the hearts were perfused for 5 minutes in an enzyme-containing solution including 1.4 mg/100 mL collagenase (Yakult Honsha, Tokyo) and 1.4 mg/100 mL protease (type XIV, Sigma) with 40 µmol/L CaCl2. The right ventricle was subsequently removed and cut into small pieces, which were incubated at 37°C, by use of a shaker bath, in a solution containing 0.2 mg/mL collagenase and 0.1 mg/mL protease. Aliquots of the superfusate were removed sequentially for a period of 10 to 50 minutes so that an optimal yield of single cells could be chosen for electrophysiological recordings.

Electrophysiological Recordings

Cells were current- or voltage-clamped using the gigaseal suction microelectrode whole-cell method with an Axopatch 200 amplifier (Axon Instruments Ltd, Burlingame, Calif.). Since results from different populations of myocytes were compared (normal versus diabetic rats), it was essential to measure the capacitance of each cell and to normalize current amplitudes by dividing by cell capacitance, giving current densities (in picoamperes per picofarad). It was also necessary to minimize the series resistance, since for large currents (such as I), significant voltage errors can occur. This was done by using large-tipped relatively low-resistance (1- to 2-MΩ) microelectrodes and electronic series-resistance compensation. Impalements yielding an initial series resistance >10 MΩ were rejected.

Results

Action Potentials

Recent electrophysiological recordings from rat ventricle have shown that a marked increase in the action potential duration (APD) develops several weeks after diabetic conditions have been induced by STZ treatment. Our initial experimental results confirmed this finding in ventricular myocytes isolated from acutely diabetic rats and also showed that the amount of APD change was a function of stimulus rate. In control myocytes (Fig 1A) and in those from STZ-treated animals (Fig 1B and 1C), as the rate of stimulation is increased (from 0.2 to 3.3 Hz), there is a broadening of the APD during both the early and late phases of repolarization. Note, however, that in myocytes from STZ-treated animals, the APD exhibits a greater sensitivity to rate changes: higher stimulation rates result in relatively more prolongation of the APD (Fig 1B). In some cases, this prolongation is very marked (Fig 1C). The histograms in Fig 2 illustrate the changes in APD at two different levels of membrane potential for four different stimulation rates, ranging from 0.03 to 2 Hz.
The passive properties of the ventricular cells from diabetic animals were not significantly different from those of the normal cells, although there was a tendency toward a slight depolarization of the resting potential and an increase in input resistance. Resting potentials (mean±SD) were −76.3±1.7 and −74.8±1.5 mV in control (n=47) and diabetic (n=37) myocytes, respectively. The mean cell capacitance in the control group was 115.9±28.9 pF, whereas in the diabetic cells it was 104.4±38.3 pF. The input resistance (measured close to the resting potential) was 62.8±28.3 MΩ in the control cells and 79.5±25.6 MΩ in the diabetic cells.

A decrease in K+ currents in ventricular tissue from rats that had been made diabetic for 6 to 8 weeks has been described previously.8,9 Therefore, we examined (1) whether this could be observed after only 4 to 6 days of diabetic conditions, and (2) whether there were changes in the rate dependence of these outward K+ current(s). In response to depolarizing voltage steps, two major time- and voltage-dependent (but Ca2+-independent) outward K+ currents have been described in rat ventricular myocytes.23 One of these is 4-AP sensitive and has been named Iᵢ. The second K+ current exhibits much slower inactivation and is partially blocked by high concentrations of tetraethylammonium. It has been denoted the delayed-rectifier K+ current (Iₖ). An inwardly rectifying K+ current (Iₖᵢ) is also present at voltages near the resting potential and can be studied under voltage clamp by applying hyperpolarizing clamp steps.

In our experimental protocols, ventricular cells were impaled while being superfused with a Tyrode’s solution containing CdCl₂ (0.5 mmol/L) to block the Ca2+ current. Current-voltage (I-V) relations for the three K+ currents were obtained by applying 500-millisecond pulses at selected rates (usually 0.2 Hz), from a holding potential of −90 mV to potentials ranging from −110 to +40 mV. Measurements of the peak outward and the steady-state outward current amplitudes were made relative to the 0 current level.

**Peak Iᵢ**

Iᵢ was recorded from a total of 50 right ventricular myocytes, 25 from control rats and 25 from STZ-treated rats. In Fig 3A, representative raw data are compared from control (left tracing) and diabetic (right tracing) animals. STZ-induced diabetes resulted in a small, but significant, reduction of the peak Iᵢ at all potentials positive to +20 mV (Fig 3B). The relatively small effect (=20% decrease) is partly due to the experimental protocol. The data used to construct I-V relations were

![Graph showing changes in peak transient outward current in ventricular myocytes from diabetic rats.](image-url)
obtained at a stimulation rate of 0.2 Hz. At faster, more physiological rates, the reduction of I in diabetic myocytes was much larger (see below). The recordings from the diabetic myocytes in Fig 3A show the largest reduction obtained at 0.2 Hz. However, approximately this amount of STZ-induced reduction in I was observed consistently when faster repetition rates of voltage-clamp depolarizations were applied.

The threshold for activation of I was unchanged in the diabetic myocytes, indicating that a shift in the activation voltage range was unlikely. In 11 diabetic myocytes, we also studied the voltage-dependent inactivation of I. These data were fitted to a Boltzmann equation (as in Reference 23), giving a voltage for 50% inactivation of −30.6 mV, with a slope factor of 5.3. These values are not significantly different from those obtained in normal rat ventricular myocytes.

**Steady-State Outward Current**

In the hyperpolarized range of potentials from −110 to −70 mV, the steady-state current is generated mainly by I. Conversely, the steady-state outward currents at membrane potentials positive to −30 mV are mainly due to a delayed-rectifier type of K current recently described by Apkon and Nerbonne. We have denoted this current I or I and use these terms interchangeably. This current is insensitive to 4-AP (results not shown). Ideally, therefore, the steady-state outward current should be studied in the presence of 4-AP to rule out a contribution of residual I at the end of the voltage steps. However, 4-AP could not be used for this purpose, since the amount of block it provides is both rate and voltage dependent (Reference 24; see below).

Fig 4 shows the mean current densities (as picoamperes per picofarad) for these two steady-state K currents over the voltage range from −110 to +40 mV in control and diabetic myocytes. Note that there were no significant differences in the two I-V curves between −110 and −30 mV, indicating that there were no STZ-induced changes in I. In contrast, at potentials positive to +20 mV, the steady-state delayed-rectifier outward current was significantly reduced (P<.05) in the diabetic group.

**Rate-Dependent Modulation of I in Control and Diabetic Myocytes**

The rate dependence of the K currents that are activated by depolarization was studied using the following protocol. Each myocyte was first rested for 30 seconds and then stimulated at selected rates. During these trains of depolarizing clamp pulses, the amplitudes of both the peak and steady-state components of outward current decreased, gradually settling to a new steady-state level within several depolarizations. The delayed rectifier (steady-state) component, I, consistently decreased more than the peak outward current, I, perhaps because of its slower time course of recovery. In our initial experiments, an attempt was made to examine the rate dependence of the steady-state (non-inactivating) component of outward current by first blocking the transient current with 4-AP. However, as mentioned previously, there was a significant "reverse use dependence" of the 4-AP-induced block; ie, at all rates of stimulation, there was a "reappearance" of I after rest.

At higher rates, this unblocking became quite prominent (results not shown). It was therefore necessary to study the rate-dependent changes in I and I by measuring the peak (I) and steady-state (I) amplitudes in the absence of 4-AP. In these protocols, at each stimulus rate the peak and steady-state current amplitudes were normalized to the respective first post-rest current amplitude. This enabled a current ratio to be plotted as a function of the rate of stimulation. Results from these experiments showed that the relative frequency-dependent reduction in outward current is larger in the diabetic myocytes. Fig 5A shows examples of original recordings from two cells: control (left tracing) and diabetic (right tracing). In Fig 5B, the mean ratios for the peak (left tracing) and steady-state (right tracing) outward currents are plotted as a function of stimulus rate. The differences in the peak current, I, recorded from diabetic and control cells were highly significant (P<.002) at all rates faster than 1 Hz. However, for the steady-state current, I, these differences were significant (P<.02) only at 3.3 Hz. Thus, K currents in the diabetic cells exhibit an enhanced rate sensitivity; ie, the attenuation of I at higher rates of stimulation is more pronounced in diabetic than in control cells. The most prominent rate-dependent effect is on the peak I. This reduction in I provides at least part of the explanation for the observed prolongation of the action potential in diabetic cells at physiological stimulation rates.

To examine this rate-dependent effect in more detail, the kinetics of recovery or reactivation of the peak I was studied. In these experiments, paired depolarizations were applied at selected intervals ranging from 10 to 1800 milliseconds. The ratio of the second to the first I amplitude (defined as the "relative I") was plotted against the interpulse interval. A ratio of 1.0 was defined as the fully recovered current at an interval of 5.0 seconds. This analysis was done for control (n=27) and diabetic (n=14) myocytes. The data in Fig 6 show...
that the reactivation kinetics of $I_r$ were slowed significantly in cells from diabetic animals. However, it should also be noted that, for intervals correlating to physiological rates of activity (200 to 600 milliseconds), there was incomplete recovery even under control conditions, as Apkon and Nerbonne have described previously.

Although the observed differences between cells from control and diabetic rats were quite small (corresponding to an $\approx$10% to 15% reduction in the cells from diabetic rats), they were highly significant ($P<.005$ for intervals of 20 to 200 milliseconds, $P<.02$ for 400 milliseconds). Small changes in net repolarizing currents are functionally important, since during the plateau and early repolarization phases of the rat ventricular action potential, the rate of change of membrane potential is very small. Thus, only very small (10 to 30 pA) net currents are present. For this reason, even very small changes in $I_r$ and/or $I_K$ can result in significant changes in the repolarization waveform.

In both groups of cells, the reactivation time course of $I_r$ was best fitted by the sum of two exponential processes, as shown previously for rat and rabbit ventricles. The reactivation data were therefore fitted to the following equation:

$$y = 1 - A \exp\left(-\frac{t}{\tau_1}\right) - B \exp\left(-\frac{t}{\tau_2}\right)$$

where $t$ is the interpulse interval, $\tau_1$ and $\tau_2$ are the two time constants, and $A$ and $B$ represent the relative contributions of the fast and slow recovery processes, respectively. The best fit gave values for $\tau_1$ of 23.9 and 19.9 milliseconds and $\tau_2$ of 4348 and 2222 milliseconds for cells from control and diabetic rats, respectively. The $A$ values were 0.418 and 0.429, and the $B$ values were 0.125 and 0.224 for cells from control and diabetic rats, respectively. Thus, the overall recovery process is markedly slower in the diabetic cells because of a much larger relative contribution of the slower component ($B$).
Fig 7. The effects of rate changes on the L-type Ca\(^{2+}\) current in a myocyte from a diabetic rat. The cell was held at -80 mV. After a 30-second rest, it was depolarized at 1 Hz with a 200-millisecond prepulse to -45 mV (which elicited a Na\(^+\) current) followed by a 300-millisecond pulse to -10 mV. Top, The two Ca\(^{2+}\) current tracings that are superimposed are the 1st postrest tracing and the 10th Ca\(^{2+}\) current tracing in this series. The latter Ca\(^{2+}\) current tracing is slightly smaller than the 1st postrest Ca\(^{2+}\) current tracing (*). At higher rates, there was a larger rate-dependent reduction in the Ca\(^{2+}\) current. The pipette contained 10 mmol/L EGTA and cesium instead of potassium, whereas the bathing solution contained 3 mmol/L cesium and 3 mmol/L 4-aminopyridine, to ensure a complete inhibition of all outward K\(^+\) currents.

It is possible that changes in the kinetics of recovery of the I\(_{Ca}\) also contribute to the action potential prolongation. In many preparations,\(^{26-28}\) I\(_{Ca}\) shows rate-dependent increases (positive staircases). This would increase the height of the plateau of the action potential and could account for its prolongation. However, this facilitation of I\(_{Ca}\) is known to be reduced significantly or abolished when intracellular Ca\(^{2+}\) is buffered.\(^{27}\) I\(_{Ca}\) was measured under conditions in which all the K\(^+\) currents were blocked by use of (1) cesium-containing pipette filling solution and (2) 3 mmol/L CsCl and 3 mmol/L 4-AP in the bathing medium. After a 30-second rest, each cell was depolarized to -10 mV at 1 or 2 Hz. This was done either from -80 mV after a 200-millisecond prepulse to -45 mV, which inactivated the Na\(^+\) current (I\(_{Na}\)), or from -45 mV. From a holding potential of -45 mV, there were pronounced negative staircases (reduced currents, as in References 27 and 28). These changes in I\(_{Ca}\) cannot account for the observed action potential prolongation. When the same pattern of depolarizations was applied from -80 mV (close to the normal resting potentials), there was either no change or a slight reduction in I\(_{Ca}\) for cells from both control and diabetic rats. Thus, under our conditions (with 10 mmol/L EGTA in the pipette), the action potential prolongation is not due to changes in I\(_{Ca}\). A typical I\(_{Ca}\) recording from a cell from a diabetic rat is shown in Fig 7.

In our final experiments, an attempt was made to gain insight into the hormonal mechanism(s) that underlies the observed changes in K\(^+\) current after induction of the diabetic state. STZ treatment is known to result in a hyperthyroid state within 2 to 3 days.\(^{15}\) This was confirmed by measurements of plasma T\(_3\); mean±SD values were 0.78±0.17 nmol/L for control animals and 0.50±0.16 nmol/L for diabetic animals (which is significantly lower, P<.001). Since it has been shown that a hyperthyroid state can speed up the reactivation of I\(_{K}\),\(^{17}\) it is conceivable that the hypothyroid state would be responsible for the observed slower reactivation. Accordingly, five rats were made diabetic, and T\(_3\) replacement consisting of daily injections of a physiological replacement dose of T\(_3\)\(^{20}\) was started 1 day after the STZ injection. T\(_3\) was administered because the peripheral conversion of thyroxine (T\(_4\)) to T\(_3\) is impaired in diabetes.\(^{15}\) Plasma measurements confirmed that the T\(_3\) replacement was sufficient to restore normal ( euthyroid) levels and that the animals were hyperglycemic (see “Materials and Methods”). Nevertheless, the kinetics of I\(_{K}\) recovery in myocytes from this group (n=34 cells) remained significantly slower than in control cells and did not differ from the data from diabetic rats. The reactivation curves for I\(_{K}\) are plotted in Fig 8A, which compares data from control and diabetic rats. The diabetic group was subdivided, so that cells obtained from animals receiving T\(_3\) replacement could be compared with cells from (1) control rats and (2) diabetic rats that did not receive T\(_3\) replacement. Note that thyroid replacement did not restore the reactivation time course of I\(_{K}\) to normal values. The reactivation values were significantly smaller in diabetic rats with T\(_3\) replacement compared with control rats (P<.05) at all intervals up to (and including) 400 milliseconds. Moreover, the values in T\(_3\) group were not different (P>.05) from the diabetic group at any stimulus interval. The normal rate dependence of the steady-state component, I\(_{K}\), also was not restored by T\(_3\) replacement under diabetic conditions, as shown in Fig 8B. Thus, at 2 and 3.3 Hz, the relative steady-state current was significantly smaller in this group (T\(_3\)) than in the control group.
Fig 9. Graphs showing prevention by 3-O-methylglucose (3-OMG) of streptozotocin (STZ)-induced effects on K+ currents in rat ventricle. Myocytes were isolated 6 to 7 days after injection of either STZ alone (100 mg/kg) or after coinjecting STZ (100 mg/kg) together with 1 mL of a 2.5 mol/L solution of 3-OMG. Note that this coinjection procedure prevents the toxic effects of STZ (ie, plasma glucose levels are normal; see text) and prevents the changes in the rate dependence of both the peak and the steady-state outward currents. The rate dependence of the peak current (top) and steady-state currents (bottom) are plotted as relative current vs rate. ○ indicates mean values from the "diabetes-protected" (3-OMG-treated) group (n=11); ∙, mean values from the diabetic (STZ-treated) group (n=6). *P<.05 and **P<.005 vs control.

(P<.05 and P<.005 for the two rates, respectively), but these values were not different (P>.05) from those obtained from the diabetic group.

Since the dose of STZ used in these experiments was at the higher end of concentrations used previously to induce diabetes,29 we needed to ensure that the observed electrophysiological effects were not due to nonspecific or toxic effects of STZ per se. This was done in a series of experiments in which STZ (100 mg/kg) injection was preceded by a bolus injection (1 mL) of a 2 to 3 mol/L solution of 3-O-methylglucose (3-OMG). This procedure has been shown to protect the pancreas from the toxic effects of STZ.30 In four rats treated in this way, the mean plasma glucose levels after 6 to 7 days was 9.3 mmol/L. Changes in the peak and steady-state outward currents were studied in 11 cells from one of these rats and compared with currents in six cells obtained from a rat that had received only STZ. Fig 9 shows that when STZ is administered with 3-OMG, the rate dependence of both peak and steady-state outward currents is not significantly different from that in normal myocytes (see Fig 5). Thus, STZ itself does not have any direct toxic effects.

To ensure that the electrophysiological changes observed were not artifacts due to the high concentrations of STZ used, we performed a further series of experiments using a lower dose (55 mg/kg) of STZ. In these experiments, animals (n=5) were killed after 7 weeks. Their mean plasma glucose concentration was 31.7±6.0 mmol/L. In 18 cells from these animals, the rate dependence of the two outward currents, I peak and I steady-state, were studied in 11 cells from one of these rats and compared with currents in six cells obtained from a rat that had received only STZ. Fig 9 shows that when STZ is administered with 3-OMG, the rate dependence of both peak and steady-state outward currents is not significantly different from that in normal myocytes (see Fig 5). Thus, STZ itself does not have any direct toxic effects.

Fig 10. Graphs comparing acute effects of 100 mg/kg streptozotocin (STZ) with long-term effects of 55 mg/kg STZ. The rate dependence of the steady-state current (I steady-state; top) and peak current (I peak; bottom) are shown for control myocytes (○), for myocytes obtained 6 to 7 days after injecting rats with the higher STZ dose (●), and for myocytes obtained 7 weeks after injecting rats with the lower STZ dose (△). Note that very similar changes were observed in the K+ currents recorded from both of these groups of cells from diabetic rats (see text for details). *P<.05 **P<.005 vs control.
condition ($P<.005$ for each group versus control). Note also that the two STZ-treated groups did not differ from each other. In each treated group, the relative size of $I_e$ was significantly smaller at 1 Hz ($P<.05$) and at 2 and 3.3 Hz ($P<.005$) than in control. Again, $I_e$ in the two groups did not differ from each other ($P>.05$).

**Discussion**

Our results demonstrate for the first time that electrophysiological changes can occur in single ventricular myocytes within 4 to 6 days after the induction of a diabetic state. Earlier work had described somewhat similar electrophysiological changes on a time scale of several weeks.

Thus, the elevation in plasma glucose levels (and the whole range of ensuing metabolic corollaries) can act as a trigger that results in significant changes in particular ionic ($K^+$) currents within just a few days. We have also shown that the same changes occur with a lower dose of STZ (55 mg/kg) after 7 weeks (Fig 10) and that it is not STZ per se that causes these changes (Fig 9). We have not determined whether it is the elevated glucose, per se, that acts as the direct signal for the observed changes in $K^+$ currents or whether some other factor in this insulin-deficient model of diabetes is responsible. Most previous studies on the deleterious effects of diabetes on cardiac function have made measurements only after a much longer diabetic period (4 to 6 weeks). Much of that work focused on the reduction in contractility and myosin ATPase activity, often attempting to correlate this with alterations in isoenzyme (V$_i$) content.

Our findings confirm earlier results obtained using multicellular preparations from mammalian ventricle, which showed that in diabetes mellitus there is a substantial increase in APD. We have examined this in detail by studying the rate dependence of the APD and of the underlying $K^+$ currents. The major change after STZ treatment is the development of a greater response (increase in APD) to rate increases. This change is not due to depolarization of the resting potential, which under some conditions may account for action potential prolongation.

One of the ionic mechanisms responsible for these changes in APD is a reduction in the magnitude and a slowing of the time course of recovery of $I_e$. Because of regional heterogeneity in rat ventricles, there is considerable variability in the configuration of the action potentials and in the magnitude of $I_e$. Nevertheless, a significant reduction in the magnitude of $I_e$, in the diabetic state could be demonstrated. Our analyses of the time course of reactivation of $I_e$ showed that the slowing of reactivation is due to an increase in the contribution of the slower component of this recovery process (see below). The nonactivating or "pedestal" component of the outward current $I_w$ was also reduced. This current change also contributes to action potential prolongation, especially at relatively fast stimulation rates. These changes are functionally important since the action potential plateau is determined by a fine balance of currents (10 to 30 pA in magnitude); therefore, even small changes in the underlying currents can have dramatic effects (as in Fig 1C).

Under our conditions (Fig 7), rate-dependent changes in $I_{Ca}$ were not involved in action potential changes; ie, when EGTA (10 mmol/L) was included in the recording pipette, there were no changes in $I_{Ca}$ as the rate was increased. However, rate-dependent increases in $I_{Ca}$ may further contribute to APD prolongation under physiological conditions when $Ca^{2+}$ buffering is weaker.

Our findings have several implications for cardiac function under diabetic conditions. First, a prolongation of APD at high rates may limit diastolic filling and ultimately reduce the stroke volume. Second, APD prolongation may reduce or abolish the normal endocardial-epicardial APD gradient. This may change the normal temporal pattern of ventricular repolarization and could result in flattening or inversion of the T wave. This change in T-wave configuration is quite commonly observed in diabetic patients. The prolongation of the QT interval in diabetic patients suggests that in the human myocardium as well there is an attenuation of $K^+$ current(s), which repolarize the action potential. Such changes may facilitate reentry, especially during tachycardia, when rate-dependent APD prolongation would be expected to be pronounced.

The effects on $K^+$ currents that we have identified are not due to the hypothyroid state that accompanies diabetes, since thyroid hormone replacement did not reduce or reverse these changes (Fig 8). Previous studies have also shown that a "physiological" replacement dose of $T_3$ failed to normalize the diabetes-induced reduction in cardiac function, as monitored by measurements of peak systolic pressure and the pressure-rate product.

Nevertheless, the changes that we have identified may reflect common mechanism(s) underlying diabetes and hypothyroid conditions. It is of interest that opposite changes (a speeding up of $I_e$ recovery and a smaller sensitivity to rate changes) have been described under hyperthyroid conditions. Therefore, it seems that, regardless of thyroid status, changes in metabolic status affect the kinetics of $I_e$ and thus can contribute to the alterations in action potential configuration.

It is interesting that the slower component of reactivation of $I_e$ is very susceptible to changes in thyroid hormone levels. Our previous work was done in rabbit ventricular myocytes under hyperthyroid conditions, in which recovery of $I_e$ is normally much slower. In larger mammals with a lower heart rate, the slower component of recovery is dominant. Recently, however, relatively fast reactivation of $I_e$ has been described in human atrial cells.

Further work may provide more information concerning the signal that triggers the observed electrophysiological alterations (eg, elevated glucose versus elevated fatty acid levels). It will also be important to establish how regulation of $K^+$ channels by catecholamines, protein kinases, etc, may be altered under diabetic conditions. Finally, it will be interesting to study whether there is a metabolic process regulating the expression of $K^+$ channels or altering their kinetic properties or whether the combinations of channel subtypes that make up the macroscopic current are changed.

**Acknowledgments**

This study was supported by operating grants to Dr Giles and Dr Severson from the Medical Research Council of Canada and the Heart and Stroke Foundation of Canada. In addition, a Medical Scientist Award (Dr Giles) and a Postdoctoral Fellowship (Dr Firek) were provided by the Alberta Research Council.
Heritage Foundation for Medical Research. We thank C. Collins and K. Burrell for skilled secretarial assistance and the Department of Laboratories of the Foothills Hospital for performing the plasma $T_3$ analyses.

References


5. Horackova M, Murphy MG. Effects of chronic diabetes mellitus on the electrical and contractile activities. $^{45}$Ca$^{+2}$ transport, fatty acid profiles and ultrastructure of isolated rat ventricular myocytes. Pflugers Arch. 1988;411:564-572.


Short-term diabetes alters K+ currents in rat ventricular myocytes.
Y Shimoni, L Firek, D Severson and W Giles

Circ Res. 1994;74:620-628
doi: 10.1161/01.RES.74.4.620
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/74/4/620

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/