Sex-dependent impairment of cardiac action potential conduction in type 1 diabetic rats

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The incidence of diabetes mellitus is increasing. Cardiac dysfunction often develops, resulting in diverse arrhythmias. These arise from ion channel remodeling or from altered speed and pattern of impulse propagation. Few studies have investigated impulse propagation in the diabetic heart. We previously showed a reduced conduction reserve in diabetic males and females. In conclusion, the slowing of cardiac impulse propagation in the STZ-diabetic rat is smaller in female diabetic females. Pretreatment of diabetic males with the angiotensin-converting enzyme inhibitor quinapril reduced Cx43 lateralization and the effects of 9 mM K+ on propagation. In conclusion, the slowing of cardiac impulse propagation in type 1 diabetes is smaller in female rats, partly due to the presence of female sex hormones. This difference is (partly) mediated by sex differences inactivation of the cardiac renin-angiotensin system.

gap junctions; connexin43

CARDIOVASCULAR DISEASE is a leading cause of mortality in the Western world (17). Significant sex differences exist in the incidence and outcomes of cardiovascular derangements (26) and in normal and pathological cardiac function (14, 18, 46). Electrical dysfunction manifests as diverse arrhythmias, some of which result from changes in ionic currents underlying the cardiac action potential (30). Sex differences in arrhythmia susceptibility are variable: ventricular arrhythmias and sudden cardiac death are more common in men, whereas supraventricular tachycardia and drug-induced torsades de pointes are more common in women (34, 35). Other arrhythmias result from abnormalities in the speed and pattern of conduction of the cardiac impulse (1, 21, 23). These arrhythmias often result from changes in intercellular coupling, mediated by the gap junction protein connexin43 (Cx43) (5, 16). Conduction abnormalities have been characterized in heart failure (1), hypertrophy (9), and ischemic/hypoxic conditions (6).

Diabetes is an additional increasingly common pathology, with significantly elevated cardiovascular risk (44, 45). Using animal models of diabetes, we have found that an upregulated cardiac renin-angiotensin system (RAS) (10, 13) contributes to the potentially arrhythmogenic attenuation of K+ currents and to action potential prolongation (40). We also identified sex differences in K+-current modulation (42), partly attributed to selective activation of the cardiac RAS. Selective activation results in lower cellular angiotensin II levels in diabetic female hearts compared with diabetic male hearts (43). Concordantly, cells from diabetic females show less oxidative stress (41).

We recently established that conduction of the cardiac impulse is compromised in a streptozotocin (STZ) model of diabetes (33). Conduction velocity in the heart depends primarily on two factors: cellular excitability and intercellular electrical coupling. The heart has a large redundancy in these parameters, termed the conduction reserve (50, 51), so that only extreme alterations in intercellular coupling (gap junctions) or in excitability lead to a substantial slowing of conduction (5, 16, 39, 51). In earlier work (33), our laboratory found that baseline conduction velocity was not significantly changed in hearts from STZ-diabetic rats. However, further perturbations, such as reduction of excitability by elevated extracellular K+, or partial uncoupling of gap junctions by heptanol, caused significantly larger effects in diabetic rats than in controls (33). This was associated with altered cellular distribution of Cx43, a major component of gap junctions, which function to facilitate rapid and synchronized impulse propagation (15, 38). The focus of the present work is on the sex dependence of this partial intercellular uncoupling in the diabetic heart. Cx43 is tightly regulated by multiple factors (15). Some of these, such as high glucose, PKC expression/activity, and oxidative stress, are implicated in diabetic pathology (19, 24, 50). Importantly, gap junction remodeling, which occurs with many cardiac pathologies (6, 38), may be triggered by elevated angiotensin (12, 22). Thus gap junction remodeling is blocked by inhibition of angiotensin formation or by receptor blockade (7, 9).

Based on the previous work from our laboratory suggesting sex-selective activation of signaling mediators (43), we hypothesized that there may be sex differences in cardiac impulse propagation in the STZ-diabetic rat. The present study compared conduction velocity and cellular Cx43 distribution in diabetic males and females.

METHODS

This study, conforming to the National Institutes of Health Guide for Care and Use of Laboratory Animals, was approved by the University of Calgary Animal Care Committee.
Animals. Age-matched male and female Sprague-Dawley rats (250–300 g) were used as controls or 7–14 days following an intravenous injection of STZ (100 mg/kg) to induce diabetes. The diabetic state was confirmed by measuring blood glucose (≥28 mM in all diabetic rats) with a glucometer (One Touch Ultra; LifeScan, Milpitas, CA). We also used ovariectomized females, made diabetic 3 wk after surgery. Rats were anesthetized by CO₂ inhalation and euthanized by cervical dislocation. Hearts were mounted and perfused (at 37°C, constant flow rate) on a Langendorff apparatus and used for either imaging or cell isolation and immunolabeling.

Experimental protocols. In all experiments, propagation of the ventricular action potential was measured (see Voltage-sensitive dye imaging) before and after perfusion for 10 min with 9 mM K⁺ or before and after 15 min perfusion with 0.75 mM heptanol. The effects of heptanol may not have reached steady state, but comparison under identical conditions and perfusion times enabled detection of intergroup differences.

Voltage-sensitive dye imaging. We used voltage-sensitive dye imaging (8) to quantify the activation pattern and conduction velocity in the left ventricular free wall. Voltage-sensitive dyes, when illuminated at appropriate wavelengths, emit a fluorescent signal proportional to the voltage across cell membranes. Recording this signal with a high-speed video camera enables recordings of electrical activation of the heart. Hearts were perfused (as in Refs. 31 and 32) at rates of 6–8 ml/min (depending on size) with perfusion pressure and temperature monitored (31). After a 20-min stabilization period, the solution was switched to one containing 1 µM di-4-ANEPPS (Molecular Probes, Eugene, OR) for 5 min.

Image data processing. Data were processed offline (31, 32). The activation time for each pixel in an image was detected as the time of maximum rate of rise of the fluorescence signal (31). Activation maps were computed for individual cycles with activation times referenced to the stimulus pulse. Signal-averaged activation maps were computed by averaging activation times for individual pixels over all cycles. Activation maps were signal averaged over 5 s of recording (20–25 cycles). An overall measure (activation time) of the speed of activation was obtained by constructing a histogram of all individual pixel activation times from each map. The width of this histogram (at 20% of peak level) was taken as a measure (activation time) of the speed of activation. The reasons for using this measure were discussed at length in a previous publication (Ref. 33 and supplemental material; note: all supplemental material can be found with the online version of this article). By comparing activation times for the same region in the same preparation before and after an intervention, we obtained a normalized measure of the change in conduction velocity, with each preparation as its own control. Activation times were normalized to the value obtained in normal Krebs solution. This measure was therefore not influenced by the exact region of interest chosen or by the size of the preparation. The preparation was paced using a unipolar stimulation electrode, located at the base of the left ventricle. This produced an approximately straight activation wavefront, traveling from base to apex of the free wall. This stimulus location was chosen to produce an activation wavefront propagating through the myocardium only, without significant involvement of the specialized conduction system. Activation times were computed for paced recordings in normal and test solutions. A more detailed analysis can be found in earlier work from our laboratory (33).

Cell isolation. Ventricular myocytes were obtained by enzymatic dispersion, as described recently (41). Hearts were perfused for 5 to 6 min (at 37°C, bubbled with 100% O₂) with a solution consisting of (in mM): 113 NaCl, 4.7 KCl, 1.2 MgSO₄, 0.6 KH₂PO₄, 0.6 Na₂HPO₄, 12 NaHCO₃, 12 KHC₂O₃, 5.5 glucose, 10 HEPES, and 30 taurine. This was followed by the same solution containing the digestive enzymes liberase blendzyme (0.25 mg/ml; Roche), trypsin (0.14 mg/ml), and 12.5 µM CaCl₂. After 7 to 8 min, both ventricles were cut into pieces. After shaking, the tissue was filtered and a suspension of cells was collected and stored at room temperature (no enzymes, 20 mM taurine, 5 mg/ml albumin, and 0.1 mM CaCl₂).

Immunofluorescence. Isolated ventricular myocytes and tissue sections were prepared for immunofluorescence microscopy as described previously (33, 41). Briefly, isolated cell suspensions or tissue sections on glass slides were fixed with 1% formaldehyde and permeabilized with 2% Triton X-100. Primary antibody (polyclonal anti-Cx43; Sigma, St. Louis, MO) was added to aliquots of a cell suspension or pipetted onto the tissue sections in a humid chamber (nonspecific binding was blocked with 1% in PBS. The sections or cell suspensions were incubated overnight (at 4°C) in primary antibody, washed 3 times (in the case of cell suspensions by low-speed centrifugation and resuspension of the resulting pellet), and then exposed to secondary antibody conjugated to a fluorochrome (Alexa fluor 488, Molecular Probes, Eugene, OR; or Cy3, Jackson Laboratories, West Grove, PA) and incubated at room temperature for 1–1.5 h. Aliquots of cell suspensions (~5 µl) were washed (3 times) and pipetted into a drop of mounting medium on glass slides, covered with coverslips, and sealed with clear nail polish. For tissue sections, mounting medium was pipetted onto a slide, and the slide was sealed with a coverslip. Control experiments with secondary antibody alone verified antibody specificity and determined background fluorescence. All sections or cell suspensions were labeled at the same time under the same conditions, and images were collected using the same camera settings. Cells labeled with the Alexa 488- or the Cy3-conjugated secondary antibody were both included in the analysis, to rule out possible artifacts due to choice of secondary antibody.

Analysis of lateralization. Fractional lateralization of Cx43 was determined from immunofluorescence images of cells or tissue sections labeled with anti-Cx43, using Photoshop and locally written software. Background light intensity and total light intensity above background were determined for each image. Areas of interest that included all of the light in images associated with intercalated discs were drawn, and the intensity of pixels within these regions was set to 0. The total remaining light intensity in the image was then determined. The ratio of the total light intensity in the image, after eliminating the light from the intercalated discs, to the total light intensity of the original image was used as an estimate of the fraction of lateralized Cx43. The remaining fraction of Cx43 was at the intercalated discs.

Statistics. In this study, we found that individual responses of perfused hearts to elevated K⁺ and to heptanol were highly variable, leading to overlap in the responses of male and female hearts. In several cases, the magnitudes of responses were considerably separated from the major clusters. Our analysis was done both with and without these outliers. When outliers were excluded, with a criterion of excluding responses that were two standard deviations away from the mean, intergroup differences were significantly different with P < 0.005 (using Student’s two-sided, unpaired t-test). The inclusion of these outliers still maintained significant differences with P < 0.05.

RESULTS

Gender differences in conduction. In the present study, we employed the same procedures used in a previous study from our laboratory (33) to compare conduction velocity (activation times) between groups. This involved measuring the slowing of conduction following either the reduction of excitability or a partial uncoupling of gap junctions. Initially, control experiments were carried out on hearts from nondiabetic female rats, demonstrating that the responses to 9 mM K⁺ and 0.75 mM heptanol in control females were not significantly different from control males. Thus the average slowing of conduction by elevation of K⁺ in control males was by a factor of 1.25 ± 0.05 (n = 6). In control females, conduction was slowed by a factor of 1.21 ± 0.05 (n = 10).
Subsequently, we studied the effects of diabetes on conduction in female hearts. In experiments using hearts from diabetic females, perfusion for 10 min with 9 mM K⁺, the average slowing was by a factor of $1.29 \pm 0.04$ ($n = 17$), which is not significantly different than in control female hearts. This lack of significant effects of diabetic conditions is in marked contrast with the effects in diabetic males, in which 9 mM K⁺ slows conduction by a factor of $1.47 \pm 0.04$ ($n = 12$). Figure 1 shows the scatter of individual responses (Fig. 1A) as well as (Fig. 1B) the mean ($\pm$SE) values of conduction slowing obtained in control and diabetic males and females. The difference between diabetic males and females is significant ($P < 0.005$ without outliers and $P < 0.05$ with outliers).

Figure 2 shows examples of activation wavefronts, obtained during pacing in normal (5.9 mM) and in elevated (9 mM) K⁺. Figure 2, A and B, was obtained from a female diabetic rat, whereas Fig. 2, C and D, was from a male diabetic rat.

Comparing the effects of 9 mM K⁺ shows the larger slowing of conduction in the male (the white bands indicate the approximate location of the propagating wavefront).

A different way of showing this is by plotting isochronal maps (33), in which the color scale represents the activation time at each point. In this representation, conduction slowing is reflected as closer isochrone spacing. Figure 3 shows such maps from a diabetic female (Fig. 3, A and B) and a diabetic male (Fig. 3, C and D) heart in normal and elevated K⁺. Conduction slowing in response to 9 mM K⁺ is clearly more pronounced (larger increase in density of isochrones) in the male.

In the next set of experiments, conduction slowing was obtained by perfusion with the gap junction uncoupler heptanol (0.75 mM) for 15 min. In 12 control male rats, 0.75 mM heptanol slowed conduction by a factor of $1.26 \pm 0.06$, whereas in nine control female hearts, conduction was slowed by a factor of $1.26 \pm 0.05$. In diabetic males, 0.75 mM heptanol slowed conduction by a factor of $1.50 \pm 0.04$ ($n = 26$). In diabetic females, 0.75 mM heptanol slowed conduction by a factor of $1.28 \pm 0.06$ ($n = 15$). This is not significantly different than in control females but is significantly ($P < 0.05$ with outliers and $P < 0.005$ without outliers) smaller than the effect obtained in diabetic males. Figure 4A illustrates the scatter of the data, as well as the mean ($\pm$SE) values (Fig. 4B).

Table 1 shows a summary of the effects of elevated K⁺ and heptanol on the degree of slowing of conduction in control and diabetic male and female hearts.
Potential mechanisms for gender differences. The results so far show that under diabetic conditions, the slowing of propagation of the cardiac impulse in diabetic female hearts, in response to both reduced excitability and to partial gap junction uncoupling, is similar to that obtained in control female hearts. This is in marked contrast with diabetic males, in which these two different experimental maneuvers have significantly larger effects than in control hearts. This implies that the conduction reserve is larger in female hearts.

In a subsequent set of experiments we tested whether this is due to a protective effect of estrogen, as suggested for many cardiac derangements (18). Ovariectomized female rats were made diabetic 3 wk after surgery. Experiments were performed 7–14 days after STZ injection. In seven hearts, elevation of K⁺ to 9 mM slowed conduction velocity by a factor of 1.53 ± 0.07, which is significantly (P < 0.05 with outliers and P < 0.005 without outliers) larger than in nonovariectomized diabetic females and not significantly different than in diabetic males. This result, illustrated in Figure 5, suggests that estrogen plays a key role in maintaining conduction velocity in diabetic females.

In the final optical mapping experiments, we attempted to investigate some of the mechanisms by which estrogen may mediate the sex differences. Earlier work from our laboratory showed that cardiac angiotensin II levels and oxidative stress are increased in diabetic male hearts (41, 43). These changes are reduced or absent in diabetic females (41–43). Earlier work from our laboratory also showed that in vivo treatment of male rats with an angiotensin-converting enzyme (ACE) inhibitor reduces or prevents diabetes-induced increase in angiotensin II and the resulting oxidative stress (41). In the present experiments, we investigated whether ACE inhibition reverses the effects of diabetes on conduction velocity in male rats. Rats received quinapril (6 mg/kg) in their drinking water for 3 wk before the onset of diabetes, and experiments were done 7–14 days after STZ injection. In seven hearts from quinapril-treated

Table 1. The effects of elevated 9 mM potassium and 0.75 mM heptanol on the relative slowing of propagation in control and diabetic male and female hearts

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<th>Elevated Potassium</th>
<th>Heptanol</th>
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<td></td>
<td>Control</td>
<td>Diabetic</td>
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<td>Male</td>
<td>1.25±0.05</td>
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<td>Female</td>
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Values are means ± SE.
were not significant at $P = 0.05$ (for the analysis shown in Fig. 6B, $P = 0.08$). Analysis of the tissue sections was complicated due to difficulties in finding equivalent image fields, in which all of the cells were similarly oriented, in the sections from the male and female hearts. Because of this we also analyzed the distribution of Cx43 in isolated cells from control and diabetic male and female rats (see DISCUSSION for potential complications). In experiments using isolated cells, as with conduction measurements, the results proved to be variable. In some cells the extent of lateralization was found to be similar to that found in diabetic males. In others, lateralization was significantly smaller than in diabetic males and comparable with that measured in control males. On averaging measurements in a large number of cells, the distribution of Cx43 immunofluorescence showed significantly less lateralization in cells from diabetic females. The mean values for the fraction of lateralized Cx43 (the balance being in the intercalated discs) was $0.44 \pm 0.01$ in cells from diabetic males ($n = 291$ cells from 10 different preparations) and $0.38 \pm 0.01$ in cells from diabetic females ($n = 279$ cells from 9 preparations; $P < 0.002$). The mean value for the fraction of lateralized Cx43 in control male diabetic male rats, elevation of $K^+$ to 9 mM slowed conduction by a factor of $1.26 \pm 0.05$, which is significantly ($P < 0.05$) less than in diabetic males not receiving quinapril (results not shown).

Gender differences in Cx43 distribution. In parallel to optical mapping, we attempted to identify whether there are sex differences in subcellular Cx43 distribution. In previous work from our laboratory we used labeled Cx43 to show that in males there is a diabetes-induced lateralization of Cx43, relative to labeling in intercalated discs (33). In the present experiments we compared Cx43 lateralization in both tissue sections and in cells from diabetic females with lateralization in diabetic males. Upon visual examination, sections from diabetic female hearts generally appeared to show less lateralization of Cx43 than that seen in sections from diabetic males. Figure 6 shows examples from the heart of a diabetic male (Fig. 6A, images a and a') and a diabetic female (Fig. 6A, images b and b'). The brackets in images a' and b' show examples of lateralized Cx43.

For quantification of the extent of lateralization, the images of the sections were analyzed as described in METHODS. Figure 6B shows the results from the analysis of fields of cells from 20 sections (10 male and 10 female). Although there appeared to be a clear trend indicating that there was less Cx43 lateralization in the female hearts than in the male hearts, the results
myocytes was 0.24 ± 0.3. Figure 7A shows phase contrast and immunofluorescent images in a cell from a diabetic male (a and a’) and in one from a diabetic female (b and b’), showing the difference in Cx43 lateralization. Figure 7B shows the mean lateralization values in cells from diabetic males and females. The extent of Cx43 lateralization is significantly (P < 0.05) smaller in cells from diabetic females.

Finally, we examined whether angiotensin II elevation in diabetic males might play a role in Cx43 lateralization (as with conduction changes in quinapril-treated male diabetic rats). Cx43 lateralization was measured in cells isolated from quinapril-treated diabetic males. Concordant with the effects on conduction velocity, the ACE inhibitor also significantly reverses the lateralization of Cx43 in male diabetic rats. Figure 8 shows immunofluorescence images from cells from a control rat (a), a diabetic rat (b), and a quinapril-treated diabetic male (c). The results we obtained with quinapril treatment are consistent with recent findings by De Mello (7).

DISCUSSION

Interpretation and significance. The present experiments show that conduction of the cardiac impulse, measured by optical mapping, is more sensitive to perturbation in diabetic male rats compared with diabetic females.

Gap junction remodeling and change in function occurs in various cardiomyopathies (38). The present results expand and highlight the previously reported complexity of gap junction regulation and its contribution to propagation of the cardiac impulse. Multiple factors regulate gap junction function, including calcium, pH, oxidative stress, protein kinases, ischemia, and hypoxia (6, 19, 28, 51, 52).

Sex-specific differences in Cx43 mRNA or protein levels have been suggested before, at baseline (36), in aging rats (49) and under ischemic conditions (26). This is attributed to a role of estrogen in modulating Cx43 expression and function. There is some indirect evidence suggesting sex differences in ventricular (47) and atrioventricular nodal conduction velocity (29), although this has not been directly measured, to our knowledge.

The present results are the first to demonstrate that female sex hormones play a direct role in affecting conduction velocity, in parallel to the redistribution of Cx43. Thus we find that conduction of the cardiac action potential in diabetic female rats is less sensitive to reduction of cell excitability and to partial gap junction uncoupling, compared with diabetic males. The abolition of these sex differences by ovariectomy strongly indicates a key modulating effect of estrogen. The parallels between functional changes (in conduction) and structural changes (Cx43 lateralization) suggest that the sex-dependent effects on conduction are largely modulated through regulation by estrogen of Cx43 function and subcellular distribution.

The results with quinapril pretreatment further suggest that much of the estrogen-dependent effect is mediated by changes in local levels of angiotensin II in the diabetic heart. Earlier work showed that gap junction remodeling is prevented by angiotensin II receptor blockade (9). A role for angiotensin II is also suggested by Kasi et al. (22), who showed that ACE
normal and pathological conditions in several species, underlie ionic currents (35, 42, 43, 48). These results, obtained in differences in action potential configuration and underlying models (34). Earlier work has provided ample evidence for sex development of cardiac arrhythmias, both in humans and in animal species. Overexpression of Cx43 in isolated cells can be seen. In this example, Cx43 was localized primarily at the intercalated discs. Scale bar = 10 μm. In b, immunofluorescence image of a second myocyte from an STZ-treated male rat; in this case, significant lateralization of Cx43 can be seen. In c, immunofluorescence image of an isolated myocyte from a diabetic male rat pretreated with quinapril. B: summary of the analysis of Cx43 lateralization in isolated cardiac myocytes from male diabetic and quinapril-treated male diabetic rats. The fraction of fluorescence associated with ICD is given by white columns; the fraction of fluorescence from LAT is given by the black columns. Lateralization in quinapril-treated diabetic rats was significantly (P < 0.05) reduced compared with lateralization in non-treated diabetic rats (see METHODS for analysis of lateralization).

Fig. 8. Cx43 lateralization in cardiac myocytes from male diabetic rats and quinapril-treated diabetic rats. A: in a, immunofluorescence image of an isolated cardiac myocyte from a male control rat labeled with polyclonal anti-Cx43. In this example, Cx43 was localized primarily at the intercalated discs. Scale bar = 10 μm. In b, immunofluorescence image of a second myocyte from an STZ-treated male rat; in this case, significant lateralization of Cx43 can be seen. In c, immunofluorescence image of an isolated myocyte from a diabetic male rat pretreated with quinapril. B: summary of the analysis of Cx43 lateralization in isolated cardiac myocytes from male diabetic and quinapril-treated male diabetic rats. The fraction of fluorescence associated with ICD is given by white columns; the fraction of fluorescence from LAT is given by the black columns. Lateralization in quinapril-treated diabetic rats was significantly (P < 0.05) reduced compared with lateralization in non-treated diabetic rats (see METHODS for analysis of lateralization).

overexpression causes conduction defects and connexin dysfunction.

As a working model, we propose that modulation of angiotensin II levels by estrogen is a major mechanism affecting Cx43 function and thus conduction velocity. Estrogen has long been recognized as a suppressor of the RAS (11, 25). Earlier work from our laboratory (43) showed that angiotensin II elevation is smaller in diabetic female hearts than in male hearts, concordant with the present results.

There is abundant evidence for sex differences in the development of cardiac arrhythmias, both in humans and in animal models (34). Earlier work has provided ample evidence for sex differences in action potential configuration and underlying ionic currents (35, 42, 43, 48). These results, obtained in normal and pathological conditions in several species, underlie sex-related differences in arrhythmias related to abnormal automaticity or premature excitation. The present work is the first, to our knowledge, to provide evidence for sex-related differences in the propagation of the cardiac action potential. This implies that a different class of arrhythmias, dependent on formation of re-entry pathways in the heart, may also show sex differences.

It should be mentioned that the loss of Cx43 in isolated cells is not always tightly linked to loss of function (53). Nevertheless, in the present experiments, sex-dependent structural and functional changes seemed to be linked. In previous work from our laboratory (33) we ruled out contributions of changes in ion channels (Na⁺ and inward rectifier K⁺) to the functional response.

A central feature of these experiments is the large variability obtained in measurements of conduction velocity changes as well as in Cx43 lateralization. Nevertheless, significant sex differences appear when averaging large numbers of experiments. The source of this variability is not clear. The variability may be smaller in males (see Fig. 1), suggesting that variations in the estrus cycle may account for some of the large variations in effects of experimental maneuvers on conduction in females, since cardiac function is known to be affected by this cycle (54). Another source of variability may reflect changes in Cx43 that occur upon cell isolation. Barker et al. (2) showed endocytosis of Cx43 after cell dissociation. This process may vary from heart to heart and contribute to the scatter in our results. The process of endocytosis may be reduced in isolated female cells, but this issue was beyond the scope of this study. However, by using large numbers of cells, we did establish differences between cells isolated from male and female hearts subjected to identical enzymatic treatment. Thus, despite internalization of Cx43 following cell isolation, cells from females appear to maintain a more normal distribution of Cx43, which presumably also enables smaller disruption in conduction.

The use of heptanol may be problematic, due to possible additional effects on membrane lipids. Although this may complicate interpretation of the data, it is important to keep in mind that experimentally, heptanol does slow conduction. This slowing is similar in control males and control females, but slowing in diabetic males is larger than in diabetic females. Furthermore, lateralization of Cx43 is larger in diabetic males than in females.

Thus our hypothesis and conclusion that conduction slowing is greater in diabetic males than females seem justified, even if the effects of heptanol include contributions from additional mechanisms, in addition to gap junction uncoupling. Furthermore, the experiments with heptanol are only one of three interlinked experiments (along with elevated K⁺ and Cx43 measurements).

It should also be noted that heptanol may not reach steady state. However, in all experiments we used the same dose and exposure time and measured larger effects in diabetic males than females.

Thus, despite the large variability in our results, the use of two independent procedures to measure changes in function (propagation), in parallel with measurement of structural changes (Cx43 lateralization), overcomes some of the deficiencies in each individual assay, so that the combination of results...
strongly supports our conclusion that significant sex differences exist.

Caution must be taken in applying results from rodent models to humans. However, it is of great interest that in both rat and human diabetes cardiac angiotensin II levels are elevated (10, 13). There are several other common features between human diabetes and the STZ rat model, including prolongation of the QT interval in the rat (4) and human (37) ECG. QT prolongation may result from attenuated repolarizing currents. Although the complement of K+ currents determining repolarization differs in humans and rats, the rapidly activating K+ current and slow component of delayed rectifier K+ current (IKs), which are prominent in human ventricle, are attenuated in a type 1 diabetic rabbit model (55). Interestingly, IKs, is attenuated by angiotensin II (3), emphasizing the possible parallels between activation of the RAS and electrophysiological derangements in rats and humans.

Finally, our study involves a type 1 diabetes model, whereas the majority of human diabetes is type 2. Nevertheless, there is growing appreciation for an overlap between the two types: both types of diabetes involve hyperglycemia, secretory defects in pancreatic β-cells, oxidative stress, and similar changes in the ECG, as well as activation of the angiotensin system. The impact on cardiovascular mortality is very similar (20). Future studies will determine how sex hormones and diabetes-associated derangements interact to modulate gap junctions and conduction velocity, thereby further elucidating potential sex differences in arrhythmias involving conduction abnormalities.

GRANTS

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