Female Mice Lacking Estrogen Receptor β Display Prolonged Ventricular Repolarization and Reduced Ventricular Automaticity After Myocardial Infarction

Thomas Korte, MD; Martin Fuchs, MD; Andreas Arkudas, BS; Sebastian Geertz, BS; Rainer Meyer, PhD; Ajmal Gardiwal, MD; Gunnar Klein, MD; Michael Niehaus, MD; Andrée Krust, PhD; Pierre Chambon, PhD; Helmut Drexler, MD; Klaus Fink, MD; Christian Grohé, MD

Background—Major gender-based differences in the incidence of ventricular tachyarrhythmia after myocardial infarction have been shown in humans. Although the underlying mechanisms are unclear, earlier studies suggest that estrogen receptor–mediated effects play a major role in this process.

Methods and Results—We examined the effect of estrogen receptor α (ERα) and estrogen receptor β (ERβ) on the electrophysiological phenotype in female mice with and without chronic anterior myocardial infarction. There was no significant difference in overall mortality, infarct size, and parameters of left ventricular remodeling when we compared infarcted ERα-deficient and ERβ-deficient mice with infarcted wild-type animals. In the 12-hour telemetry ECG recording 6 weeks after myocardial infarction, surface ECG parameters did not show significant differences in comparisons of ERα-deficient mice versus wild-type controls, infarcted versus noninfarcted ERα-deficient mice, and infarcted ERα-deficient versus infarcted wild-type mice. However, infarcted ERβ-deficient versus noninfarcted ERβ-deficient mice showed a significant prolongation of the QT (61 ± 6 versus 48 ± 8 ms; P < 0.05) and QTc intervals (61 ± 7 versus 51 ± 9 ms; P < 0.05) and the JT (42 ± 6 versus 31 ± 4 ms; P < 0.05) and JTc intervals (42 ± 7 versus 33 ± 4 ms; P < 0.05). Furthermore, infarcted ERβ-deficient versus infarcted wild-type mice showed a significant prolongation of the QT (61 ± 6 versus 53 ± 8 ms; P < 0.05) and QTc intervals (61 ± 7 versus 53 ± 7 ms; P < 0.05) and the JT (42 ± 6 versus 31 ± 5 ms; P < 0.05) and JTc intervals (42 ± 7 versus 31 ± 5 ms; P < 0.05), accompanied by a significant decrease of ventricular premature beats (7 ± 21/h versus 71 ± 110/h; P < 0.05). Finally, real-time polymerase chain reaction–based quantitative analysis of mRNA levels showed a significantly lower expression of Kv4.3 (coding for I\(_{\text{Na}}\)) in ERβ-deficient mice (P < 0.05).

Conclusions—Estrogen receptor β deficiency results in prolonged ventricular repolarization and decreased ventricular automaticity in female mice with chronic myocardial infarction. (Circulation. 2005;111:2282-2290.)

Key Words: estrogens ■ receptors ■ mice ■ myocardial infarction ■ arrhythmia
estrogen receptor α (ERα) and estrogen receptor β (ERβ). These transcription factors can activate downstream target genes such as the endothelial-inducible isofoms of NO synthase as well as connexin 43 in the heart.

In the present study, we used an in vivo, closed-chest mouse model to test the hypothesis that ERα and ERβ per se influence ventricular repolarization and ventricular automaticity in a female mouse model with chronic anterior MI and studied the expression of potassium channels as molecular targets of ERα and ERβ underlying these differences.

Methods

Mice

The generation of all animals included in this study has been described before. A total of 71 female mice were investigated: 16 had ERα deficiency (oERKO) and 18 were WT animals, and 15 had ERβ deficiency (βERKO) and 15 were WT animals. oERKO and βERKO mice had a C57BL/6 genetic background. Because of the limitations of the breeding protocol (both oERKO and βERKO homozygous litters are infertile and had to be bred with heterozygous litters), the total number in the groups studied was controlled and was within physiological limits (data not shown). The Hannover Medical School ethics committee for animal experiments approved the study protocol, and the investigation conformed with the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health.

Coronary Artery Ligation

Six weeks before electrophysiological examination, a total of 47 mice (10 oERKO versus 13 WT; 15 βERKO versus 9 WT) underwent left anterior descending artery ligation for induction of an anterograde MI, and 24 mice (6 oERKO versus 5 WT; 7 βERKO versus 6 WT) underwent sham operation. The surgical procedure was performed as described previously.

Tissue Collection

Mice were euthanized after invasive electrophysiological study, and the hearts were fixed in situ as we have described previously.

Morphometry

The infarcted size was measured in 1 section of each of the 3 upper slices and averaged. The infarct size was performed in picrosirius red–stained slices (0.1% solution in saturated aqueous picric acid) with the use of a computerized morphometry system (Q500MC, Leitz). The infarct size was measured as the affected percentage of the total endocardial circumference of the left ventricle.

Ambulatory ECG Telemetry

Six weeks after MI, the mice underwent ambulatory ECG recordings with the use of implantable PhysioTel TA100E-A3 radiotransmitters (DataScience International) as we have described previously. All baseline surface ECG parameters were measured manually with online calipers by 2 investigators independently (T.K., S.G.), as has been defined in detail elsewhere. For each parameter, the mean of 10 consecutively measured beats was calculated. Both investigators were blinded to the genotype of the mice studied. Rate-corrected QTc and JTc intervals were calculated with the use of the following formula proposed by Mitchell et al: QTc = QT / (R-R/100) and JTc = JT / (R-R/100). Custom-made software was used to detect the R peaks of the ECG signal and to calculate the R-R intervals (Chart 3.6). The Hannover Medical School ethics committee for animal research and the government approved the study protocol, and the investigation conformed with the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health.

Invasive Electrophysiological Study Protocol

The mouse invasive electrophysiological study methodology has been previously described in detail by us and others. Mice were studied 1 day after Holter recording. Briefly, animals underwent endotracheal intubation and were ventilated. An octopolar mouse electrophysiology catheter (NuMED, Inc) was placed via the left jugular vein for pacing and endocardial electrogram recording. A standard atrial and ventricular pacing protocol was used to determine the electrophysiological parameters as previously reported. Atrial and ventricular refractoriness and atrioventricular (AV) effective and functional refractory periods were obtained with the use of a standard programmed atrial stimulation protocol. In addition, rapid atrial pacing and double and triple atrial and ventricular testing were applied to assess atrial and ventricular arrhythmia inducibility. As defined for Holter recordings, induced ventricular tachycardia was defined as nonsustained if the duration was <30 seconds.

Real-Time Polymerase Chain Reaction and Semiquantitative Measurement of Target Gene Expression

The comparative description of the expression of voltage-gated potassium channels Kv1.5 and Kv4.3 in the left ventricular tissue of the mouse was performed by SYBR GREEN real-time polymerase chain reaction (PCR) with the use of the ABI PRISM 7700 Sequence Detector (Applied Biosystsems). In this 96-well thermal cycler with laser fluorescence detection, 40-cycle PCRs were run with “Rox” as internal reference, a calibrator for normalizing variable measuring conditions, and a heating scheme following the SYBR GREEN I protocol. The ribosomal 18s served as endogenous control (“housekeeping gene”). Intercalating in double-stranded DNA SYBR GREEN (Quaigen) indicates the gain of new amplicons within each cycle. Suitable primer pairs were designed by using PRIMER EXPRESS Software and checked by BLAST search for their specificity. By melting curve analysis, only target gene amplicons were verified when the presence of primer dimer or spurious products was ruled out. The stable efficacy of the chosen primer pairs was tested in different concentrations. Relative gene expression was calculated because of conditions at that stage of PCR when amplification was logarithmic and thus could be correlated with an initial copy number of gene transcription. The relative Kv gene expression is shown as a percentage of part of the full amount of measured Kv cDNA in each sample.

Mouse Genetic Primer

Mouse genetic primers were as follows: Kv4.3: forward primer GCTC-CAGCGGAAGAACAA, reverse primer GTCTGGAACC TGTCGTTCACTT; Kv1.5: forward primer GGCACCACGTCGAT- GAT, reverse primer ACATGCGCACGAAACGGTAACGA.

Data Acquisition and Analysis

Surface ECGs, endocardial electrograms, and telemetry electrogram recordings were acquired on a multichannel amplifier and converted to a digital signal for analysis (MacLab System, AD Instruments). Signals were recorded at a sampling rate of 1000 Hz.

Statistical Analysis

All continuous variables, such as ECG intervals and cardiac conduction properties, were compared with controls, with data presented as mean±SD. Mortality and the incidence of inducible or spontaneous...
ous arrhythmia were compared by the χ² test or Fisher exact test. For comparison of the infarct size and electrophysiological parameters between 2 groups, the Student t test or ANOVA was used, when applicable. For comparison of ventricular automaticity during ambulatory ECG recording, VPBs per 12 hours were counted and compared between 2 groups by use of the unpaired Student t test. A probability value of <0.05 was considered statistically significant.

Comparisons among ERα animals were as follows: (1) αERKO versus WT (to test solely the effect due to ERα deficiency); (2) αERKO versus αERKO+MI (to test solely the effect of MI on animals with receptor deficiency); and (3) αERKO+MI versus WT+MI (to test the effect of ERα deficiency on animals with MI).

Comparisons among ERβ animals were as follows: (1) βERKO versus WT (to test solely the effect due to ERβ deficiency); (2) βERKO versus βERKO+MI (to test solely the effect of MI on animals with receptor deficiency); and (3) βERKO+MI versus WT+MI (to test the effect of ERβ deficiency on animals with MI).

Results

Animals, Mortality, and Infarct Size

Absolute numbers of animals with induction of MI or sham operation are given in Table 1. There was no statistically significant difference in mortality during the 6 weeks after MI when we compared αERKO+MI and βERKO+MI with infarcted WT animals. MI resulted in a significant increase in the ratio of heart weight to body weight in comparison to sham-operated controls. There were no significant differences in infarct size and ratio of heart weight to body weight when we compared αERKO+MI and βERKO+MI with infarcted WT animals.

Ambulatory ECG Telemetry

ECG Data

The total number of ambulatory ECGs performed and the results of the ECG data are summarized in Table 2. There was a significant prolongation of QT, QTc, JT, and JTc when we compared βERKO with βERKO+MI and βERKO+MI with infarcted WT animals (Figures 1 and 2).

There was no significant difference in duration of P, PR, QRS, QT, QTc, JT, and JTc when we compared αERKO and βERKO with WT controls and compared WT controls with

---

**TABLE 1. No. of Total Mice, Cumulative Deaths, Infarct Size, Cardiac Weights, and Dimensions**

<table>
<thead>
<tr>
<th></th>
<th>Total Mice</th>
<th>BW</th>
<th>HW/BW</th>
<th>Total Deaths</th>
<th>Infarct Size, %</th>
<th>Septum Thickness, mm</th>
<th>LV Diameter, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>αERKO sham MI</td>
<td>5</td>
<td>28±5</td>
<td>5.6±1</td>
<td>...</td>
<td>...</td>
<td>1.4±0.4</td>
<td>2.8±0.8</td>
</tr>
<tr>
<td>WT sham MI</td>
<td>13</td>
<td>27±5</td>
<td>8.6±1</td>
<td>4</td>
<td>60±1</td>
<td>1.4±0.4</td>
<td>3.9±1</td>
</tr>
<tr>
<td>αERKO+MI</td>
<td>6</td>
<td>29±5</td>
<td>6.1±1.8</td>
<td>...</td>
<td>...</td>
<td>1.3±0.1</td>
<td>3.8±0.6</td>
</tr>
<tr>
<td>βERKO sham MI</td>
<td>10</td>
<td>28±6</td>
<td>8±2.6</td>
<td>3</td>
<td>60±2</td>
<td>1.4±0.2</td>
<td>3.8±1</td>
</tr>
<tr>
<td>βERKO+MI</td>
<td>6</td>
<td>24±3</td>
<td>6±0.1</td>
<td>...</td>
<td>...</td>
<td>1.3±0.1</td>
<td>3.6±0.2</td>
</tr>
</tbody>
</table>

*HW indicates heart weight (measured in milligrams); BW, body weight (measured in grams); and LV, left ventricle.*

---

**TABLE 2. Surface ECG Conduction Intervals in Mice During Ambulatory ECG Recording**

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>P, ms</th>
<th>PR, ms</th>
<th>QRS, ms</th>
<th>QT, ms</th>
<th>QTc, ms</th>
<th>JT, ms</th>
<th>J TC, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>αERKO WT sham MI</td>
<td>4</td>
<td>95±7</td>
<td>16±1</td>
<td>36±3</td>
<td>22±4</td>
<td>51±6</td>
<td>53±6</td>
<td>30±3</td>
</tr>
<tr>
<td>αERKO WT+MI</td>
<td>9</td>
<td>91±4</td>
<td>16±3</td>
<td>32±4</td>
<td>23±7</td>
<td>52±1</td>
<td>59±10</td>
<td>34±6</td>
</tr>
<tr>
<td>αERKO+MI</td>
<td>6</td>
<td>95±7</td>
<td>16±3</td>
<td>38±1</td>
<td>20±4</td>
<td>53±8</td>
<td>54±9</td>
<td>33±5</td>
</tr>
<tr>
<td>αERKO+MI</td>
<td>7</td>
<td>98±7</td>
<td>17±3</td>
<td>38±3</td>
<td>25±4</td>
<td>61±9</td>
<td>62±10</td>
<td>36±8</td>
</tr>
<tr>
<td>βERKO WT sham MI</td>
<td>6</td>
<td>91±6</td>
<td>15±2</td>
<td>37±3</td>
<td>16±3</td>
<td>47±5</td>
<td>50±6</td>
<td>31±3</td>
</tr>
<tr>
<td>βERKO WT+MI</td>
<td>5</td>
<td>96±6</td>
<td>16±2</td>
<td>36±5</td>
<td>22±6</td>
<td>53±8</td>
<td>53±7</td>
<td>31±5</td>
</tr>
<tr>
<td>βERKO+MI</td>
<td>5</td>
<td>90±4</td>
<td>15±3</td>
<td>36±3</td>
<td>17±5</td>
<td>48±8</td>
<td>51±9</td>
<td>31±4</td>
</tr>
<tr>
<td>βERKO+MI</td>
<td>10</td>
<td>100±5</td>
<td>15±2</td>
<td>38±5</td>
<td>20±3</td>
<td>61±6*</td>
<td>61±7*</td>
<td>42±6*</td>
</tr>
</tbody>
</table>

*R-R indicates R-R interval; P, duration of P wave; PR, duration of PR interval; QRS, duration of QRS interval; QT, duration of QT interval; QTc, rate-corrected duration of QT interval; JT, duration of JT interval; and JTc, rate-corrected duration of JT interval.*

*p < 0.05 compared with WT+MI and βERKO sham MI.*
and without MI. There also was no significant difference when we compared αERKO with αERKO+MI and αERKO+MI with infarcted WT animals.

**Baseline Heart Rate and Arrhythmia Recording**

The mean R-R during the 12-hour Holter recording was not significantly different when we compared αERKO and βERKO with controls and αERKO+MI and βERKO+MI with infarcted WT animals.

No VPBs or ventricular tachycardias were documented in noninfarcted WT, αERKO, and βERKO animals during 12-hour Holter recording. In infarcted αERKO mice 14±30 (range, 0 to 83) VPBs per hour (2 of 7 animals) and in infarcted WT animals 51±99 (range, 0 to 250) VPBs per hour (3 of 9 animals) were documented (P>0.05). In infarcted βERKO animals 7±21 (range, 0 to 82) VPBs per hour (3 of 9 animals) and in infarcted WT animals 71±111 (range, 0 to 333) VPBs per hour (4 of 6 animals) were documented (P<0.05; Figure 3). In 1 infarcted WT animal, recurrent nonsustained ventricular tachycardia was documented (Figure 4).

**Electrophysiological Study**

The total number of animals with completed electrophysiological study and the results of surface ECG parameters and of the electrophysiological data are summarized in Table 3.

**Cardiac Conduction Properties and Electrophysiological Data**

There was no significant difference in all groups compared with regard to sinus node function and atrioventricular conduction (AV interval, AV Wenckebach cycle length, AV 2:1, AV effective refractory periods, AV functional refractory periods). Furthermore, there was no statistically significant
difference in any of the groups compared with regard to ventricular refractoriness.

**Programmed Stimulation and Arrhythmia Inducibility**

With the use of standard programmed electrical stimulation protocols and burst atrial and ventricular pacing, provocation of ectopic or reentrant rhythms was attempted. No animals experienced spontaneous ventricular arrhythmias during placement of the catheter. No nonsustained ventricular tachycardia/sustained ventricular tachycardia was inducible. There was no statistically significant difference in ventricular tachycardia inducibility in any of the groups compared. Inducibility of atrial tachycardia/atrial fibrillation is summarized in Table 3; there was no statistically significance in any of the compared groups.

**Expression of Different K⁺ Channels in the Mouse Ventricle**

To establish whether the prolongation of repolarization in infarcted female βERKO mice is due to differential expression of fast- and slow-rectifying potassium channels, we analyzed mRNA expression of selected K⁺ channels with real-time PCR using RNA harvested from the intact left ventricle of female αERKO and βERKO animals and their controls. The K⁺ channels examined included Kv1.5 (coding...
for $I_{Na}$ and Kv4.3 (coding for $I_{K,1}$). We chose these channels because earlier reports suggest that the expression and function of these 2 potassium channels are likely to be dependent on the sex hormone receptor status (ie, estrogen receptors).13,34 Whereas the transcript levels of Kv1.5 showed no significant difference, there was a significantly lower expression of Kv4.3 in female ERKO mice, ventricular repolarization and increased left ventricular diameter in female mice with knockout of ERB.

**Discussion**

In infarcted female βERKO mice, ventricular repolarization is significantly prolonged and ventricular spontaneity is significantly decreased. This finding is accompanied by a significant and specific lower expression of Kv4.3 in βERKO animals. Thus, ERB plays a significant role in ventricular repolarization and automaticity in the female mouse heart after MI, which is mediated, at least in part, by downregulation of Kv4.3 expression.

**Mortality, Infarct Size, and Left Ventricular Remodeling**

This study showed no evidence that infarct size, ventricular remodeling, and mortality are significantly altered by ERα and ERβ. Thus, the electrophysiological differences shown in female βERKO mice with chronic MI do not appear to be related to infarct size or the extent of left ventricular remodeling in this subgroup of animals and the time point studied.

The role of estrogen, particularly 17β-estradiol, has been studied extensively in different models of myocardial ischemia, whereas the role of the respective estrogen receptors remains to be elucidated. We recently showed a reduction in chronic infarct size and cardiomyocyte apoptosis in ovariec-tomized female mice treated with 17β-estradiol.35 However, estrogen can increase post-MI ventricular remodeling and mortality. Cavasin et al36 found a decreased ejection fraction and increased left ventricular diameter in female mice with

**TABLE 3. Electrophysiological Data Summary**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>SNRT, ms</th>
<th>AV, ms</th>
<th>AWCL, ms</th>
<th>AV 2:1, ms</th>
<th>AVERP, ms</th>
<th>AVFRP, ms</th>
<th>VERP, ms</th>
<th>VT, n</th>
<th>AT/AF, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>αERKO</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT sham MI</td>
<td>4</td>
<td>263±71</td>
<td>43±17</td>
<td>97±12</td>
<td>60±1</td>
<td>63±14</td>
<td>102±6</td>
<td>25±14</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>WT + MI</td>
<td>9</td>
<td>190±22</td>
<td>44±5</td>
<td>89±7</td>
<td>59±5</td>
<td>56±1</td>
<td>96±8</td>
<td>26±14</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>αERKO sham MI</td>
<td>6</td>
<td>230±14</td>
<td>57±5</td>
<td>100±7</td>
<td>64±6</td>
<td>59±7</td>
<td>98±5</td>
<td>23±4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>αERKO + MI</td>
<td>7</td>
<td>193±46</td>
<td>51±8</td>
<td>95±6</td>
<td>63±5</td>
<td>55±4</td>
<td>102±6</td>
<td>33±14</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>βERKO</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT sham MI</td>
<td>6</td>
<td>236±36</td>
<td>37±22</td>
<td>96±4</td>
<td>60±2</td>
<td>54±7</td>
<td>97±1</td>
<td>32±12</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>WT + MI</td>
<td>5</td>
<td>205±3</td>
<td>61±13</td>
<td>90±5</td>
<td>70±5</td>
<td>60±6</td>
<td>96±4</td>
<td>28±5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>βERKO sham MI</td>
<td>5</td>
<td>192±33</td>
<td>54±4</td>
<td>94±5</td>
<td>69±3</td>
<td>60±7</td>
<td>99±6</td>
<td>40±14</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>βERKO + MI</td>
<td>9</td>
<td>168±25</td>
<td>45±18</td>
<td>90±1</td>
<td>60±5</td>
<td>75±35</td>
<td>105±26</td>
<td>32±8</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

SNRT indicates sinus node recovery time; AV, AV interval; AWCL, AV Wenckebach cycle length; AVERP, AV effective refractory period; AVFRP, AV functional refractory period; VERP, ventricular effective refractory period; VT, ventricular tachycardia; AT, atrial tachycardia; and AF, atrial fibrillation.

**Figure 4.** Ambulatory telemetry recording of an awake, unrestrained WT mouse with MI. Sinus rhythm (SR) with a cycle length of 83 ms and recurrent runs of VPBs are shown.
Role of ERβ for Ventricular Repolarization and Spontaneity and Potassium Channel Expression in Noninfarcted and Infarcted Female Mouse Heart

In a previous study we and others described a prolongation of repolarization and increased ventricular vulnerability in C57 BL/6 WT mice with chronic MI.30,42 Huang et al43 found a significantly prolonged ventricular action potential duration and increased ventricular vulnerability in the infarcted rat heart, accompanied by a significant decrease in expression of both Kv2.1 and Kv4.3. Kääb et al44 showed reduced expression and function of Kv4.3 as a potential mechanism of action potential prolongation in failing human heart with chronic MI.

The mechanisms underlying gender-specific differences in cardiac repolarization are still largely unknown. Hormonal regulation of cardiac K⁺ channel gene expression may affect electrical activity. The issue of the role of sex steroid hormones in the regulation of K⁺ channel expression is of major importance and has been the focus of previous studies.45–49 However, the results of animal studies with regard to the electrophysiological effects of sex hormones have been divergent, and it is not clear from previous data whether sex steroid hormones consistently alter cardiac repolarization.50–52 Trepnair-Boulay et al13 found a significantly prolonged ventricular repolarization as measured by action potential duration in female mice compared with male mice, which was accompanied by a significantly decreased expression of Kv1.5 and of its corresponding K⁺ current, Iₖur, in the female ventricle. Saba et al22 found that 17β-estradiol prolongs AV nodal conduction and the right ventricular effective period, and they argue that hormonal status affects aspects of cardiac electrophysiological function. Furthermore, Drici et al23 did not find gender differences with regard to QT interval, action potential duration, dispersion of refractory periods, and conduction velocities in Langendorff-perfused WT mice but described inducibility of significantly longer periods of polymorphic ventricular tachycardia in female mice uncovered by halothane, accompanied by a pronounced expression of KCNE1. Song et al34 were the first to show a direct influence of estrogen on the transcription, ie, expression, of Kv4.3 in the myometrium of female rats.

This study demonstrates, for the first time, a significant prolongation of repolarization (QT, QTc, JT, JTc) in infarcted female mice compared with noninfarcted transgenic animals and infarcted WT animals. Furthermore, only infarcted βERKO mice had significantly fewer VPBs in 12-hour ECG monitoring. From the data of the present study, it can be hypothesized that prolongation of repolarization caused the reduction of ventricular spontaneity in the infarcted βERKO animals, although an altered dispersion of repolarization might also have contributed to this effect.52 Future studies will have to address this aspect.

To further characterize the underlying mechanisms, we studied the expression of Kv1.5 and Kv4.3 in αERKO and βERKO animals because these channels play an important role in cardiac repolarization and earlier reports suggest that their expression and function might be altered by sex hormones.13,34 We showed a decreased expression of Kv4.3 (coding for Iₖur) in the βERKO left ventricle but not in the αERKO left ventricle. The expression of Kv1.5 was unchanged in both αERKO and βERKO animals. These data suggest a downregulation of the expression of Kv4.3 in the female mouse heart via ERβ. The influence of ERβ on potassium channel expression is not apparent during electrophysiological examination of the βERKO mouse but becomes apparent after MI of βERKO animals. The prolonged repolarization is not due to the chronic MI because in this model prolonged repolarization is not apparent in infarcted WT controls and is not apparent in infarcted αERKO animals and their controls. Future studies will have to focus on how ERβ regulates potassium channel expression and to what extent regulation via ERβ is estrogen dependent.

Limitations

There are limitations in the translation of the results of this study to other species and to human physiology. K⁺ channels may play different roles in repolarization, and the effect of sex hormones on repolarization might significantly differ among species.52 To date, in vivo mouse electrophysiology has been proven to be a helpful tool to understand the underlying mechanisms involved in the pathology of clinically relevant cardiac electrophysiology. This animal model serves as the transgenic model of choice because of a combination of technical difficulties in transgenic techniques for larger animals, as well as cost, reproduction, and ethical issues.53 Future research is needed to directly characterize

Figure 5. Significantly reduced relative mRNA expression of Kv4.3 in the female βERKO heart compared with WT animals. Rel. indicates relative.
gender-based differences found in cardiac repolarization comparing estrogen receptor–deficient female and male mice.

Significance of the Present Study

The present study provides new insight into the role of estrogen receptor β in the pathophysiology of cardiac arrhythmias after MI. Our study suggests regulation of potassium channel expression via ERβ, which significantly influences ventricular repolarization and automaticity in the female heart after MI. The work thus improves our understanding of the fundamental mechanisms by which sex hormones influence cardiac repolarization and highlights our awareness of gender differences in the control of K⁺ channel gene expression.

Acknowledgments

This study was supported by Deutsche Forschungsgemeinschaft, Deutsche Herzstiftung, and institutional grants by BONFOR. We thank Olivier Smithies for sharing the transgenic animals with knockout of estrogen receptor β. We thank Hanne Bock, Martina Lennarz, Charlotte Halstrick, and Axel Allera for expert technical assistance and advice.

References


45. Pham TV, Sosunov EA, Gainullin RZ, Danilo P Jr, Rosen MR. Impact of sex and gonadal steroids on prolongation of ventricular repolarization and arrhythmias induced by I\textsubscript{K}-blocking drugs. Circulation. 2001;103:2207–2212.
Female Mice Lacking Estrogen Receptor β Display Prolonged Ventricular Repolarization and Reduced Ventricular Automaticity After Myocardial Infarction

Thomas Korte, Martin Fuchs, Andreas Arkudas, Sebastian Geertz, Rainer Meyer, Ajmal Gardiwal, Gunnar Klein, Michael Niehaus, Andrée Krust, Pierre Chambon, Helmut Drexler, Klaus Fink and Christian Grohé

_Circulation_. 2005;111:2282-2290; originally published online May 2, 2005; doi: 10.1161/01.CIR.0000164262.08004.BB

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/111/18/2282

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ 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_ is online at:
http://circ.ahajournals.org//subscriptions/