Mice Display Sex Differences in Halothane-Induced Polymorphic Ventricular Tachycardia

Milou-Daniel Drici, MD, PhD; Linda Baker, PhD; Patricia Plan; Jacques Barhanin, PhD; Georges Romey, PhD; Guy Salama, PhD

Background—Molecularly engineered mice are extensively used as models of cardiovascular diseases, yet little is known about sex differences in the electrophysiology of mouse hearts.

Methods and Results—This study investigated the influence of sex on drug-induced polymorphic ventricular tachycardia (PVT) in Langendorff-perfused male and female mice hearts (n=54) by injecting a bolus of halothane (1.75 mmol/L) in the perfusate while recording ECGs or optical action potentials (APs). There were no statistically significant differences between male and female hearts (n=54) with respect to mean RR (193±5 ms), PR (47±1 ms), QT intervals (101±3 ms), optical AP durations (APD$_{75}$=23.11±4.2 ms), dispersion of refractory periods, and conduction velocities (n=5 male and 5 female). Halothane induced PVTs lasting a mean duration of 90 seconds; in female hearts, 55% of PVTs lasted longer than the median, whereas in male hearts 17% exceeded the mean (P<0.05). The total duration of PVTs exposed a marked sex difference, 378±144 seconds in female versus 27±10 seconds in male hearts (P<0.05). In optically mapped male hearts, halothane reduced APD$_{75}$ (17.61±1.6 ms) and then elicited VTs (n=6 of 6), but in female hearts, halothane elicited PVTs (n=1 of 6) or arrested the hearts (n=5 of 6). Except for KCNE1, Northern blots (KCNQ1, MERG, Kv1.5, connexins 40 and 43, TREK1, and TASK1) did not detect sex differences.

Conclusions—This mouse model reveals sex difference in response to a pharmacological challenge yet does not display sex differences in standard electrophysiological parameters. Differences in KCNE1 may contribute to sex differences uncovered by halothane. (Circulation. 2002;106:497-503.)

Key Words: action potentials • tachycardia • sex • electrophysiology • mapping

Female sex is associated with two thirds of the cases of drug-induced polymorphic ventricular tachycardia (PVTs), such as Torsades de Pointes (TdP), which reflects how sex differences may impair women’s health. Clinical and experimental studies show that female sex is associated with a greater drug-induced QT prolongation, which facilitates the emergence of TdP. Part of this difference has been ascribed to sex-specific regulation of ionic channel expression by sex steroids. However, a detailed characterization of sex-related alterations of channel expression remains difficult because of spatial heterogeneities in ion channels resulting in dispersions of action potential durations (APDs) and repolarization across the ventricles. Studies have explored the effects of androgen and estrogen replacement in oophorectomized animals and shed new light on how they influence the QT interval. Sex was shown to influence the QT adaptation to heart rate but in mice, difficulties in measuring APDs or QT dispersion or eliciting arrhythmia have led to the notion that mice are of limited use in this area.

The present report reveals sex differences in perfused spontaneously beating mouse hearts by exposing the hearts to halothane while recording surface electrograms and optical maps of electrical activity elucidate the nature of halothane-induced arrhythmias.

Methods

Animal Preparations

Sex differences in halothane-induced arrhythmias were studied in mouse hearts perfused in a Langendorff apparatus adapted for ECG or optical mapping. Briefly, mice (Charles River Laboratories, Paris, France) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and maintained under anesthesia throughout the experiments. Male and female mice (8-16 weeks old, n=6 per group) were perfused in the Langendorff apparatus, with a bolus of halothane (1.75 mmol/L) added to the perfusate. ECG recordings were obtained from the epicardial surface, and optical mapping was performed as previously described. Halothane was continuously infused at a concentration of 1.75 mmol/L, and the halothane concentration was monitored continuously using a halothane analyzer (Baker Instruments, NJ). 

The current study was conducted in accordance with the guidelines established by the American Heart Association and the ethical principles of the Declaration of Helsinki, and the protocol was approved by the institutional animal care and use committee.

Received February 22, 2002; revision received April 30, 2002; accepted May 1, 2002.

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Circulation is available at http://www.circulationaha.org

DOI: 10.1161/01.CIR.0000023629.72479.24
Wilmington, Mass) were anesthetized with sodium pentobarbital (35 mg/kg IP) plus heparin (400 UI/kg). The heart was removed via a midsternal thoracotomy, cannulated at the aorta, and retrogradely perfused at constant flow rate (2.3 mL/min) with Tyrode’s solution containing (in mmol/L) NaCl 112, KCl 5, NaHCO3 25, KH2PO4 1, MgSO4 1.2, CaCl2 1.8, and glucose 50, gassed with 95% O2 and 5% CO2 at pH 7.38 and 37°C. Pilot experiments (n=5) were performed to determine the stability of the model and showed no signs of rundown for >3 hours with respect to heart rate, ECG, and AP characteristics. ECG studies consisted of 12-week-old male and female OF1 mice (n=54) weighing 23 to 32 g.

**Optical Apparatus**

In parallel experiments, hearts were stained with the voltage-sensitive dye di-4-ANEPPS (10 µL of 2 mmol/L in DMSO), as previously described. Light from a halogen lamp was collimated, passed through an interference filter (520±30 nm), and focused on the left epicardium by epi-illumination. Fluorescence was collected with a camera lens passed through a long-pass filter (>630 nm), and an image of the heart was focused on a 16×16-element photodiode array to simultaneously monitor APs from 256 diodes. Each diode (0.9×0.9 mm2, 0.11 mm dead space) recorded APs from hundreds of cells (330×330 µm2 area and a depth of ~70 µm). The photocurrent from each diode was passed through an IV converter (3-MΩ feedback resistor), amplified, digitized, and stored in computer memory. The perfused hearts were mounted in a chamber designed to abate movement artifacts without the use of chemical uncoupplers, which modify the mouse AP.12

**Northern Blot Analysis**

Ventricle RNAs were isolated from 3-week-old and 2-month-old OF1 male and female littermate mouse hearts. Poly(A)+ RNA (2 µg) were separated by electrophoresis on 1% agarose gel and transferred onto nylon membranes (Hybond N, Amersham). Blots were probed with 32 P-labeled specific cDNA fragments of the different cardiac-channel subunits, including KCNQ1, KCNE1, MERG, and Kv1.5, as well as gap-junction subunits connexins 40 and 43 and the 2P channel TREK1 and TASK1 in Express Hybrid solution (Clontech) at 60°C for 16 hours, washed stepwise to a final stringency of 0.2 SSC, 0.3% SDS at 60°C, and exposed to a Fuji BAS-1500 imager for radioactivity quantification. Variations in loading were corrected by -actin probing for internal quantification. Each channel signal was divided by the corresponding -actin signal, yielding a ratio that allowed comparison according to sex.

**Electrogram Recordings**

Signals were recorded with 4 Ag+/-AgCl electrodes from unpaced hearts, which flank the epicardium in a simulated Einthoven configuration. Electrodes were connected to an adjustable band-pass differential amplifier (ORTEC Inc). ECG channels were amplified and filtered (1 to 2 kHz), stored, and analyzed with the use of pCLAMP (Axon Instruments). The PR interval was measured from the beginning of the P wave to the beginning of the R wave and the QT from the beginning of the Q (or the base of the R wave if not possible) to the end of the T wave, defined as the point at which the voltage returns to the isoelectric baseline. For each heart, a set of 3 consecutive RR-QT interval pairs was obtained from ECG recordings. This investigation conformed to the guidelines of the Universities of Nice and Pittsburgh and to the Guide for Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1996).

**Halothane Challenge to the Isolated Heart**

A Tyrode solution containing 1.75 mmol/L halothane was prepared by diluting Tyrode saturated with 17.5 mmol/L Halothane (Laboratories) and verified by gas chromatography. A 200-µL bolus of 17.5 mmol/L halothane was injected in the 1.8-mL reservoir of Tyrode that serves as a compliance chamber. Continuous perfusion with fresh Tyrode washed out the halothane such that the bolus exposed the heart to a maximum concentration of 1.75 mmol/L halothane that decreased rapidly.

Pilot measurements monitored the occurrence of arrhythmia after a 200-µL bolus of halothane; if no arrhythmia occurred, a 300-µL bolus and then a 400-µL bolus were injected after a 5-minute delay. The bolus of 200 µL was chosen because it provoked a ventricular arrhythmia in 5 out of 5 female hearts. In other experiments, an arrhythmia was induced and then propranolol (1 µmol/L) was perfused for 10 minutes before repeating the halothane challenge.

**Analysis of Optical Data**

Simultaneously recorded optical APs were used to determine the spatial heterogeneities of APDs, activation, repolarization patterns, and refractory periods. The activation time point at each diode was taken as the maximum first derivative of the fluorescence upstroke (dF/dtmax), and APDy was calculated from the activation minus the repolarization time point (ie, the time when the downstroke recovers 75% to baseline).12,15 Hearts were under sinus rhythm or paced with electrodes on the right or left ventricles at the apex or base of the free wall. Mean APD3 (mean±SD) was averaged from 9 diodes on the center of the left ventricle and compared for statistical significance between male and female hearts (CL=200 ms). Refractory periods were measured by pacing the heart (S1 to S2=200 ms) for 10 beats and then applying a premature impulse (S3) at decreasing S1 to S2 intervals from 200 to 100 ms in 5-ms steps, from 100 to 50 ms in 2-ms steps, and then from 50 ms until refractory period in 1-ms steps.12 Isochronal maps of activation were generated from local activation time points and conduction velocities from the sum of local velocity vectors.12,15 APs with S/N ratios <10/1 or excessive movement artifact were not included in the analysis. Isochronal maps of activation were generated with the Delaunay’s triangulation algorithm. Fast Fourier Transforms (FFTs) were calculated to characterize the frequency distribution and spatial organization of VT.

**Statistical Analysis**

Analysis of ECG waveforms was reported as mean±SEM. Global descriptive statistics were performed. Statistical analysis according to sex included the proportion of drug-induced arrhythmia that lasted ≥90 seconds (median value of the sample) (analyzed with Fisher’s exact test) and the duration of the anesthetic-induced arrhythmia (analyzed with 1-way ANOVA). Because the variability of the data increased with the duration of the arrhythmia and in some hearts an occasional zero value arose (absence of arrhythmia), a logarithmic transformation of the variable was performed according to the formula $y=\log(1+x)$, where x is the total duration of the anesthetic-induced ventricular arrhythmia (in seconds). Continuous variables, such as APD90, RR, QT, and PR interval values and their increase from baseline, were analyzed with 1-way ANOVA, Student’s t test, or Mann-Whitney-Wilcoxon rank-sum test when applicable (all tests performed with Statview 4.5 and SuperAnova 1.11, Abacus Corp). A value of $P<0.05$ was considered statistically significant.

**Results**

**Sex Differences in ECG Waveform**

ECG recordings of Langendorff-perfused hearts began 10 minutes after the start of each experiment. The excellent stability of ECG signals made it possible to reliably measure the P, PR, and QT intervals in a total of 54 hearts. As illustrated in Figure 1, isolated hearts in sinus rhythm had the following mean values (in ms): RR, 193±25; P-wave duration, 21±1; PR, 47±1; and QRS, 15±0.5 (n=54). The average QT interval was 101±3 ms, yielding a corrected QT (Fridercia) of 175±4 ms (Figure 1A). Females tended to have a slower heart rate than males and a longer QT and QTc,
base), n = 5 hearts, in ms.

velocity in m/s calculated from the average conduction velocity in 5 hearts paced at 200 ms CL; and ARP, gradients of refractoriness (difference between apex and base), n = 5 hearts, in ms.

average CL during VT was 57 ± 2 ms (n = 44 hearts). The mean duration of these arrhythmias (median, 90 seconds) was approximately equal to the time taken for the bolus of halothane to pass and wash out through the coronary circulation. After washout, arrhythmias ceased and the heart returned to sinus rhythm (Figure 1D) at a slower rate (RR = 232 ± 8 ms) and with longer PR intervals (75 ± 3 ms, P < 0.0001), which gradually returned to control in ~3 minutes. Table 2 summarizes the changes in electrophysiological parameters caused by halothane.

### Sex and Arrhythmias

Even though the time elapsed from the bolus injection to the onset of arrhythmia did not differ between sexes (male, 38 ± 3 seconds versus female, 40 ± 2 seconds, P > 0.7), the occurrence and the duration of PVTs did vary with sex (Figure 2A). Male hearts were more resistant (25%) to halothane-induced PVTs than were their female counterparts (0%, P < 0.05), and the durations of arrhythmias markedly differed according to sex (Figure 2B). Male hearts (17%) had PVTs lasting longer than the median value of 90 seconds, whereas most female hearts (55%) had arrhythmias exceeding it (P < 0.05). The duration of PVTs was longer in female (378 ± 44 seconds) than in male (27 ± 10 seconds) hearts (n = 49, P < 0.05) (Figures 2B and C). The cycle length of arrhythmias tended to be longer in females (60 ± 3 ms) than in males (51 ± 5 ms), but the difference fell within experimental error (P = 0.07). Propranolol (1 μmol/L) was remarkably effective at protecting the hearts from halothane-induced arrhythmias in both sexes (n = 5).

### Sex Differences in APDs and Refractory Periods

Optical recordings detected a tendency for shorter APD_{75} in male compared with female hearts, although the difference was not statistically significant. The mean spontaneous CL

### Table 1. Electrophysiological Parameters of Male and Female Mouse Hearts

<table>
<thead>
<tr>
<th></th>
<th>RR</th>
<th>QT</th>
<th>QTC</th>
<th>APD_{75}</th>
<th>ΔRP</th>
<th>Θ_{mean}</th>
<th>Start PVT</th>
<th>Incidence of PVT</th>
<th>PVT &gt; 90 s</th>
<th>Duration of PVT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>193 ± 8</td>
<td>99 ± 4</td>
<td>172 ± 6</td>
<td>21.49 ± 1.43</td>
<td>9.6 ± 0.89</td>
<td>0.47 ± 0.06</td>
<td>38 ± 3</td>
<td>89%</td>
<td>17%</td>
<td>27 ± 10</td>
</tr>
<tr>
<td>Females</td>
<td>196 ± 7</td>
<td>103 ± 4</td>
<td>178 ± 6</td>
<td>22.62 ± 0.78</td>
<td>10.8 ± 2.5</td>
<td>0.45 ± 0.07</td>
<td>40 ± 2</td>
<td>100%*</td>
<td>55%</td>
<td>378 ± 144†</td>
</tr>
</tbody>
</table>

**Notes:**
- RR, QT, and QTC values represent means ± SD in ms; QTC = QT/RR^{1/2}.
- APD_{75} indicates optical APDs measured at 75% recovery to baseline, at 200 ms CL, averaged over 9 diodes per heart (n = 5 hearts) in ms; Θ_{mean} mean conduction velocity in m/s calculated from the average conduction velocity in 5 hearts paced at 200 ms CL; and ARP, gradients of refractoriness (difference between apex and base), n = 5 hearts, in ms.
- Statistically significant values between male and female hearts are found for the incidence (*P < 0.05) and duration †(P < 0.05) of PVT.
was 225±12 ms with an APD$_{75}$=23.11±4.2 ms (n=5 male and 5 female, 9 diodes/heart) in the absence of halothane (Figure 3A). When paced at a CL of 200 ms, ventricular APD$_{75}$ of male and female hearts (n=5 hearts each) were not statistically different. Refractory periods were heterogeneous across the epicardium, being short at the apex and progressively longer toward the base. The gradient of refractoriness (difference between apex and base) had a tendency of being greater in female than in male hearts (10.8±2.5 versus 9.6±0.89 ms), but without statistical significance (Table 1). Halothane (200-µL bolus) elicited a tachycardia (CL=65 ms) and a shortening of APD$_{75}$ to 17.61±1.6 ms (n=5) (Figure 3C). An activation pattern of an arrhythmic beat (Figure 3D) shows that activation propagated from right to left (bright to dark) in 5 to 6 ms. The stability of activation patterns and ECG from the opposite side of the heart indicated that the arrhythmia was a reentrant monomorphic circuit around the perimeter of the heart.12

The nature of arrhythmias elicited by halothane was investigated by mapping optical voltage oscillations during VTs. Halothane elicited more severe arrhythmias in female than in male hearts, resulting in complete arrest of female hearts (10.8±2.5 versus 9.6±0.89 ms), but without statistical significance (Table 1). Halothane (200-µL bolus) elicited a tachycardia (CL=65 ms) and a shortening of APD$_{75}$ to 17.61±1.6 ms (n=5) (Figure 3C). An activation pattern of an arrhythmic beat (Figure 3D) shows that activation propagated from right to left (bright to dark) in 5 to 6 ms. The stability of activation patterns and ECG from the opposite side of the heart indicated that the arrhythmia was a reentrant monomorphic circuit around the perimeter of the heart.12

The nature of arrhythmias elicited by halothane was investigated by mapping optical voltage oscillations during VTs. Halothane elicited more severe arrhythmias in female than male hearts, resulting in complete arrest of female hearts but brief MVTs (n=4 of 6) or PVTs (n=2 of 6) in male hearts. MVTs (960 beats/min) had a dominant frequency (mean 13.9±3.3 ms Hz, n=4) in their FFT spectra (Figure 3E) and Poincaré maps of interaction intervals with stable CL (65.0±3.24, n=9 beats), with all points falling in a tight cluster indicative of stable reentry (Figure 3F). Hearts in halothane-induced PVTs exhibited regions with MVTs (Figure 4A) with a dominant frequency (Figure 4B) and regions with more complex voltage oscillations (Figure 4C) and FFT spectra (n=2 of 6) (Figure 4D). These results indicate that on the anterior surface of the heart, there are regions with out-of-phase monomorphic VTs and other regions with polymorphic VTs.

**Northern Blots**

As in previous work, the level of KCNE1 mRNA was high in 3-week-old mouse hearts and decreased with age, reaching a low but detectable level at 12 weeks.11 The signal was sex-balanced in 3-week-old mice (ratio of male to female, 1.1) but more pronounced in adult female mice by 2- to 3-fold. The amount of messenger RNA for the other delayed rectifier subunits, including KCNQ1, Kv1.5 (encoding I$_{Ks}$), MERG (the mouse counterpart of HERG), and connexin 40 and 43, TREK1, and TASK1 were not influenced by sex.

**Discussion**

The manipulation of genes in the mouse has yielded important animal models to study how specific gene products modify the phenotype of complex physiological systems. Transgenic and gene-targeted mice have been developed to study cardiac arrhythmias; some are engineered to model human arrhythmias or human diseases in which arrhythmias occur.17 Other models are made to manipulate steroid hormone receptors to investigate how sex differences influence the cardiac phenotype.18 Once a molecularly engineered
mouse is made, its phenotype must be characterized and
correlated to an aspect of human physiology or pathophysi-
ology. There are striking differences between mice and men
with respect to heart rate, ventricular AP shape and durations,
ionic currents involved in cardiac repolarization, and size of
hearts.17 Despite these differences, there are striking parallels
in mice and larger mammals with regard to gradients of
repolarization and refractoriness, the spatial distribution of
ionic channels, and the induction of reentry, although differ-
ent ion channels are involved.12

Sex Differences Exposed by Halothane
Halothane has long been known to exert variable effects on
cardiac rhythm19 and was shown to decrease heart rate and
increase PR and QT intervals in dogs.20 Several cardiac
channel currents are altered by halothane, namely a ubiqui-
tous blockade of \( I_{Na} \), \( I_{Ca-L} \),21 potassium channels, including
\( I_{K} \),22,23 and derangements of gap junctions that could contrib-
ut to arrhythmia.24 In mammalian species, gap junctions are
formed by isoforms of connexins where connexin 43 is
expressed throughout the heart, connexin 40 in atrial muscles,
and connexin 45 in the conduction system.25 Connexin 43 has
been implicated in the pathogenesis of ventricular arrhythmia
in mice.24 It is unlikely that sex differences in connexin
explain these PVTs because no differences in connexin
mRNAs were observed and normal P waves were recorded
throughout such arrhythmia. Similarly, an eventual role of
\( I_{Kr} \), the rapid component of the delayed rectifier \( I_{K} \), is
not obvious, because specific \( I_{K} \) blockers (dofetilide and E4031)
do not modify the mouse ECG. 2P channels (TREK 1 and 2
and TASK 1) could be targeted by halothane but would be
protective rather than arrhythmogenic because of their
tendency to induce cell hyperpolarization. Finally, with the
exception of KCNE1 mRNA, which is expressed at lower
levels in males (1 of 3) than in females, no other sex
differences were seen in Northern blots. The duration of
halothane-induced PVTs in male hearts (\( \approx 1 \) minute, max-
imum 3 minutes) roughly equals the time that halothane

Figure 3. Halothane-induced MVTs. A, APs controls. Example of optical APs
recorded from one diode. B, Activation map from control heart. In all activation
maps, the first site to depolarize is depicted in “light gray” (ie, time \( t=0.0 \)
ms) and subsequent depolarizations in increasingly darker shades; isochronal
lines are 1 ms apart. C, APs during MVT. APs recorded from a diode after the
addition of halothane. D, Activation map during an arrhythmia after the addition
of halothane. E, FFT spectra of an arrhythmia. The FFT spectrum was analyzed
over a 1.25 second time interval after the injection of halothane to mouse heart.
The power spectrum of voltage oscillations during an arrhythmia displayed a
dominant frequency of 15 Hz. F, Poin-
caré map of inter-activation intervals dur-
ing an arrhythmia. Plot \( I_{n+1} \) versus \( I_n \),
where \( I_n \) is the inter-activation interval of
beat \( n \) of VT. The interaction intervals
demonstrated the stability of the VT
since the VT cycle length fell in the nar-
row range of 64 to 68 ms.
lasts in the coronary circulation. This is markedly different in female hearts where arrhythmias last far longer (ie, 14 to 44 minutes), emphasizing the importance of this observation, even if the mechanism of such a difference remains unclear.

Relevant Model of Sex Differences

Several models have been used for evaluating sex differences in QT interval and arrhythmia, including dogs and rabbits. Mice display a sex-dependent QT adaptation to heart rate with a steeper slope in females resulting in a longer QT during bradycardia in female mice. The present study sheds new light on the relevance of mouse hearts as a model of sex differences by revealing sex differences in the occurrence of PVTs in this species. Differences in the expression cardiac ionic channels or their modulation can account for sex-dependent PVTs, yet Northern blot experiments do not suggest so, with the exception of KCNE1.

Acknowledgments

Supported by National Institutes of Health grants HL57929 and HL59614 to Dr Salama, a postdoctoral fellowship from the American Heart Association to Dr Baker, and the Délegation à la Recherche Clinique (DRC) of the Centre Hospitalier Universitaire de Nice to Dr Drici.

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Circulation. 2002;106:497-503; originally published online July 1, 2002;
doi: 10.1161/01.CIR.0000023629.72479.24
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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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:
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