Deletion of the Serotonin Receptor Type 3A in Mice Leads to Sudden Cardiac Death During Pregnancy

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Background: The serotonin receptor type 3 (Htr3) blocker is associated with QT prolongation and torsades de pointes. However, little is known about effects of Htr3 on the heart arrhythmia.

Methods and Results: An electrophysiological study Involving knock-out (KO) female mice lacking functional Htr3a (*Htr3a*$^{-/-}$) and their wild-type littermates during non-pregancy (NP) and late pregnancy (LP) was performed. Htr3a mRNA was present in the wild-type, but not in the *Htr3a*$^{-/-}$ mouse hearts. Serotonin and tryptophan hydroxylase 1 (Tph1), a rate-limiting enzyme of serotonin synthesis in hearts, is increased during pregnancy. The heart weight and size were increased in the pregnant mice regardless of a mutation. The QTc intervals were prolonged after pregnancy in both the wild (NP: 171.2±16.8 vs. LP: 247.7±14.3 ms; P<0.001) and *Htr3a*$^{-/-}$ mice (NP: 187.9±18.7 vs. LP: 275.6±11.0 ms, P<0.001). Compared with wild-type LP mice, *Htr3a*$^{-/-}$ LP mice had increased spontaneous ventricle tarchycardia (VT; 56% vs. 0%, P=0.002), VT inducibility (66% vs. 25%, P=0.002) and mortality (56% vs. 0%, P=0.002). Pharmacologic administration of serotonin and Htr3 agonists (m-CPBG) decreased the QT interval in wild mice, but not in *Htr3a*$^{-/-}$ mice.

Conclusions: Htr3a is present in mouse hearts. Serotonin and Tph1 were increased during pregnancy. The deletion of Htr3a was related to fatal arrhythmias and sudden cardiac death during pregnancy, and its activation reversed the QT prolongation. (*Circ J* 2015; **79:** 1807–1815)

Key Words: Fatal arrhythmia; Pregnancy; QT prolongation; Serotonin receptor type 3

Serotonin, 5-hydroxytryptamine (5-HT), was first isolated as a vasoconstrictor from the blood, and was later identified in the central nervous system (CNS). It is found in 3 main areas of the body: the intestinal wall, platelets, and the CNS. The functions of 5-HT in the CNS as a neurotransmitter are numerous and appear to involve control of appetite, sleep, memory, behavior (including sexual and hallucinogenic behavior), and endocrine regulation.[1] Tryptophan hydroxylase 1 (Tph1) is a rate-limiting enzyme of 5-HT synthesis in peripheral tissues. Peripherally, 5-HT, which is stored in platelets, appears to play a major role in homeostasis, blood pressure regulation, cardiovascular functions,[1] motility of the gastrointestinal tract,[2] and carcinoid tumor pathology. The role of 5-HT in the heart has been demonstrated by cardiac dysfunction in

Tph1 knock-out (KO) mice and/or 5-HT2b receptor KO mice.[3,4] These biological actions of 5-HT are mediated by numerous cognate receptors. It now appears that there are at least 15 receptor subtypes that belong to 4 populations: 5-HT1/5, 5-HT2, 5-HT3, and 5-HT4/6/7 subtypes.[5]

Unlike the other serotonin receptor genes in the mouse genome, which encode G-protein-coupled serotonin receptors, *Htr3a* encodes subunits of the ligand-gated cation channels, and is essential to form a functional Htr3 channel.[6–8] However, the precise role of Htr3 as an ion channel expressed in the heart has not been fully evaluated. Htr3 antagonists, including granisetron (Kytril) and ondansetron (Zofran), are widely used for the treatment of postoperative and chemotherapy-induced nausea/vomiting. They have been reported to widen the QRS

complex, and prolong the JT, QT, and PR intervals. Htr3 antagonists at high concentrations induce cardiac dysrhythmias with the risk of QT prolongation.[9–12]

We hypothesized that serotonin may have a role in preventing QT prolongation through Htr3a. To prove this hypothesis, we identified the presence of Htr3a, serotonin, and Tph1 in the heart. Pregnancy is a well-known condition during which the heart undergoes various hemodynamic and structural changes, with an increase in cardiovascular events.[13,14] It was reported that the serum serotonin level increased 2-fold during pregnancy.[15] Therefore, we evaluated the change in serotonin and Tph1 in the heart during pregnancy. We also analyzed the QT interval changes and arrhythmias in wild type and *Htr3a*$^{-/-}$ mice during pregnancy. Finally, we demonstrated that the pharmacological administration of a serotonin and Htr3 agonist (m-CPBG) could reverse the QT prolongation.

Methods

This investigation conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (Animals were cared for in strict accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. All experimental protocols were reviewed and approved by the institutional animal care and Use committee of Yonsei University College of Medicine and Cardiovascular Research Institute). This study protocol was approved by the Institutional Animal Care and Use Committee of Yonsei University College of Medicine and Cardiovascular Research Institute, and conforms to the guidelines of the American Heart Association.

Generation of Htr3a-Targeted KO Mice
Mice were housed on a 12-h light/dark cycle in climate-controlled, pathogen-free barrier facilities. Htr3a-targeted KO mice (B6.129X1-*Htr3a*$^{tm1jul/J}$) were purchased from Jackson laboratory. *Htr3a*$^{-/-}$ mice were backcrossed with C57BL/6J mice for more than 14 generations and were used for experiments when they were age of 8–12 weeks. Mating was confirmed by the presence of a vaginal plug the next morning, and was designated day 0 of gestation (G0). RNA extraction, reverse transcription polymerase chain reaction (RT-PCR), and real time RT-PCR were performed as previously described.[15] The following primer sequences were used: *Htr3a* primers 5'-AAATCAGGGCGAGTGGGAGCTG-3' and 5'-GACACGATGATGAGGAAGACTG-3', and Tph1 primers 5'-ACCATGATTGAAGACAACAAGGAG-3' and 5'-TCAACTGTTCTCGGCTGATG-3'.

Physiological Tests
All physiological tests were performed during non-pregnant (NP) and late pregnant (LP; G16~18) stages. All procedures were performed after an anesthethic (isoflurane/O$_2$) was administered via inhalation. Body temperature was maintained at 37±0.5°C by using a heating pad.

Echocardiography A high-performance cardiovascular ultrasound system with a 15L8 15MHz linear array transducer at a frame rate of 100 frames/s was used to perform the echocardiography. Two-dimensional views were acquired in the short-axis and parasternal long-axis view. M-mode recordings were acquired in the short-axis view at the level of the papillary muscles. The left ventricular (LV) chamber dimensions (LV internal dimension diastole, LV internal dimension systole, and wall thickness) were measured from the M-mode recordings. The LV fractional shortening (FS) and ejection fraction (EF) were calculated as follows:

FS (%)=((LV internal dimension diastole–LV internal dimension systole)/LV internal dimension diastole)×100

EF (%)=((LV end-diastolic volume–LV end-systolic volume)/ LV end-diastolic volume)×100

Electrocardiography The ECG leads I, II and III were recorded using a data acquisition system (MP100 and Acknowledge software, Biopac Systems Inc). The QT interval was measured from the start of the QRS complex to the end of the T wave; the end of T wave was defined as the point of return to the isoelectric line. The corrected QT interval (QTc) was calculated by dividing the QT interval by the square root of the preceding R-R interval (**Figure S1**). To determine if pharmacologic activation of Htr3 could shorten the QT interval, we measured the QT intervals after an intra-peritoneal injection of serotonin (100 μmol/L) and a Htr3 agonist, m-CPBG (200 nmol/L).

Electrophysiological Studies Electrophysiological studies were performed in the mice as previously described.[16,17] A jugular vein cutdown was performed to gain access to the right internal jugular vein. An octapolar catheter (Millar Instruments, TX, USA) was positioned in the right atrium and ventricle via the jugular vein. Programmed electrical stimulation was performed using a standard protocol with 100-ms drive trains and single extrastimuli to measure the function of the atrioventricular node and conduction properties of the atrial and ventricular tissue. The sinus node function was determined by measuring the sinus node recovery time (SNRT) following 10 s of pacing at 3 cycle lengths (120, 100 and 80 ms). Burst pacing was performed with eight 50 ms bursts and four 30 ms burst episodes for up to a total burst pacing duration of 1 min. Programmed electrical stimulation for induction of arrhythmias was performed with single, double, and triple extrastimuli for up to a minimum coupling interval of 10 ms, as previously described.[16] After the interventional study, including ventricular pacing, the mouse hearts were harvested and weighed.

Optical Mapping
Optical mapping was performed in 6 mice from each group. After performing a median sternotomy, the hearts were rapidly excised and immersed in cold Tyrode's solution (composition in mmol/L: 125 NaCl, 4.5 KCl, 0.25 MgCl$_2$, 24 NaHCO$_3$, 1.8 NaH$_2$PO$_4$, 1.8 CaCl$_2$, and 5.5 glucose). The ascending aorta was immediately cannulated and perfused with 37°C Tyrode's solution equilibrated with 95% O$_2$ and 5% CO$_2$ to maintain a pH of 7.4. For performing the optical mapping, the contractility was inhibited by 10–17 μmol/L of blebbistatin.[18,19] Then, the hearts were stained with RH237 (Invitrogen, California, USA), and they were excited with quasi-monochromatic light (520±30 nm) from 2 green LED lamps. Emitted light was collected by a camera (MiCAM Ultima; BrainVision, Tokyo, Japan) with a 610-nm long pass filter at 1 ms/frame and 100×100 pixels, with a spatial resolution of 0.5×0.5 mm^2/pixel. The action potential duration (APD) was measured from the (dF/dt) max to 90% (APD$_{90}$) and 70% recovery to baseline (APD$_{70}$). The mean APD was calculated for each heart by averaging the APD from a region of the ventricle consisting of 10×10 pixels or the 100 APD from each heart. To determine if the activation of Htr3 could shorten the APD, we measured the APD$_{90}$ and APD$_{70}$ after infusion of serotonin (100 μmol/L), m-CPBG (200 nmol/L) and Htr3 antagonist, ondansetron (1 μmol/L), in wild mice.
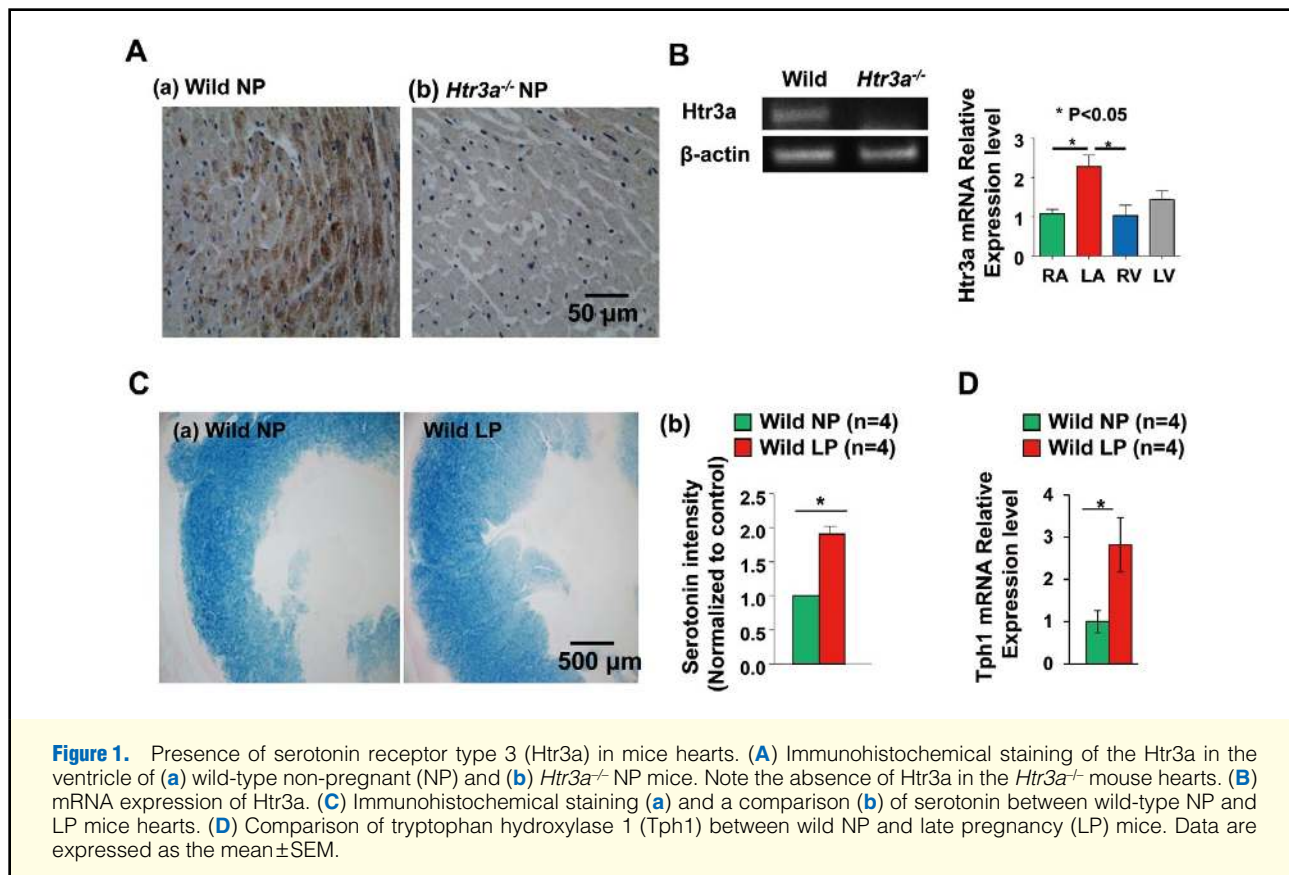
Figure 1. Presence of serotonin receptor type 3 (Htr3a) in mice hearts. (**A**) Immunohistochemical staining of the Htr3a in the ventricle of (**a**) wild-type non-pregnant (NP) and (**b**) *Htr3a⁻/⁻* NP mice. Note the absence of Htr3a in the *Htr3a⁻/⁻* mouse hearts. (**B**) mRNA expression of Htr3a. (**C**) Immunohistochemical staining (**a**) and a comparison (**b**) of serotonin between wild-type NP and LP mice hearts. (**D**) Comparison of tryptophan hydroxylase 1 (Tph1) between wild NP and late pregnancy (LP) mice. Data are expressed as the mean±SEM.

Histology and Immunohistochemical Staining

For the histology, 5 hearts from each group were fixed in 4% (w/v) paraformaldehyde in phosphate buffered saline for 4 h at 4ºC, before being embedded in paraffin. Tissue slides were stained with Hematoxylin and Eosin. Immunohistochemical staining was performed using polyclonal anti-Htr3a antibody (Sigma Aldrich), and anti-serotonin antibody (ImmunoStar). To visualize the bound antibodies, we used a VECTASTAIN® ABC Kit (Vector labs) and a DAB Substrate Kit (Vector laboratories).

Statistical Analysis

Data were expressed as the mean±SEM. An analysis of variance with a post-hoc test, a Tukey's test, was used to compare the means of the continuous variables that were approximately normally distributed among the groups. The categorical variables are reported as the count (percentage) and were compared by using a Fisher's exact test. The SPSS statistical package (SSPS Inc, Chicago, IL, USA) was used to perform all statistical evaluations. A P value of ≤0.05 was considered statistically significant.

Results

Htr3 Expression in Mouse Hearts

In the immunohistochemical staining, Htr3a was detected in the left ventricle of wild type mice, but not in that of *Htr3a⁻/⁻* mice (**Figure 1A**). Htr3a mRNA was expressed in the wild type, but was not observed in the *Htr3a⁻/⁻* mouse hearts. The Htr3a (n=4) was higher in the left atrium (LA) than in the right atrium (RA) (2.29±0.5 vs. 1.08±0.22, P=0.02) and in the right ventricle (RV) (2.29±0.5 vs. 1.03±0.5, P=0.02) (**Figure 1B**).
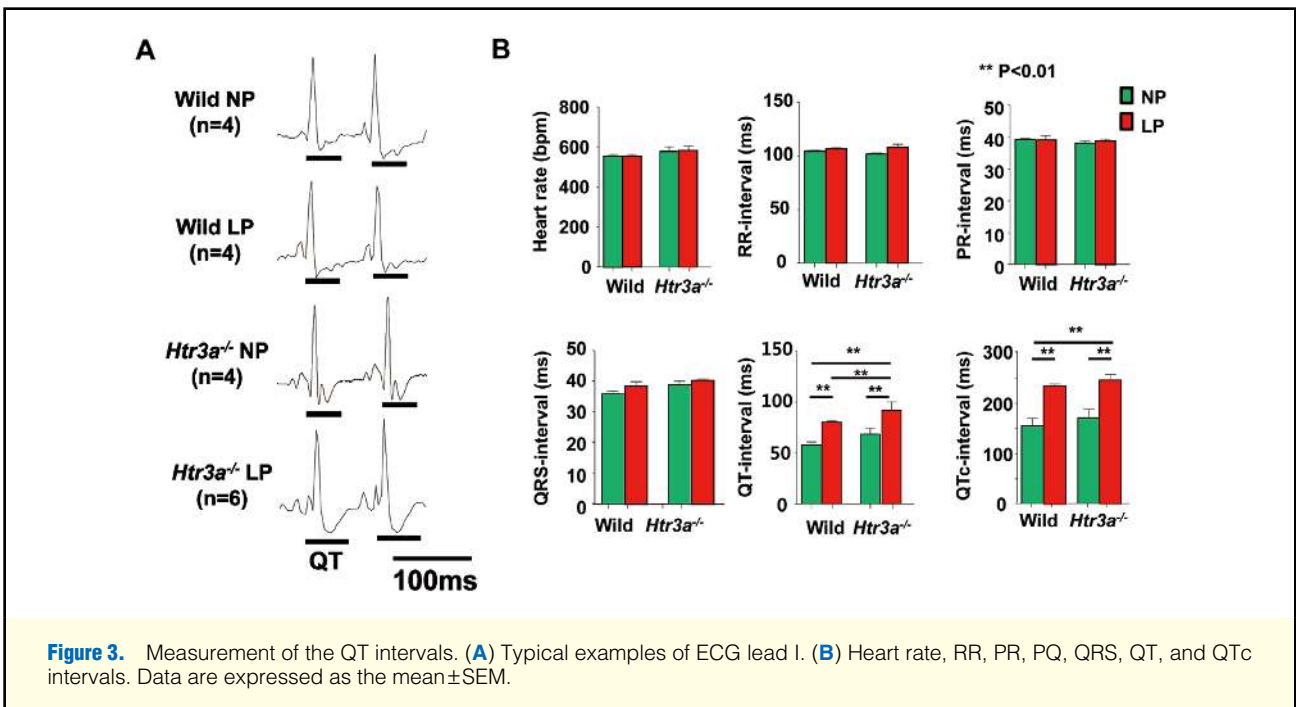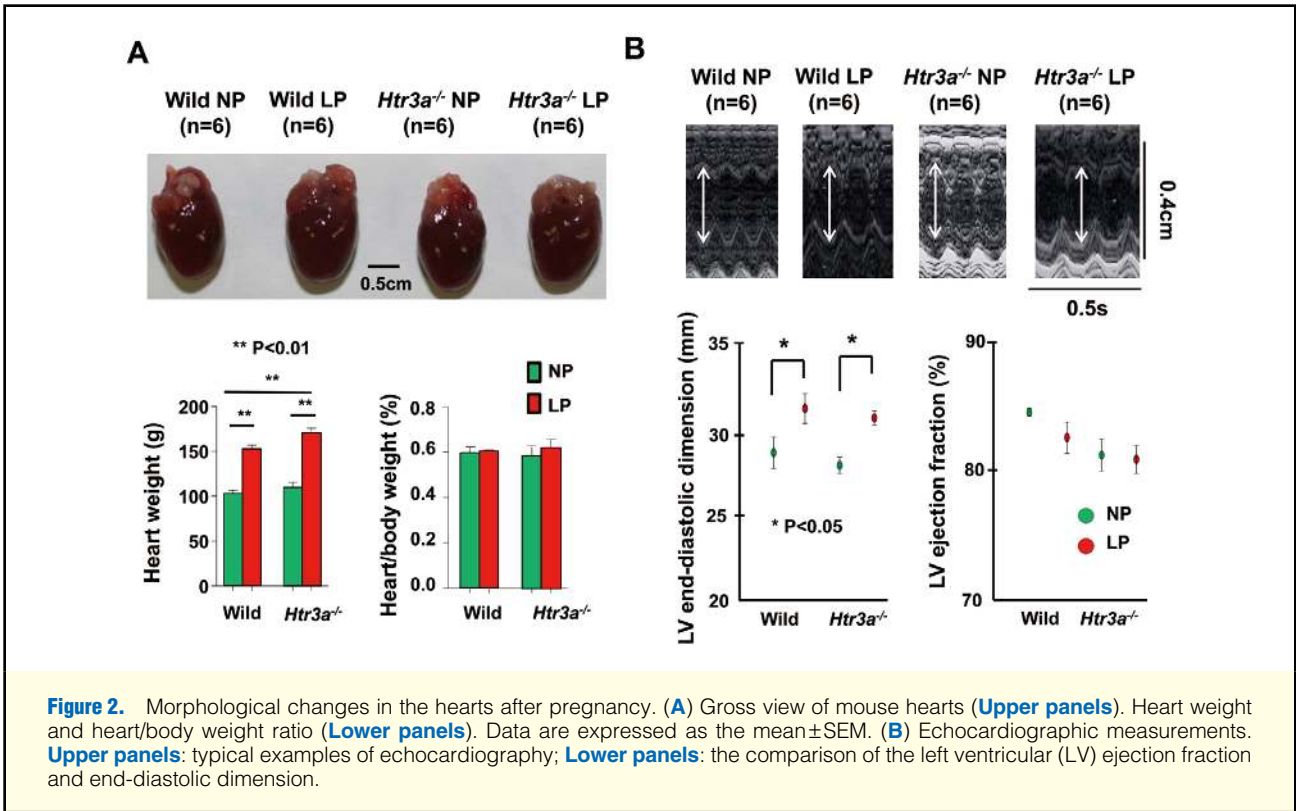
Serotonin was stained in the wild-type mouse hearts at baseline and during pregnancy (**Figure 1C-a**). Compared with the wild NP mice, the expression level of serotonin (**Figure 1C-b**) and Tph1 mRNA (**Figure 1D**) was significantly increased in the wild LP mouse hearts.

Morphological Changes

The LP mice had an increased heart weight compared with the NP mice regardless of any mutations (P<0.001). However, the heart/body weight did not differ among the groups (**Figure 2A**). An echocardiography was performed on 6 mice in each group. An echocardiographic analysis also showed structurally normal hearts in the *Htr3a⁻/⁻* mice (n=6). The LP mice (n=6) had an increased LV end-diastolic dimension compared with the NP mice regardless of the genetic background (**Figure 2B**, **Table S1**). There was no significant difference in the LV EF among the groups (**Figure 2B**).

Htr3⁻/⁻ Mice Showed a Prolonged QT Interval During Pregnancy

Figure 3A shows the ECG lead II of the mice from each group. The heart rate, the interval between R-wave and R-wave (RR), and the interval between P-wave and R-wave (PR) intervals did not differ between the wild-type and *Htr3a⁻/⁻* mice during the NP and LP stages. Compared with the NP wild-type mice (n=4), the QT (NP: 58.0±2.8 vs. LP: 80.5±1.0 ms, P<0.001) and QTc intervals (NP: 171.2±16.8 vs. LP: 247.7±14.3 ms, P<0.001) were prolonged in the LP wild-type mice (n=4). The QT (NP: 68.8±5.4 vs. LP: 92.0±8.4 ms, P<0.001) and QTc intervals (NP: 187.9±18.7 vs. LP: 275.6±11.0 ms, P<0.001) were longer in the LP *Htr3a⁻/⁻* mice (n=6) than in the NP

Figure 2. Morphological changes in the hearts after pregnancy. (**A**) Gross view of mouse hearts (**Upper panels**). Heart weight and heart/body weight ratio (**Lower panels**). Data are expressed as the mean±SEM. (**B**) Echocardiographic measurements. **Upper panels**: typical examples of echocardiography; **Lower panels**: the comparison of the left ventricular (LV) ejection fraction and end-diastolic dimension.

Figure 3. Measurement of the QT intervals. (**A**) Typical examples of ECG lead I. (**B**) Heart rate, RR, PR, PQ, QRS, QT, and QTc intervals. Data are expressed as the mean±SEM.

*Htr3a⁻/⁻* mice (n=4) (**Figure 3B**).

Spontaneous VT and Sudden Death in *Htr3a⁻/⁻* Mice During Pregnancy

Figure 4A shows the example of spontaneous ventricular tachycardia (VT, asterisks in **Middle panel**) and ventricular fibril-

lation (VF, **Lower panel**) in the *Htr3a⁻/⁻* mice during pregnancy. Ten (56%) out of 18 LP *Htr3a⁻/⁻* mice and 3 (19%) out of 16 NP *Htr3a⁻/⁻* mice had spontaneous VT. However, VT was not observed in the wild-type NP (n=12) and LP mice (n=12). The LP *Htr3a⁻/⁻* mice had a higher spontaneous VT rate than both the NP (P=0.002) and LP wild-type mice (P=0.002) (**Figure 4B**).
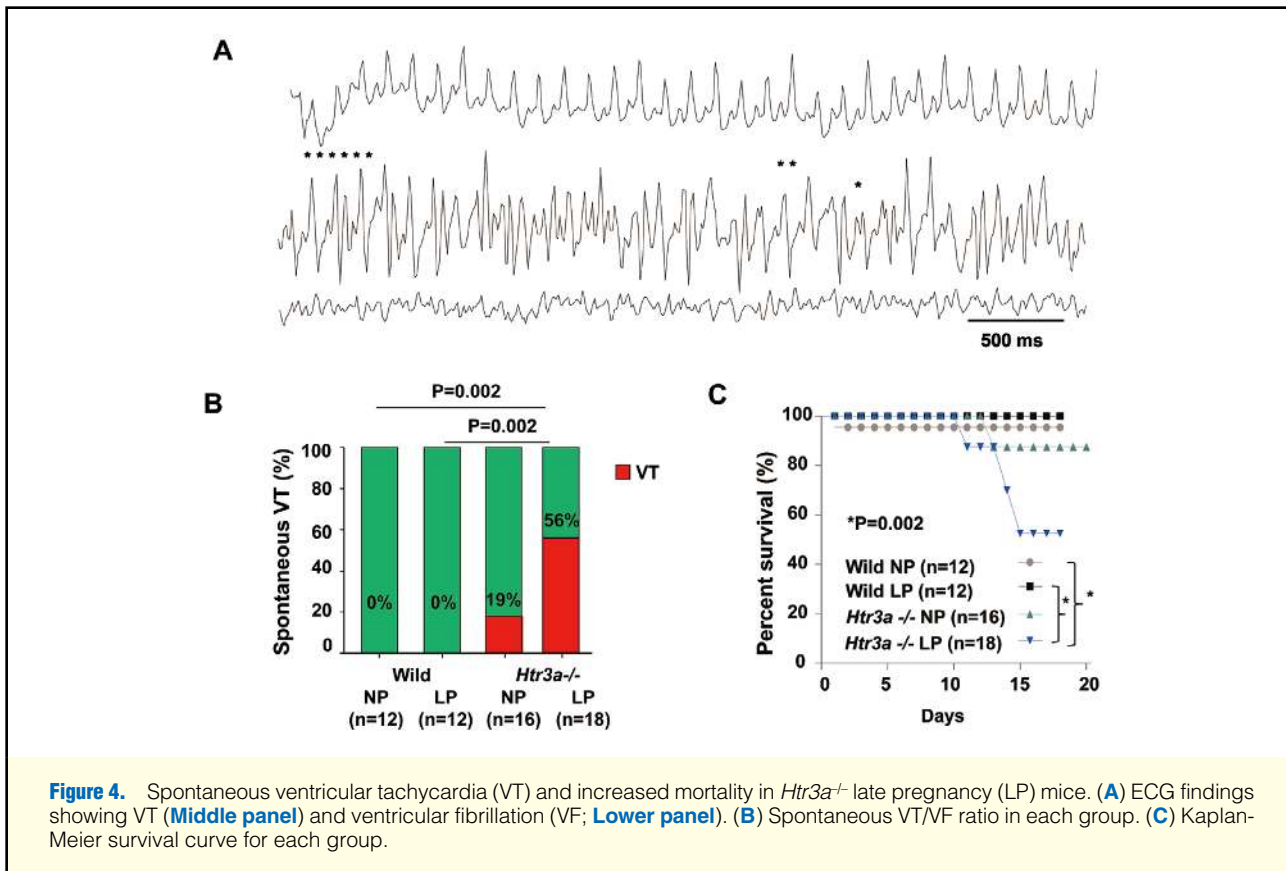
Figure 4. Spontaneous ventricular tachycardia (VT) and increased mortality in *Htr3a*^{−/−} late pregnancy (LP) mice. (**A**) ECG findings showing VT (**Middle panel**) and ventricular fibrillation (VF; **Lower panel**). (**B**) Spontaneous VT/VF ratio in each group. (**C**) Kaplan-Meier survival curve for each group.

Table. Electrophysiologic Study of Mice

| Parameter	Wild NP (n=3)	Wild LP (n=3)	*Htr3a*^{−/−} NP (n=6)	*Htr3a*^{−/−} LP (n=3)*
SNRT, ms	147.0±14.2	114±12.3	186.7±32.1	314.7±0.0[†]
cSNRT, ms	168.0±4.0	127.0±24.0	296.7±36.2[†]	353.7±32.7[†]
AVBCL, ms	22.7±4.6	28.7±12.1	34.7±14.2	–
AVNERP, ms				
S_1=100 ms	16.3±0.6	16.3±0.6	16.3±1.0	–
S_1=90 ms	16.0±0.0	16.0±0.0	16.0±0.0	–
S_1=80 ms	16.0±0.0	16.0±0.0	15.5±0.8	–
VABCL, ms	22.3±1.5	31.3±10.3	31.0±17.0	–
VERP, ms				
S_1=100 ms	16.3±0.6	16.0±0.0	15.0±6.3	–
S_1=90 ms	16.0±0	15.3±0.6	15.8±6.5	–
S_1=80 ms	16.0±0	15.3±0.6	16.2±6.6	–

AVBCL, atrioventricular block cycle length; AVNERP, atrioventricular node effective refractory period; (c)SNRT, (corrected) sinus node recovery time; LP, late pregnancy; NP, non-pregancy; VABCL, ventriculoatrial block cycle length; VERP, ventricular effective refractory period. *Several parameters could not be measured in the *Htr3a*^{−/−} LP group because of the induction of ventricular fibrillation. [†]Significant difference from the control: P-value <0.05. The AVNERP and VERP were measured using a S_1 pacing CL of 100 ms. The SNRT was measured after 110 ms pacing.

Moreover, 10 (56%) out of 18 LP *Htr3a*^{−/−} mice died suddenly at the end of gestation, thus having a lower survival rate than the NP (P=0.002) and LP wild-type mice (P=0.002, **Figure 4C**).

Electrophysiological Characteristics
The electrophysiological characteristics are presented in **Table**. Compared with the control (n=4), the SNRT (147.0±14.2 vs. 314.7±14.6 ms, P=0.001) and corrected sinus node recov-ery time (cSNRT) (168.0±4.0 vs. 353.7±32.7 ms, P=0.001) were significantly longer in the *Htr3a*^{−/−} LP mice (n=4). Because of VT or VF induction, the exact values of the atrioventricular block cycle length (AVBCL), atrioventricular node effective refractory period (AVNERP), ventriculoatrial block cycle length (VABCL), ventricular effective refractory period (VERP) could not be measured in the *Htr3a*^{−/−} LP mice (**Table**).
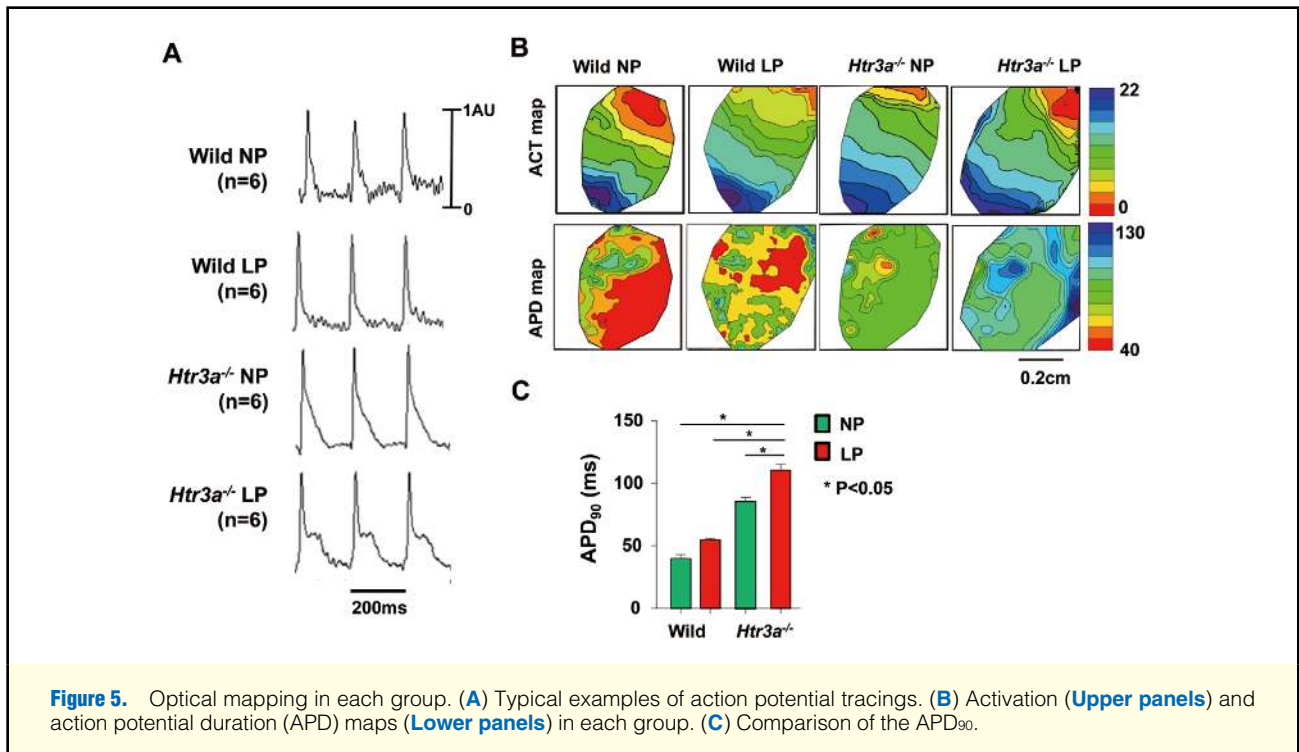
Figure 5. Optical mapping in each group. (**A**) Typical examples of action potential tracings. (**B**) Activation (**Upper panels**) and action potential duration (APD) maps (**Lower panels**) in each group. (**C**) Comparison of the APD$_{90}$.
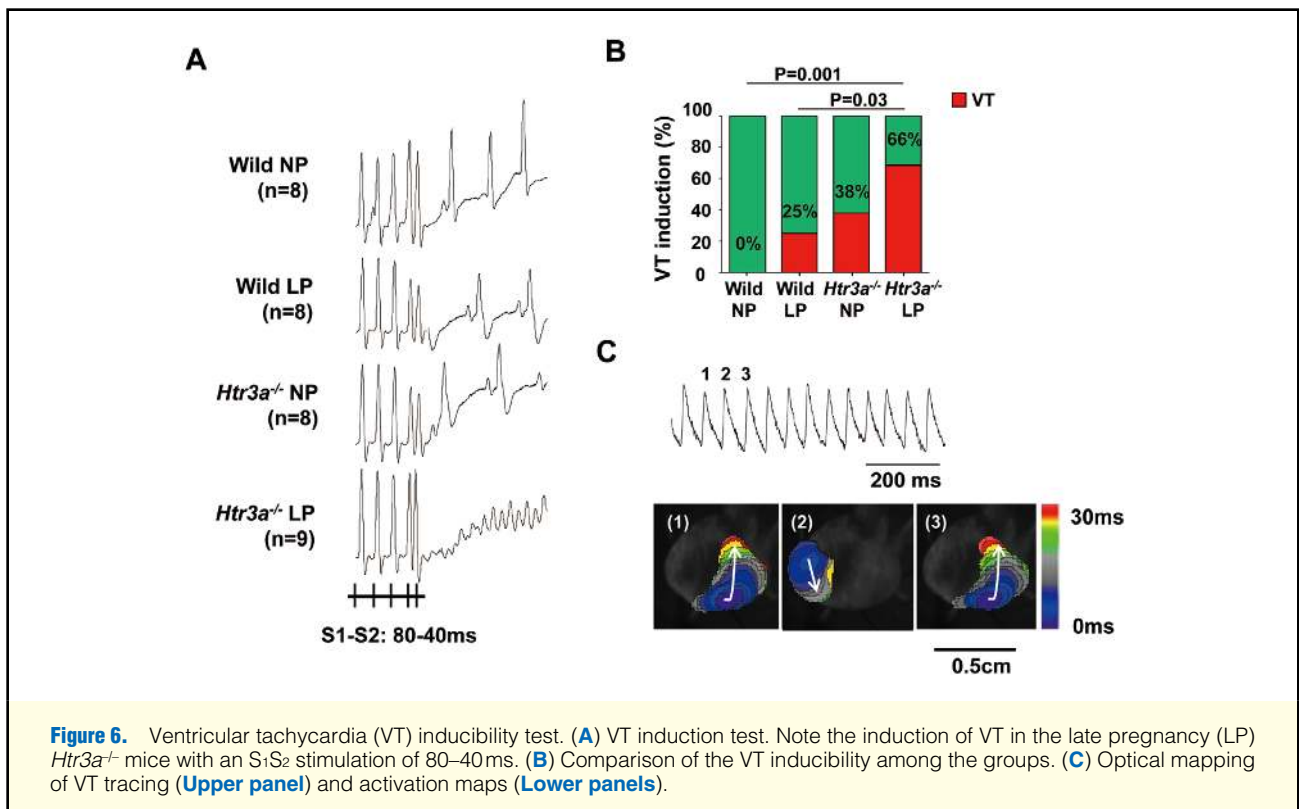

Figure 6. Ventricular tachycardia (VT) inducibility test. (**A**) VT induction test. Note the induction of VT in the late pregnancy (LP) *Htr3a$^{-/-}$* mice with an S$_1$S$_2$ stimulation of 80–40 ms. (**B**) Comparison of the VT inducibility among the groups. (**C**) Optical mapping of VT tracing (**Upper panel**) and activation maps (**Lower panels**).

Optical Mapping Findings

Figure 5A shows the action potential traces at the base of the LV during pacing cycle lengths of 200 ms in Langendorff-perfused mouse hearts. The APD was prolonged during pregnancy in the wild-type mice and *Htr3a$^{-/-}$* mice. The Htr3a deletion induced prolongation of the APD in both the NP and LP status. **Figure 5B** shows the activation map. The total ventricular activation time was significantly more increased in
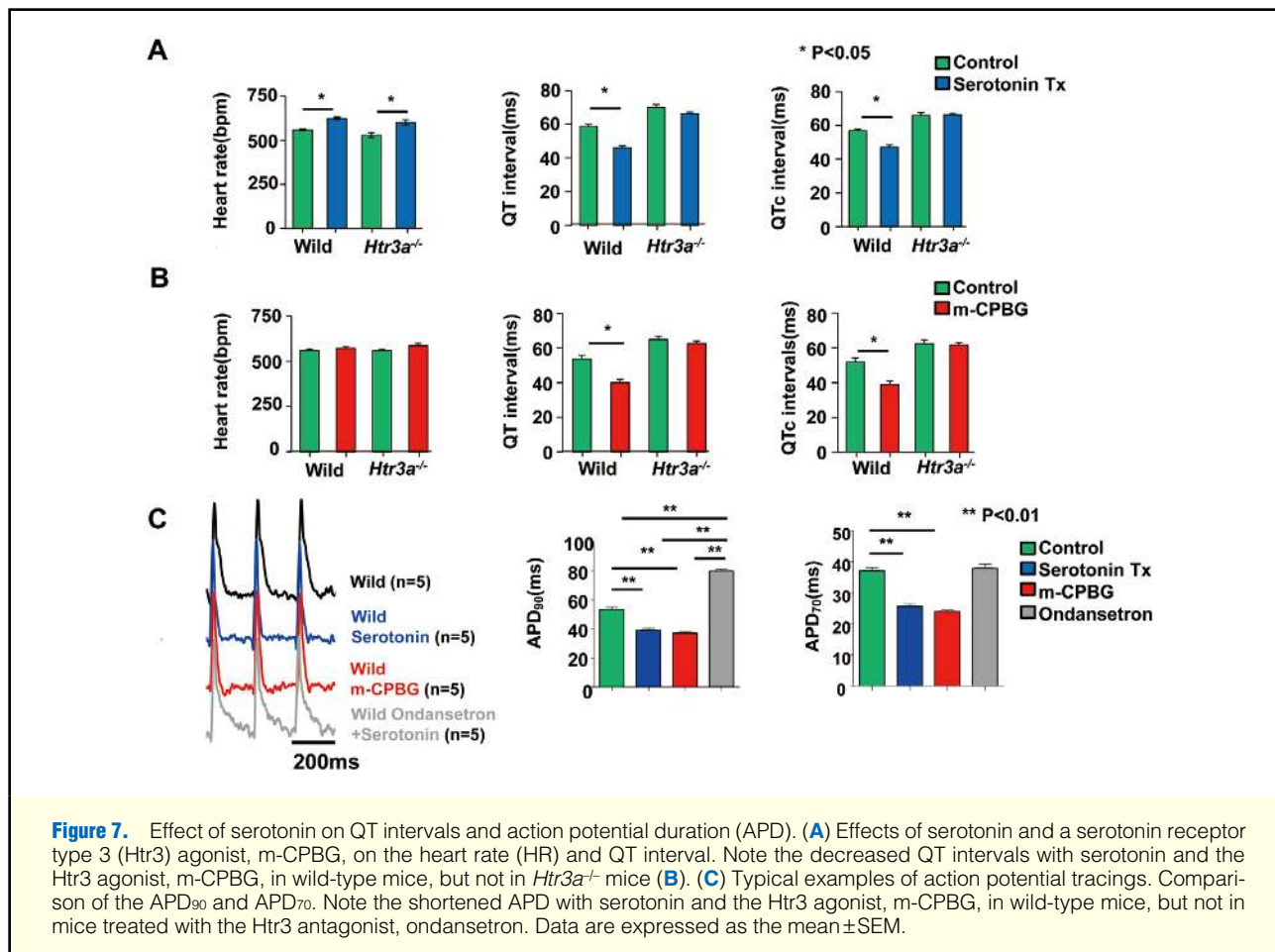
Figure 7.   Effect of serotonin on QT intervals and action potential duration (APD). (**A**) Effects of serotonin and a serotonin receptor type 3 (Htr3) agonist, m-CPBG, on the heart rate (HR) and QT interval. Note the decreased QT intervals with serotonin and the Htr3 agonist, m-CPBG, in wild-type mice, but not in $Htr3a^{-/-}$ mice (**B**). (**C**) Typical examples of action potential tracings. Comparison of the APD$_{90}$ and APD$_{70}$. Note the shortened APD with serotonin and the Htr3 agonist, m-CPBG, in wild-type mice, but not in mice treated with the Htr3 antagonist, ondansetron. Data are expressed as the mean±SEM.

the LP $Htr3a^{-/-}$ hearts (29.0±2.2 ms) than in the NP wild-type hearts (11.0±1.0 ms, P<0.001). The APD$_{90}$ of the LP $Htr3a^{-/-}$ hearts (114.1±7.9 ms) was longer than that of the NP wild-type hearts (76.6±2.1 ms, P<0.001), LP wild-type hearts (77.0±5.3 ms, P<0.001), and NP $Htr3a^{-/-}$ hearts (80.5±3.2 ms, P<0.001; **Figure 5C**).

Increased VT Vulnerability in $Htr3a^{-/-}$ Mice During Pregnancy
The typical examples of induced VF are presented in **Figure 6A**. **Figure 6B** shows the comparison of the VT inducibility test among the 4 groups. VT was induced in none of the NP wild-type mice (n=8), in 2 (25%) of the LP wild-type mice (n=8), in 3 (38%) of the NP $Htr3a^{-/-}$ mice (n=8), and in 6 (66%) of the LP $Htr3a^{-/-}$ mice (n=9). The LP $Htr3a^{-/-}$ mice had a higher VT inducibility than the NP wild-type mice (P=0.001) and LP wild-type mice (P=0.002). **Figure 6C** shows an example of a VT rotating around the LV in a counterclock wise manner.

Activation of the Htr3 Shortens the QT Interval and APD
In order to determine if the activation of the $Htr3$ could shorten the QT interval, we examined the effects of serotonin and an Htr3 agonist on the QT interval. An intra-peritoneal injection of serotonin (100 μmol/L) shortened the QTc interval in the wild-type mice (QTc, from 58.7±2.1 to 46.3±2.1 ms, P=0.001). However, serotonin failed to shorten the QTc interval in the $Htr3a^{-/-}$ mice (QTc, from 65.7±3.7 to 66.3±1.8 ms, P=0.81) (**Figure 7A**). The Htr3 agonist, m-CPBG, also short-

ened the QT interval in the wild-type mice (QTc, 51.9±2.3 to 39.1±1.8 ms, P=0.009), but not in the Htr3a$^{-/-}$ mice (QTc, 62.5±2.2 vs. 61.7±1.5 ms, P=0.70; **Figure 7B**). Interestingly, in both the wild-type and $Htr3a^{-/-}$ mice, the heart rate was increased by serotonin, but not by the Htr3 agonist (m-CPBG). This finding suggested that an increase in the heart rate induced by 5-HT was independent of Htr3.

We then examined whether the Htr3 agonist actually reduced the QT interval during a prolonged QT status. To prolong the QT interval, we used erythromycin, which is known to induce QT prolongation in mice.[20] The Htr3 agonist, m-CPBG (200 nmol/L), reduced the prolonged QT interval induced by erythromycin (200 μmol/L) (QTc from 51.9±2.0 to 43.2±2.1 ms, P=0.04; **Figure S2**).

Finally, we evaluated the effect of Htr3 activation on APD using optical mapping. The serotonin (100 μmol/L) and Htr3 agonist, m-CPBG (200 nmol/L), shortened both APD$_{90}$ and APD$_{70}$. However, the APD shortening effect of serotonin was abolished in mice treated with the Htr3 antagonist, ondansetron (1 μmol/L) (**Figures 7C,D**).

Discussion
Main Findings
The main finding of this study was that Htr3 was present in mouse hearts. Serotonin and Tph1 in the heart are increased during pregnancy. Second, although the deletion of Htr3a did not cause any electrophysiological changes in the non-pregnant

state, pregnant *Htr3a*^{-/-} mice had sudden death and spontaneous VT with electrophysiological changes of the QT, APD prolongation, and an increased VT inducibility. Third, the pharmacological administration of serotonin and the Htr3 agonist shortened the QT intervals in the wild-type mice, but not in the *Htr3a*^{-/-} mice. Our results suggest that Htr3 has a role in preventing QT prolongation, especially in mouse hearts during pregnancy.

Serotonin and the Heart

Serotonin is a biogenic amine that mediates neuronal transmission both in the central and peripheral nervous systems. Outside of neurons, serotonin is present mostly in platelets and can be transferred to all organs. Many studies have reported on the relationship between serotonin and the heart function. *Tph1*^{-/-} mice exhibited a larger variability in the cardiac output, stroke volume, and end systolic pressure-volume relationship as compared with the control mice.[4] Htr2b is associated with valvular heart disease and heart embryogenesis.[21,22] With respect to heart arrhythmias, under acute coronary ligation conditions, ventricular ectopic beats and VT/VF are increased by serotonin injection in rats.[23] Htr4 mediates atrial tachycardia,[24] and a Htr4 blockade has a beneficial effect on atrial fibrillation.[25] Recently, it was reported that mouse cardiac tissue contained mRNA for various 5-HT receptor types, including Htr3.[26] However, the role of Htr3, a serotonin-gated non-selective cation channel, has not been demonstrated in the heart.[27] In this study, we also confirmed the presence of Htr3a in mouse hearts. Moreover, we demonstrated that serotonin and Htr3 in mouse hearts were increased during pregnancy.

Influence of Htr3 on the QT Interval and APD

In this study, serotonin and the Htr3 agonist, m-CPBG, shortened the QT intervals in the wild-type but not in the *Htr3a*^{-/-} mice. Moreover, Htr3 activation shortened the APD in the wild-type but not in the mice treated with the Htr3 antagonist, ondansertron. The serotonin and Tph1 levels of the mice hearts was increased during pregnancy. The QT prolongation was more prominent in the LP *Htr3a*^{-/-} than in the LP wild-type mice. These results strongly support the role of serotonin in preventing QT prolongation through Htr3. Consistently, the QT and APD shortening effect of serotonin was abolished after the Htr3 deletion.

The maternal heart significantly adapts to the circulatory needs of pregnancy, but the effect of pregnancy on ventricular repolarization is poorly understood. Pregnancy induces electrocardiogram disturbances such as longer QT-interval prolongation. Eghbali et al[28,29] reported that fast transient outward potassium current ($I_{to,f}$) and slow delayed rectifier potassium current (I_{Kslow}) are downregulated in pregnancy. Especially, cardiac Kv 4.3 channel gene expression was downregulated by approximately 3- to 5-fold, and was paralleled by a reduction in the transient outward K⁺ currents, a longer action potential, and by prolongation of the QT interval. Because the role of I_{Ks} in repolarization is smaller than that of $I_{to,f}$ in mice,[30] the mechanism of the QT shortening by serotonin might be related to the increase in the I_{tof} channel. Consistently, the QT shortening effect of serotonin and the Htr3 agonist was not observed in the *Htr3*^{-/-} mice. The second possible answer might be the blocking of I_{Ks}. The blocking of the cardiac HERG K⁺ channel was suggested as the possible underlying mechanism for the prolongation of the cardiac repolarization.[31] However, because the role of I_{Ks} in repolarization is small in mice,[30] the possibility of I_{ks} involvement is relatively low. The third possible answer might be the direct involvement of non-selective cat-

ion channels, such as Htr3. Recently, non-selective cation channels were reported to regulate the resting membrane potential.[32,33] Htr3, a serotonin-gated non-selective cation channel, can contribute to the formation of the resting membrane potential.

Influence of Htr3 on SNRT

In this study, the prolongation of the SNRT and cSNRT was observed in the *Htr3*^{-/-} LP mice. The prolongation of the SNRT was related with sinus bradycardia. However, in this study, we could not find a difference in the heart rate between the 4 groups. This discrepancy might be explained by the fact that, in general, pregnancy is associated with a faster heart rate, which is a risk factor for arrhythmias. El Khoury et al reported that an increase in the I_f current density contributes to the acceleration of sinoatrial node automaticity and explains, in part, the higher heart rate observed in pregnancy.[34] Another possible explanation might be the low diagnostic efficacy of SNRT.[35,36] Finally, the mechanism of overdrive suppression is related to the rebound increase in the parasympathetic tone after overdrive stimulation.[37]

Clinical Implication

The role of serotonin in the heart has not been completely determined. Htr3 antagonists, which are widely used as antiemetics, have been reported to prolong the QT intervals and to be related to tosades de pointes in high concentrations.[9–12] This finding suggests that serotonin and Htr3 might be related to cardiac repolarization. Recently, it was reported that mouse cardiac tissue contained mRNA for Htr3.[26] Consistently, this study demonstrated that the pharmalogical administration of serotonin and a Htr3 agonist decreased the QT interval in the wild type, but not in the *Htr3*^{-/-} mice. Pregnancy naturally induces physiologic hypertrophy and QT prolongation. We found the level of serotonin and a rate-limiting enzyme of serotonin, Tph1, were increased in the mouse heart during pregnancy. No wild-type mice had cardiovascular events during pregnancy. However, surprisingly, the *Htr3*^{-/-} mice had a marked fatal arrhythmia and sudden death during pregnancy. Conclusively, this study suggests that serotonin has a role in shortening the repolarization period via Htr3, and this role is extremely important during pregnancy.

Study Limitations

This study demonstrated that serotonin and Tph1 were increased during pregnancy. Peripherally, serotonin is stored in platelets; however, we could not exactly present the source of the serotonin that affects the heart. Pregnancy induces electrocardiogram disturbances such as longer QT-interval prolongation, which is related to the reduction in the Ito current and Kv4.3 channel gene downregulation. By using *Htr3*^{-/-} LP mice, we demonstrated that serotonin has an important role via Htr3 in preventing further prolongation of the QT interval during pregnancy. However, the evaluation of major repolarizing outward potassium current in mouse such as I_{tof} using the patch-clamp method is important to reveal the exact mechanism. Finally, because all 10 mice with spontaneous ventricular tachycardia died suddenly, those mice might have died of ventricular arrhythmias. However, we could not provide the exact cause of death in those mice because we did not record telemetry monitoring in all mice before death.

Conclusions

Htr3a is present in mouse hearts. Serotonin and Tph1 were

increased during pregnancy. The deletion of Htr3a was related to fatal arrhythmias and sudden cardiac death during pregnancy, and its activation reversed the QT prolongation.

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Supplementary Files

Supplementary File 1

Figure S1. Corrected QT interval is calculated by dividing the QT interval by the square root of the preceding R-R interval.

Figure S2. Serotonin receptor type 3 (Htr3) agonist actually reduced the QT interval in a prolonged QT status.

Table S1. Echocardiographic measurements

Please find supplementary file(s);
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