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Cardiac Outcomes after Perinatal Sertraline Exposure in Mice

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Abstract

Selective serotonin reuptake inhibitors are prescribed to 6–10% of pregnant women in the United States. Using an intrauterine plus neonatal exposure model to represent exposure throughout human pregnancy, we hypothesized sertraline exposure would impact intracardiac serotonin signaling and lead to small left heart syndrome in the absence of maternal psychopathology. C57BL/6 adult female mice received sertraline (5 mg/kg/d IP) or saline throughout pregnancy to time of delivery. Pups maintained exposure on postnatal days 1–14 to encompass the developmental window analogous to human gestation. Sertraline-exposed mice had increased cardiac hydroxyproline content, decreased 5-HT_{2B} receptor mRNA levels, and increased 5-HT_{2A} receptor and serotonin transporter mRNA levels on postnatal day 21 ($p < 0.05$). These changes were associated with diminished exercise capacity at 6 weeks ($p < 0.05$) and decreased adult shortening fraction and stroke volume at 5 months of age. Isolated cardiomyocytes from neonatal sertraline-exposed mice had significantly decreased proliferation, cross-sectional area and phosphorylation of Akt ($p < 0.05$ versus neonatal control mice). Perinatal sertraline exposure alters neonatal cardiac development and produces long standing changes in adult cardiac function and exercise capacity. Further studies are needed to assess if similar findings are present in the growing population that has been exposed to SSRIs during development.

Keywords

selective serotonin reuptake inhibitors; cardiac development; cardiac function; serotonin receptors

INTRODUCTION

In 2005, GlaxoSmith Kline reported an increased risk of congenital heart disease in infants of mothers taking paroxetine, a selective serotonin reuptake inhibitor (SSRI)¹. The US Food and Drug Administration subsequently changed the paroxetine product label to pregnancy category D indicating studies in pregnant women have demonstrated a risk to the fetus¹. Since that time, studies have been completed to assess the association between SSRI exposure and congenital heart disease. While many studies and several meta-analyses support an association between SSRI exposure and congenital heart disease,^{2–9} interpretation of these results has been clouded by confounding maternal depression^{7,10–12}.

Despite lingering concerns, SSRI use during pregnancy has been steadily increasing and SSRIs are now prescribed to 6–10% of pregnant women in the United States.^{13–17} Sertraline is the most commonly prescribed antidepressant in America, with over 41 million prescriptions in 2013 alone.¹⁸

Animal studies have highlighted the importance of serotonin (5-HT) and specific 5-HT receptors in normal cardiac development for years. Sari and Zhou demonstrated physiologic 5-HT concentrations permit optimal proliferation while both lower and higher concentrations decreased cardiomyocyte proliferation.¹⁹ Cote et al. demonstrated that mice lacking tryptophan hydroxylase, a critical enzyme for 5-HT synthesis, developed cardiac insufficiency.²⁰ Mice with mutations in the serotonin transporter protein (5-HTT), which is the pharmacologic site of action of SSRIs, demonstrate dilated cardiomyopathy and myocardial hypokinesis.²¹ The 5-HT_{2B} receptor is considered critical in normal cardiac development. Administration of a 5-HT_{2B} receptor antagonist to mouse embryos causes defects in the myocardium and an absence of trabeculae.²² 5-HT_{2B} knockout mice exhibit cardiomyopathy with a loss of ventricular mass due to a reduction in number and size of cardiomyocytes.²³ Sadler et al. nicely reviews the biologic plausibility for the origin of heart defects caused by SSRIs.²⁴ Animal studies do show that paroxetine exposure decreases cardiomyocyte proliferation.¹⁹ Likewise, intrauterine fluoxetine exposure in mice leads to life-long reductions in left ventricular wall thickness and increased mortality. Offspring suffered in a dose-dependent manner from a severe form of dilated cardiomyopathy.²⁵

We previously demonstrated that neonatal sertraline exposure decreases adult left ventricular chamber size.²⁶ We utilized a neonatal model in those studies to capture the terminal proliferative phase of cardiomyocyte development. It is important to note that cardiomyocyte proliferation and growth in mice are robust for 14 days after delivery, whereas human cardiomyocyte proliferation is essentially complete at birth.^{27,28} We also showed suggestions of increased fibrosis by Masson Trichrome in sertraline-exposed mice which may contribute to the small left heart syndrome.²⁹ When sertraline-exposed mice were stressed by coronary artery ligations, the sertraline-exposed mice had less ventricular dilation post-myocardial infarction.²⁹ To further investigate our findings of small left heart syndrome, we developed a novel intrauterine plus neonatal exposure model to fully encompass exposure throughout pregnancy. We hypothesized that sertraline exposure would lead to changes in serotonin signaling during development resulting in long-standing reductions in left ventricular function. This is the first study we are aware of assessing cardiac outcomes in mice following two critical exposure windows that together translate to the therapeutic use of SSRIs during pregnancy.

METHODS

Animal model

All procedures were approved by the University of Iowa Animal Care and Use Committee. To fully encompass SSRI exposure throughout human pregnancy, we used an intrauterine plus neonatal exposure animal model and injected mice with sertraline, the most commonly prescribed SSRI in pregnant women. C57BL/6 adult female mice (Jackson Laboratory, Bar Harbor, ME) were randomized to receive intraperitoneal saline (10 ml/kg/d) or sertraline (5

mg/kg/d). Female mice continued to receive daily injections while breeding and throughout pregnancy to time of delivery. Pups were culled into litters of 6 and cross-fostered to avoid a carryover effect of maternal SSRI exposure on fostering. To maintain a similar exposure throughout the period of cardiac proliferation in mice, pups received intraperitoneal saline (3 ml/kg/d) or sertraline (1.5 mg/kg/d), approximately 1/3rd of the maternal dose to reflect placental transfer during pregnancy,³⁰ on postnatal (PN) days 1–14. The neonatal exposure matched the prenatal exposure. Pups were weighed daily during injections, on PN 21, and at 20 weeks of age. Plasma sertraline levels were assayed in the pups after the final injection as previously described.²⁶ Plasma was stored at –20°C until analysis. Analysis by gas chromatography was performed at NMS Labs (Willow Grove, PA). Studies were performed in male and female mice, except for telemetry which was only performed in male mice.

Histology

Mice were euthanized according to the University of Iowa Animal Care and Use Committee protocol. Hearts were dissected from control and sertraline exposed mice on PN 14, after completing the exposure window. The hearts were examined by histology with hematoxylin-eosin staining and Masson's trichrome stains. Morphometric analysis for blue staining in the trichrome-stained slides was performed using the Aperio slide scanner and Image Scope software. A color deconvolution algorithm was developed and optimized to recognize the blue stain indicative of collagen. The entire heart was analyzed and any valvular tissue was subtracted from the analysis to account for its high collagen content.

Hydroxyproline Quantification

Mice were euthanized according to the University of Iowa Animal Care and Use Committee protocol. Hearts were dissected and atria removed from control and sertraline-exposed neonatal mice on PN day 21. Tissue weights were obtained. Myocardial tissues were homogenized. Cardiac hydroxyproline (OHPRO) content was measured using a commercial colorimetric assay from Bio Vision (#K555-100, Mountain View, CA) according to the manufacturer's instructions. Hydroxyproline content, an indicator of collagen content, is measured as µg hydroxyproline per wet heart weight in mg.

Serotonin receptor and serotonin transporter mRNA levels

Mice were euthanized according to the University of Iowa Animal Care and Use Committee protocol. The heart was dissected and atria removed on PN 21 from control and sertraline-exposed mice. Tissues were snap frozen and stored at –80° C. TRIzol Reagent (Life Technologies, Grand Island, NY) was used according to manufacturer's instructions to isolate RNA. Quantitative real-time reverse transcriptase PCR utilized the TaqMan reagent and instrumentation systems (Applied Biosystems, Foster City, CA). Taqman gene expression assay primer/probe sets for mouse 5-HT receptors and 5-HTT were purchased from Applied Biosystems. Given that the reaction efficiencies for the assays are matched by design, we used the CT method for quantification.

Protein Expression

Left ventricular tissue protein samples (40–60 µg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to nitrocellulose membrane which was blocked with 5% bovine serum albumin for 1 hr and incubated for 24 h at 4° C overnight with a primary antibody 5-HT_{2B} (1:2000, BD Biosciences, Franklin Lakes, NJ), 5-HT_{2A} (1:2000, Millipore, Billerica, MA), and 5-HTT (1:400 Santa Cruz Biotechnology, Santa Cruz, CA).

Exercise Testing

All mice were accustomed to the rodent treadmill (Exer-4; Columbus Instruments, Columbus, OH) by running on the treadmill for 5 minutes at 10 meters/min at 0% incline for two consecutive days. To encourage the mice to run, the treadmill is equipped with an electrical shock grid at the rear of the treadmill. On the day of the exhaustion test, mice ran for 5 minutes at 10 meters/minute with a 10% incline. The speed was subsequently increased by 2 meters/min every two minutes as tolerated. Exercise testing terminated when mice sat on the shock grid for 10 seconds. Total distance, total time, and highest speed were measured. Exercise testing was repeated 2, 4, 9, and 14 weeks after initial testing.

Echocardiograms—Baseline echocardiograms (Visual Sonics Vevo 2100 High Resolution Imaging System and Software, Visual Sonics, Inc) were performed under anesthesia as previously described at 20 weeks in mice who received saline and sertraline injections.²⁶ Measurements and calculations were made in accordance with the American Society for Echocardiography Guidelines.³¹ Volumes were calculated based on the formulas of Teicholz et al as validated in Kronik et. al study.³² Echocardiograms were also obtained in mice at the completion of endurance training.

Telemetry recordings—At 20 weeks of age, electrocardiogram telemeters (TA-F10: Data Sciences International, St. Paul, MN) were implanted in male mice as previously described.²⁶ After a 7 day recovery, heart rate, locomotor activity, and temperature were sampled for 10 seconds every 5 minutes over 60 hours for baseline measurements. Activity was measured as the number of horizontal movements per minute. One SSRI mouse was excluded from analysis as significant portions of data were not recorded.

Cardiomyocyte Isolation

Mice were euthanized according to the University of Iowa Animal Care and Use Committee protocol. Hearts were dissected from 2–4 day old neonatal mice who received saline and sertraline and placed in ice cold PBS. Larger vessels and atria were removed carefully, followed by incubation in ice-cold HBSS for 10 minutes. Hearts were moved to a 24 well plate with 1 heart/well and repeatedly dissociated with 0.25% Trypsin-EDTA at 37 °C in 15 minute intervals. The solution was mixed and supernatant collected until tissue was dissociated. Cardiac cells were pre-plated to separate the cardiomyocytes from the adherent cardiac fibroblasts. Cell cultures were incubated in serum free media with or without 5 ng/ml sertraline for 24 hours to match the in vivo exposure. Cells were also exposed to 10^{−4} M 5-HT (diluted in serum-free media containing 1% BSA, R&D Systems). Cardiomyocytes were lysed and ethanol fixed for Western blot analysis as described above. Primary

antibodies for β -actin (1:10000), total (1:5000) and phosphorylated Akt (1:1000) and total (1:2000) and phosphorylated ERK (1:2000)(Cell Signaling Technology, Danvers, MA) were assessed. Band densities were quantified by Li-COR Biosciences Odyssey Scanner (Lincoln, NE).

EdU incorporation assay was used to assess cardiomyocyte proliferation. Labeling cells with EdU was performed using a commercial kit (iClick EU Andy Fluor 488 Imaging Kit, Applied BioProbes, GeneCopoeia, Inc., Rockville, MD, USA) following the manufacturer's instructions. EdU solution was added to each well and cells were cultured for an additional 48 hours at 37 ° C. The cells were fixed with 4% paraformaldehyde for 15 minutes at room temperature and permeabilized with 0.5% Triton X-100 for 20 minutes at room temperature. After washing three times with PBS, 0.5 mL of iClick reaction cocktail was added to each well and the cells were incubated for 30 minutes at room temperature protected from light. Cells were incubated with α -myomesin antibodies and DAPI and visualized under confocal microscopy.

To quantify cardiomyocyte cross-sectional area, cardiomyocytes were isolated from 6, 10, and 13 day old mice. Sixty μ L of ethidium bromide was added to 300 μ L subsets of cardiomyocyte cells followed by examination under confocal microscopy. The myocyte suspension was pipetted onto a cover slip, mounted on a Perspex frame and allowed to settle for 1–2 minutes. Cell imaging was performed on a Leica TCS NT laser scanning confocal microscope (Leica Microsystems, Heidelberg, Germany) equipped with an argon laser coupled to an inverted microscope. A C-Apochromat (X63) water immersion objective lens was used. The cross sectional areas of the saved optical sections were calculated using ImageJ software.

Data analysis—All values are presented as means $\pm$ standard error of the mean. Statistical comparisons were performed by two-tailed *t*-tests. Baseline echocardiogram data and post-training echocardiogram data were compared by 2-way ANOVA factoring for exposure and training. Telemetry data and treadmill testing were also compared by 2-way repeated measures ANOVA factoring for exposure (saline vs. sertraline) and time. Post hoc analysis (Holm-Sidak test) was performed if statistically significant differences were detected. *P* 0.05 was considered significant.

RESULTS

Exposure Model

Dam weights at initiation of breeding and pup weights at time of birth were not different between the control and sertraline-exposed mice. Sertraline levels in pups at the end of 14 days were < 10 ng/mL (N=8), consistent with umbilical levels in humans.³⁰ There were no differences in heart weights or body weights at the time of weaning (PN day 21) between the control mice and sertraline-exposed mice (Table 1). No significant differences were observed in heart weights or body weights (Table 1) at 20 weeks of age between control and sertraline-exposed mice.

Cardiac Findings

No significant differences were observed between control and sertraline-exposed mice on PN 14 by hematoxylin-eosin staining or Masson's trichrome staining (Figure 1). Morphometric analysis on the Masson's trichrome stained sections using the Aperio slide scanner suggested a trend toward increased fibrosis in sertraline exposed mice (Control 6.2 % total positive blue area, Sertraline 7.5 % total positive blue area) thus hydroxyproline quantification was performed. Hydroxyproline, a marker of collagen content, was quantified in neonatal hearts to assess for fibrosis. At the time of weaning, sertraline-exposed mice had significantly increased hydroxyproline content compared to control mice (Figure 2a). Coupled with this finding, sertraline-exposed mice had changes in intracardiac serotonin receptor expression. Sertraline-exposed mice had significantly decreased cardiac 5-HT_{2B} receptor mRNA levels (Figure 2b), and increased 5-HT_{2A} and serotonin transporter (5-HTT) mRNA levels (Figure 2c and 2d, respectively) compared to control mice on PN day 21. No significant differences in receptor mRNA levels were observed in 5-HT_{1A}, 5-HT_{2C}, or tryptophan hydroxylase isoforms 1 and 2 (TPH-1, TPH-2) on PN day 21 (data not shown). Figure 3 demonstrates a representative sample of the immunoblot for the 5-HT_{2B} receptor antibody in left ventricle tissue samples, showing a reduction in protein expression corresponding to the significant decrease in mRNA expression. There was no appreciable difference in protein quantification for the 5-HT_{2A} or 5-HTT in left ventricular tissue from control or sertraline-exposed mice.

Hemodynamic Assessments

Sertraline-exposed mice had significantly decreased exercise capacity at 6 weeks of age on initial exercise testing (Figure 4). Following repeated training, no differences in exercise capacity were observed between the groups (Figure 4) and all mice had decreased exercise capacity with increasing age.

Adult mice exposed to sertraline in the perinatal period had significantly decreased stroke volumes and shortening fraction (Table 2) at baseline compared to control mice. This was associated with decreases in left ventricular volumes in diastole (Table 2, $p=0.07$) but data did not reach statistical significance. No differences were observed in intraventricular septum measurements in diastole (Control 0.74 ± 0.03 mm, Sertraline 0.74 ± 0.03 mm, $p=0.99$), left ventricular posterior wall thickness in diastole (Control 0.83 ± 0.03 mm, Sertraline 0.87 ± 0.03 mm, $p=0.4$), left ventricular mass (Control 124 ± 5.7 mg, Sertraline 120.2 ± 7.1 mg, $p=0.6$), or other measured parameters. There were no anatomical defects. Sertraline-exposed mice had similar measurements on echocardiograms after exercise testing compared to adult baseline (Table 2) with persistently reduced shortening fraction post-endurance training compared to control mice (Table 2). Post-training, control mice had significant reductions in left ventricular volumes in diastole and stroke volumes compared to control baseline measurements (Table 2).

There were no differences observed in heart rate (control 533.6 ± 13.7 beats per minute (bpm), SSRI 530.1 ± 15.0 bpm, $N=8$ control, 6 SSRI, $p=0.78$) or activity level (control 4.3 ± 0.4 horizontal movements/min, SSRI 4.5 ± 0.9 horizontal movements/min, $N=8$ control, 6 SSRI, $p=0.74$) between adult control and sertraline-exposed male mice.

Cardiomyocyte Findings

To begin mechanistic investigations into the reduced ventricular volumes, studies were performed on cardiomyocytes. Isolated cardiomyocytes from 2–4 day old control and sertraline-exposed mice showed significant changes in phosphorylated Akt to total Akt at baseline conditions and after exposure to serotonin (Figure 5a and 5b). No differences were observed in phosphorylated ERK to total ERK between the groups at baseline or in the presence of 5-HT (Figure 5c and 5d). By imaging, isolated cardiomyocytes from sertraline exposed mice showed decreased proliferation shown by decreased EdU-positive nuclei (Figure 6). Sertraline-exposed mice had significantly smaller cardiomyocyte area on PN 6, 10, and 13 (Day 6 Control $398 \pm 6 \mu\text{m}^2$, Sertraline $364 \pm 5 \mu\text{m}^2$ *; Day 10 Control $791 \pm 21 \mu\text{m}^2$, Sertraline $729 \pm 16 \mu\text{m}^2$ *, Day 13 Control $1803 \pm 69 \mu\text{m}^2$, Sertraline $1513 \pm 38 \mu\text{m}^2$ *, N= 1–3 mice/group, 200 cells/mouse, *p<0.05)

DISCUSSION

SSRIs are now prescribed to 6–10% of pregnant women in the United States with limited knowledge regarding the long term effects on child health.^{13–17} The association of SSRIs and congenital heart disease continues to be controversial and is difficult to interpret in the setting of maternal depression. Animal models play a critical role in answering the question of whether SSRIs exert independent effects on cardiac development. To our knowledge, this is the first study using both intrauterine and neonatal exposure in mice to mimic exposure throughout human pregnancy. The major finding of this study is that sertraline-exposed mice have long-standing reductions in adult stroke volumes and shortening fraction. We further demonstrated early alterations in cardiomyocyte development, collagen content and serotonin receptor expression precede the functional cardiac changes.

Sertraline-exposed mice had significant changes in neonatal intracardiac serotonin signaling which included decreased mRNA levels of the 5-HT_{2B} receptor and increased mRNA levels of the 5-HT_{2A} receptor and 5-HTT. Left ventricular tissues from sertraline-exposed mice also demonstrated decreased protein expression of the 5-HT_{2B} receptor but no difference in protein expression was observed for the 5-HT_{2A} receptor or 5-HTT. To assess the impact of these changes, we performed studies on isolated cardiomyocytes from saline or sertraline-exposed mice and evaluated cardiomyocyte signaling pathways, proliferation, and size. Isolated cardiomyocytes from sertraline-exposed mice had significantly decreased cardiomyocyte area and decreased phosphorylation of Akt, as well as decreased proliferation. We speculate that the decreased 5-HT_{2B} receptor mRNA levels play a significant role in the development of diminished adult stroke volumes and reduced cardiac function by impacting cardiomyocyte development. Supporting our hypothesis, genetic deletion of the 5-HT_{2B} receptors leads to cardiomyopathy and incomplete cardiac development attributed to trabecular defects with decreased cardiomyocyte size and number.²³ In addition, Sari and Zhou demonstrated that in vitro SSRI exposure decreases proliferation of fetal rat cardiomyocytes.¹⁹ The mechanism responsible for this decreased cardiomyocyte proliferation is unknown; however it is known that the 5-HT_{2B} receptor belongs to the G protein-coupled receptor family which activates several downstream signal transduction pathways crucial to cardiomyocyte proliferation and growth.^{23,33} Recent

advances have demonstrated the importance of Akt and ERK in cardiomyocyte proliferation^{34–38} and studies suggest SSRIs have antiproliferative effects through inhibition of both Akt and ERK activation.^{39,40} We demonstrated that sertraline exposure leads to decreased activation of Akt while no changes were observed in phosphorylation of ERK. Another consideration for our observed changes in cardiomyocytes is alterations in 5-HTT expression. 5-HTT is a key protein which plays a critical role in regulating availability of 5-HT. 5-HTT is expressed in fetal cardiomyocytes and mediates their proliferation, but data suggest only a transient effect of 5-HTT on cardiomyocytes in the developmental period.¹⁹ We found increased expression of the serotonin transporter which we suspect is secondary to our pharmacologic inhibition of 5-HTT from sertraline. Additional time points would be beneficial to determine how long the increased 5-HTT expression persists.

An alternative hypothesis for the diminished adult stroke volumes and reduced function is increased fibrosis. As we noted in an earlier study, sertraline-exposed mice had suggestions of increased fibrosis by Masson Trichrome although fibrosis was not quantified and could be affected by sampling bias.²⁹ In this study, no obvious changes were observed by histology but we had suggestions of increased fibrosis by morphometric analysis for blue staining in trichrome-stained slides. To better quantify the amount of collagen content in sertraline-exposed mice, we measure hydroxyproline, a marker of collagen content. Hydroxyproline content was significantly increased in sertraline-exposed mice on PN day 21. These data demonstrate structural changes in the neonatal heart and we cannot discount a key role of increased cardiac fibroblast proliferation in the development of decreased stroke volumes. In association with the increased fibrosis, we also found increased intracardiac mRNA levels of 5-HT_{2A} receptor. Several studies have demonstrated the 5-HT_{2A} receptor as an important mediator of cardiac hypertrophy.^{41,42} 5-HT_{2A} receptors trigger a hypertrophic response induced by 5-HT stimulation.⁴¹ Lairez et al demonstrated overexpression of the 5-HT_{2A} receptor in cardiomyocytes was transient and occurred during the early phase of hypertrophy in a model of transverse aortic constriction.⁴³ However, no differences were observed in baseline echocardiograms with regards to intraventricular septum dimensions or left posterior wall thickness. In addition, 5-HT_{2B} receptor blockade has been shown to reduce cardiac hypertrophy⁴⁴ and we demonstrated decreased mRNA levels of the 5-HT_{2B} receptor.

Another important consideration is the impact of 5-HT transporter inhibition and excess 5-HT on other monoamine systems such as the dopaminergic system. Although SSRIs are considered selective, the potential interactions with dopamine are important to mention. Dopamine neuronal cell bodies and terminal sites are influenced by 5-HT. Although the impact of perinatal SSRI exposure on the dopaminergic system has not been thoroughly investigated, especially with regards to cardiac development, we do know that early fluoxetine exposure during adulthood increases dopamine levels and D2 receptors in the prefrontal cortex, hippocampus, and other brain areas.^{45–47} Additionally, 5-HT₂ receptors influence dopamine activity.⁴⁸ Gain and loss of function studies involving tyrosine hydroxylase, the rate limiting step in conversion to dopamine, demonstrate a dynamic pattern during primitive heart tube formation. Tyrosine hydroxylase was an important regulator of the heart patterning⁴⁹. Future studies should assess the impact of SSRIs on 5-HT, dopamine, and dopamine precursors.

We have assessed the clinical relevance of the reduced left heart volumes and shortening fraction. We investigated the impact of sertraline exposure on heart rate and exercise tolerance. Even in patients without cardiac malformations, small hearts can lead to severe symptoms such as palpitations, dyspnea, exercise intolerance, and postural orthostatic tachycardiac syndrome, limiting one's quality of life.^{50,51} Furthermore, heart rate and maximum oxygen capacity have an inverse relationship⁵² and we previously demonstrated increased heart rates with neonatal sertraline exposure.²⁶ We found that sertraline-exposed mice had significantly decreased exercise capacity in early life at 6 weeks of age. No differences were detected between the groups with repeated exercise testing. No heart rate differences were observed in this model of SSRI exposure. The reduced stroke volume and shortening fraction noted in our model may have limited maximal early-life exercise. It is unclear why decreased exercise capacity did not persist over time as the changes in cardiac function were long-standing. One hypothesis for this is that differences in exercise capacity may not be detected because exercise capacity itself diminished with increasing age. An additional stressor (ischemic injury, high fat diet) may help determine the implications of the observed alterations.

One important difference between our prior neonatal exposure model and our new intrauterine plus neonatal exposure model was that no heart rate difference was currently observed. We suspect that the previously demonstrated increased heart rates were due to the relatively high exposure since we directly injected 5 mg/kg/d into the pups with no placental barrier to offer protection and our measured levels after 14 days of neonatal sertraline injections were similar to maternal levels. Our new model was established to more accurately depict clinical exposure to low dose sertraline (50 mg–100 mg/d) throughout pregnancy and levels at the end of the exposure are consistent with umbilical levels.³⁰ Because our new model had similar decreased stroke volumes with no increase in heart rate, we hypothesize that the long standing changes in cardiac function are due to a direct effect of altered serotonin signaling on the developing heart. Our results suggest the timing and extent of SSRI exposure may influence long term cardiac prognosis. Studies are underway to further investigate the serotonergic signaling pathway and its role in cardiac development.

CONCLUSION

We have demonstrated that perinatal sertraline exposure leads to decreased adult stroke volumes and cardiac function. In association with these findings, sertraline-exposed mice have important changes in neonatal heart fibrosis, altered serotonin receptor expression, and decreased early-life exercise capacity. Further investigations showed critical changes in the cardiomyocytes isolated from SSRI exposed mice including decreased Akt phosphorylation, proliferation, and cardiomyocyte cross-sectional area. Prospective clinical studies are needed to determine if the decreased stroke volumes and cardiac function are also seen in humans exposed to intrauterine SSRIs. Our data and that of others suggest the potential for dose-dependent effects of exposure ranging from structural morphologic changes to physiologic alteration and potential adult pathology. Although maternal antidepressant therapy has a critical role in the preservation of fetal and maternal health, further studies are needed to determine the potential long-term risks of intrauterine SSRI exposure during a key developmental period.

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Figure 1. Subgross photomicrographs of the hearts from mice treated with sertaline (A) and saline (C) and stained with Masson's trichrome to highlight collagen. Bars = 1 mm. Higher magnification of the black boxes in sertaline-exposed heart (B) and saline exposed heart (D). Bar = 100 μ m.

Figure 2.

Quantification of (A) intracardiac hydroxyproline content (N= 9 saline, 10 sertraline) and intracardiac mRNA levels of the (B) 5-HT_{2B} receptor, (C) 5-HT_{2A} receptor, and (D) serotonin transporter (5-HTT) on postnatal day 21 (N=15 saline, 16 sertraline). *P<0.05 compared to controls, **P<0.01 compared to controls.

Figure 3.

A representative sample of 5-HT_{2B} receptor expression in left ventricular tissues from sertraline-exposed mice (Ser) and control mice (Sal) as determined by Western blot.

Figure 4.

Total exercise distance measured during exhaustion tests for mice at 6, 8, 10, 15 and 20 weeks of age. Exercise distance is defined as total distance completed on treadmill with gradually increased incline and speed. N=11 saline, 12 sertraline. *P<0.05 compared to controls.

Figure 5.

Quantification from western blots of phosphorylated to total Akt (A) and phosphorylated to total ERK (C) in 2–4 day old isolated cardiomyocytes from control and sertraline-exposed mice at baseline and after stimulation with 5-HT (B,D). N= 11 saline, 8 sertraline. *p<0.05 compared to controls.

Figure 6.
Confocal microscopy of isolated cardiomyocytes from saline exposed (top) and sertraline-exposed (bottom) mice. Panels show myomesin stain (red), DAPI stain (blue), EdU stain (green), and merged image.

Table 1

Body weights and heart weights at time of weaning and 20 weeks of age.

	Control	Sertraline
PN day 21 heart (mg)	54.3 ± 3 (N=9)	55.6 ± 2 (N=10)
PN day 21 body (g)	8.7 ± 0.2 (N=20)	9.0 ± 0.2 (N=22)
Adult heart (mg)	134.2 ± 12 (N=8)	128.2 ± 7 (N=6)
Adult body (g)	30.4 ± 1.4 (N=20)	27.3 ± 0.9 (N=22)

Table 2

Echocardiograms at baseline and after repeated exercise training.

Baseline	Control	Sertraline
	N = 20	N = 22
LVIDd (mm)	4.2 ± 0.1	4.0 ± 0.1
LVVd (μL)	79 ± 3	71 ± 7
LVVs (μL)	30 ± 3	31 ± 2
Stroke volume (μl)	49 ± 2	39 ± 2 **
Shortening Fraction (%)	34 ± 2	29 ± 1 *

Post-exercise training	Control	Sertraline
	N = 10	N = 9
LVIDd (mm)	3.5 ± 0.2	4.0 ± 0.1
LVVd (μL)	59 ± 7 †	71 ± 7
LVVs (μL)	22 ± 4	32 ± 5
Stroke volume (μl)	37 ± 4 †	39 ± 2
Shortening Fraction (%)	39 ± 4	30 ± 2 *

* P < 0.05 compared to controls,

** P < 0.01 compared to controls.

† P < 0.05 compared to control baseline. LVIDd = left ventricular internal diameter in diastole, LVVd = left ventricular volume in diastole, LVVs = left ventricular volume in systole.