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Function of the Serotonin Transporter in Vasculature of the Female Rat: comparison with the male

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Summary

1. The serotonin transporter (SERT) handles serotonin (5-hydroxytryptamine, 5-HT) and is blocked by the antidepressant SERT inhibitors fluoxetine and fluvoxamine. While the importance of SERT in the central nervous system is clear, SERT also functions in the peripheral vasculature. We tested the hypothesis that vasculature from female rats has increased SERT function compared to male rats because females are more responsive to SERT inhibitors.
2. In addition to *in vitro* experiments, we imposed the challenge of a 5-HT infusion *via* mini-pump (7 days) to investigate how males *vs* females handle chronically elevated levels of 5-HT. The SERT knockout (KO) and wild type (WT) rat were used.
3. Blood vessels from the female (aorta, carotid artery, jugular vein and vena cava) took up 5-HT acutely *in vitro* in a SERT-dependent fashion (measured by HPLC). In isometric contractility experiments using isolated tissue baths, SERT affected contractility as evidenced by the 8-fold increase in potency of 5-HT in fluvoxamine-incubated WT aortae compared to control; fluvoxamine did not alter 5-HT-induced contraction in aortae from the SERT KO female rat. Infusion of 5-HT resulted in an increase in tissue 5-HT that was reduced to a larger extent in blood vessels from the female *vs* male SERT KO rat. Contraction to 5-HT in aortae from 5-HT-infused SERT KO rats was abolished compared to SERT WT rats.
4. Collectively, these data suggest that SERT function, when challenged with 5-HT, is modestly more important in the vasculature of the female *vs* male rat.

Keywords

selective serotonin reuptake inhibitors; contractility; platelet

Introduction

5-hydroxytryptamine (5-HT) is a hormone, synthesized primarily in the enterochromaffin cells of the intestine, with a myriad of physiological actions. The best-known action of 5-HT is its involvement in the mood disorder depression. The general hypothesis of depression is that synaptic levels/release of 5-HT are inappropriately low¹. Effective use of the SERT

inhibitors fluoxetine and fluvoxamine have supported this idea, in that these inhibitors prolong the biological accessibility of 5-HT to its receptors by preventing reuptake into a presynaptic neuron. Women are more likely to have depression than men and pre-menopausal women respond better to treatment with inhibitors of 5-HT uptake than same aged men^{2–14}. These findings suggest that SERT function centrally may be different in men and women.

We have investigated SERT function in a different way. Our interest lies in understanding the mechanisms by which 5-HT affects the cardiovascular system. The functions of 5-HT are complex in that 5-HT affects multiple organs that can participate in cardiovascular function, including the blood, brain, heart, kidney, adrenals and blood vessels. Our focus presently is on the vasculature. We have recently discovered that peripheral blood vessels express SERT protein and can take up 5-HT^{15–18}. Importantly, functional SERT reduces arterial contraction to 5-HT, supported by the leftward shift in 5-HT-induced contraction when vessels are incubated with a pharmacological inhibitor of SERT¹⁸. These data suggest that SERT plays role in vascular response to 5-HT.

Because of the findings in depression, we tested the overall hypothesis that SERT function was increased in the vasculature of female rats compared to male rats. Endpoints of tissue 5-HT concentration (HPLC) and contractility (isometric contraction) were measured. We used the SERT knock out (KO) rat and its wild type (WT) control. The SERT KO rat, created by Edwin Cuppen using ENU-mutagenesis, has a stop codon in the SERT gene that prevents expression of the full length SERT protein¹⁹. This animal, coupled with classical pharmacological methods, allowed us to discover that SERT is important to 5-HT uptake in the female vasculature and may be modestly more important in the female vs male upon 5-HT challenge.

Methods

Animal Use

Female and male SERT knockout (SERT-KO) and Wistar-based wild type rats (WT; all 8–10 weeks) were used in these studies. These rats were bred at *Michigan State University* under a breeding license obtained from genOway®. All animal procedures were performed in accordance with the Institutional Animal Care and Use Committee at *Michigan State University*. Animals were used untreated, or underwent telemeter or pump implantation (below).

Radiotelemetry

In some animals, radiotelemeters (DSI PhysioTel PA series transmitter model PA-C40) were implanted subcutaneously in the lower abdomen and catheters introduced into the left femoral artery under isoflurane anaesthesia. Pressure sensing tips were advanced into the thoracic aorta. All rats were given 7 days to recover prior to any measurement. Mean arterial pressure, heart rate and general activity were recorded at 10-minute intervals (10 second recording). A 24-hour period is reported.

Western blot analysis

Homogenates of aortae from female WT, SERT KO and normal male Sprague-Dawley rat were taken through standard Western analyses, and transferred to Immobilon P¹⁶. Blots were probed overnight with goat C-20 antibody (1:200, directed towards C-terminus, Santa Cruz Biotechnology, Santa Cruz, CA USA) or goat N-14 antibody (1:200, directed towards N terminus of SERT, Santa Cruz Biotechnology). A rabbit anti-goat secondary linked to horseradish peroxidase (Santa Cruz Biotechnology) was used to develop using ECL reagents

from Amersham (Piscataway, NJ, USA). Blots were reprobed with an antibody that recognizes smooth muscle α -actin (EMD Biosciences, La Jolla, CA, USA) as a measure of equivalent protein loading.

Pump Implantation

Under isoflurane anesthesia, osmotic pumps with a release rate of 10.0 μ l/h and duration of 7 days (model 2ML1; Alzet, Cupertino, CA) were implanted between the scapulae. 5-HT (25 μ g serotonin creatinine sulfate/kg/min s.c.) was placed in the pump; this is a dose previously established as tolerated in the rat²⁰. On the 7th day after pump implantation, rats received pentobarbital (i.p.). Whole blood was drawn *via* cardiac puncture for measurement of platelet poor and platelet rich 5-HT concentration. Tissues were also collected.

Plasma and platelet 5-HT measurements

Five milliliters of blood were collected from the left cardiac ventricle and transferred into a EDTA anticoagulant vacutainer tube. Pargyline and ascorbic acid (10 μ M each) were added as inhibitors of monoamine oxidase and general oxidation, respectively. The tubes were centrifuged at 160 g for 30 min at 4°C to obtain platelet-rich plasma. Two milliliters of supernatant containing plasma and a buffy coat layer were pipetted into EDTA-coated plastic tubes and mixed with a 1:1 dilution of 0.5 M EDTA. Pargyline and ascorbic acid (10 μ M each) were added. The tubes were centrifuged at 1,350 g for 20 min at 4°C for platelet-poor plasma samples (poor) as measures of free 5-HT. To the remaining pellet (platelet layer), 1 ml of platelet buffer [mM 145 NaCl, 5 KCl, 1 CaCl₂, 1 MgSO₄, and 10 D-glucose and 1 μ M ADP] was added. Pargyline and ascorbate were added. The tubes were vortexed and allowed to sit on ice for 15 min for platelets to become activated and degranulate. The tubes were centrifuged at 730 g for 10 min at 4°C. Trichloroacetic acid (10%) was added to deproteinate samples, and the samples sat on ice for 10 min. The samples were centrifuged at 4,500 g for 20 min at 4°C and then ultracentrifuged at 280,000 g for 2 hours. These samples were considered platelet rich plasma (rich) for measures of platelet 5-HT.

Samples were injected onto a C18 reverse phase analytical column (ESA Biosciences, Chelmsford, MA) protected by a precolumn cartridge filter. This column was coupled to a single coulometric electrode conditioning cell positioned before autosampler in series with dual electrode analytical cells (ESA Biosciences, Chelmsford, MA) positioned after the analytical column. The conditioning electrode potential was set at 0.35 V, while the coulometric analytical electrodes were set at 0.0 and 0.2 V. Amounts of the monoamine oxidase metabolite 5-hydroxyindole acetic acid (5-HIAA) and 5-HT were determined by comparing peak areas in samples with those obtained from standards. Values are reported as a concentration relative to protein content. Protein content was determined by the Lowry method and used for tissue normalization when reporting 5-HT and 5-HIAA. 5-HT and 5-HIAA concentrations in blood were expressed as ng/ml. The lower limit of sensitivity for detection of 5-HIAA and 5-HT was 0.5 pg/ μ l sample.

5-HT Uptake Assay

At room temperature, tissues were placed in 1.5-mL plastic centrifuge tubes containing physiological salt solution (PSS) [mM: NaCl (130.00); KCl (4.70); KH₂PO₄ (1.18); MgSO₄·7H₂O (1.17); CaCl₂·2H₂O (1.60); NaHCO₃ (14.90); dextrose (5.50); and CaNa₂EDTA (0.03), pH 7.2]. In some experiments, the tissues were incubated either with vehicle (deionized water) or the SERT inhibitor fluvoxamine (1 μ M) for 30 minutes. 5-HT (1 μ M) or vehicle (deionized water) was then added for 15 minutes (at room temperature, this is a time of active uptake;^{17, 18}). The blood vessels from untreated rats were dipped several times in drug-free PSS to avoid extracellular 5-HT contamination and placed in 75 μ L of 0.05 mM sodium phosphate and 0.03 mM citric acid buffer (pH 2.5) containing 15%

methanol (tissue buffer). Blood vessels obtained from 5-HT-infused rats were isolated, cleaned and directly placed in tissue buffer. Samples were frozen (-80°C) until assay. Samples were thawed, sonicated for 3 seconds. Supernatant was collected and transferred to new tubes. Tissue pellets were dissolved in 1.0 M NaOH and assayed for protein. Concentrations of 5-HIAA and 5-HT in tissue supernatants were determined by HPLC as described above.

Isometric Contraction

Aortic rings of endothelium-intact thoracic aorta from WT and SERT-KO rats (untreated or 5-HT-infused) were used for measurement of isometric contractile force as described previously¹⁶. Briefly, aortic rings were mounted into 50-ml tissue baths on Grass isometric transducers (FT03; Grass Instruments, Quincy, MA) connected to a ADI Instruments PowerLab, placed under optimum passive force (4000 mg for aorta, determined previously), and allowed to equilibrate for 1 hour before a challenge with a maximal concentration of phenylephrine (10^{-5} M). After this initial challenge, tissues were washed until tone returned to baseline and half-maximal concentration of phenylephrine (10^{-7} M) was added followed by acetylcholine (10^{-5} M). Endothelium-intact tissues were considered those that relaxed more than 70% to acetylcholine. Cumulative concentration response curves to 5-HT (10^{-9} to 10^{-5} M) were generated after incubation for 60 min with vehicle or fluvoxamine (1 μM); cumulative concentration response curves to phenylephrine (10^{-9} to 10^{-5} M) were also constructed. In some experiments, tissues were also contracted with half-maximal concentration of phenylephrine (10^{-7} M) followed by curves to acetylcholine (10^{-9} to 10^{-5} M). Contraction to the EC_{50} concentration of phenylephrine achieved a similar percentage contraction in all groups ($\sim 50\%$). All data were captured in the program Chart.

Data Analysis

Contraction was evaluated from the viewpoint of absolute tension level and is reported in milligrams tension. Relaxation is reported as a percentage of initial contraction to a half-maximal concentration of phenylephrine. Agonist potency values [concentration producing 50% of the maximal response (EC_{50} or $\text{pD}_2 = -\log \text{EC}_{50}$)] were calculated by linear regression within GraphPad Prism (5.0). If a maximum contraction was not obtained, the EC_{50} value reported was estimated and the true EC_{50} value is equivalent or greater than the reported value. Unpaired *t* test with Welch's correction when appropriate was used for *in vitro* studies comparing two groups. In concentration-response curves, repeated measures two-way ANOVA followed by Bonferroni post hoc test was used. In all cases, a *P* value of <0.05 was considered significant. All results are presented as means $\pm$ SEM.

Results

Physiological parameters of SERT-KO and WT male and female rats, expression of SERT protein and plasma measures

Table 1 lists basic physiological parameters for the male and female SERT KO rats. At the time of experimentation, the body weight of SERT-KO rats was significantly reduced when compared to WT rat, whether female or male. Regardless of strain, age-matched females were smaller than males. Despite breeding well and looking healthy, the number of pups/breeding was smaller in the SERT-KO rats (8.3 ± 0.5 ; $n=29$ litters) compared to WT rats (10.4 ± 0.5 ; $n=26$ litters; $p < 0.05$). Heart rate and general activity were not different between genders (male vs female) or strain (WT vs SERT KO). Mean arterial blood pressure was modestly but significantly reduced in the SERT KO vs WT of both genders (Table 1).

Figure 1 demonstrates results of Western analysis probing for SERT protein in homogenates of aortae from WT and SERT KO female rats. As expected, the N-14 antibody, which

recognizes the amino terminus of the protein, was detected in all samples at the appropriate molecular weight (~70 kDa). By contrast, the C-20 antibody detected SERT in the WT and SERT KO homogenates differently. In the SERT KO, the signal was significantly reduced (> 70% reduction by densitometry) and this is expected given that the mutation in the SERT gene makes for a loss of the C-terminus. The loading control α -actin was similar in all lanes, indicating that the loss of the C-20 response was not because of reduced protein.

The basal (untreated) concentration of free plasma 5-HT (platelet *poor* plasma) and platelet bound 5-HT (platelet *rich* plasma) was greater in WT rats (Figure 2A) compared to SERT-KO rats (compare y-axes; Figure 2B). The female WT rat contained modestly but not significantly elevated free plasma 5-HT (poor: 45.1 ± 8.8 ng/ml) compared to male WT rat (poor: 30.3 ± 7.6 ng/ml), paralleled by a significantly smaller platelet rich 5-HT concentration compared to male (Figure 2A). No such differences were observed in the KO rats, but it is notable that most measures of 5-HT and 5-HIAA were lower in samples from the KO vs WT rats.

Basal parameters in vasculature of WT and SERT-KO female rats

5-HT and 5-HIAA content in the vasculature of WT and SERT-KO rats—In aorta, vena cava, carotid artery and jugular vein of WT female rats, basal levels (obtained after exposure to vehicle) of 5-HT and 5-HIAA could be measured (Table 2, top). Veins possess a greater amount of 5-HT than arteries ($P < 0.05$). 5-HIAA content was lower in blood vessels from SERT-KO rats compared to the same blood vessels in WT rats in females (Table 2, top; $P < 0.05$).

Acute 5-HT uptake in the vasculature of WT and SERT-KO rats: role of SERT

Uptake of exogenous 5-HT was first evaluated in aorta, vena cava, carotid artery, and jugular vein of female WT rats after incubation *in vitro* with exogenous 5-HT (1 μ M, 15 minutes). This single time point was chosen based on the knowledge this was active uptake (not saturated) that could readily be measured. These experiments were done in the absence of monoamine oxidase (MAO) inhibition such that we could observe the natural handling of 5-HT. This allows comparison with results from later experiments in which 5-HT (but not pargyline) was chronically infused. 5-HT exposure induced a significant increase in 5-HIAA but not 5-HT content in all vessels from female WT rats (Table 2, bottom).

Two separate tactics were taken to examine the role of SERT in uptake of 5-HT by the vasculature. First, the contribution of SERT to 5-HT was evaluated by measuring the effect of the SERT inhibitor fluvoxamine on 5-HT uptake in aortae of female WT rats treated with vehicle or the SERT inhibitor fluvoxamine. Only aorta were used because each aorta could be divided into four segments that were necessary for this experiment; other tissues did not provide enough mass to allow for confident measures of 5-HT and 5-HIAA when divided in such a manner. Aortic rings from female WT rats were exposed to 5-HT (1 μ M, 15 minutes) following a 30-min incubation with vehicle or fluvoxamine (1 μ M). In the presence of fluvoxamine (1 μ M), 5-HIAA content was reduced when compared to uptake of 5-HT in the presence of vehicle (vehicle control = 0.41 ± 0.15 ng/mg protein, 5-HT control = 2.7 ± 0.40 ng/mg protein; fluvoxamine control = 0.42 ± 0.13 ng/mg protein, fluvoxamine + 5-HT = 1.37 ± 0.17 ng/mg protein; $N=8$, $p < 0.05$ by ANOVA, fluvoxamine + 5-HT different from 5-HT control).

Second, the acute uptake of 5-HT in blood vessels from SERT KO rats was investigated in an *in vitro* protocol. Compared to female WT rats, the 5-HIAA content of blood vessels from female SERT-KO rats exposed to 5-HT (1 μ M, 15 min) was significantly smaller when compared to vessels from WT rats but was not abolished (Table 2, bottom).

Isometric contraction in aortae from WT and SERT KO rats—This series of experiments includes tissues from the male, while the above experiment (Table 2) did not as the comparative uptake data had already been published^{15, 16}. Pharmacological parameters for the response of the vasculature from these rats, considered control, are in Table 3. 5-HT induced a concentration-dependent contraction in aorta from WT (Figure 3A) and SERT-KO rats (Figure 3B). 5-HT was more potent in aortic rings from female SERT-KO and male SERT-KO than from their own WT control (Table 3; pD_2 value comparison, $P < 0.05$). By contrast, the maximal contraction to 5-HT was reduced in aortae from female SERT-KO rats vs female WT rats ($P < 0.05$); a reduction in 5-HT-induced maximal contraction was also observed in aorta from the male. The maximum contraction induced by 5-HT was not significantly different between aortae from female and male rats within the same group (WT vs WT, KO vs KO).

The contractile potency of 5-HT in aortae from female WT and SERT-KO rats was evaluated after incubation with vehicle or fluvoxamine (1 μ M, 30 minutes). Fluvoxamine caused a left-shift in the concentration response curve to 5-HT in aortae from female WT rats (pD_2 vehicle 5.9 ± 0.07 vs. fluvoxamine 6.7 ± 0.02 , $P < 0.05$). As expected, fluvoxamine did not modify 5-HT-induced contraction in aortae from SERT-KO rats nor did it modify maximum contraction (not shown).

While 5-HT-induced contraction was clearly modified in aorta from SERT KO rats, contraction to the α_1 adrenergic receptor agonist phenylephrine was not changed. Phenylephrine induced a concentration-dependent contraction with no significant differences in potency or E_{max} between aortae from female and male WT (Figure 3C, Table 3). E_{max} and pD_2 values of phenylephrine-induced contraction were similar between aortae from female and male SERT-KO and male SERT-KO rats (Figure 3D), and these responses were not significantly different from those obtained in WT rats within the same gender. Similarly, acetylcholine-induced aortic relaxation, as a general measure of endothelial cell function, from female WT and male WT rats (contracted half-maximally with phenylephrine) was similar (Figure 3E). ACh-induced relaxation was also observed in aortae from female and male SERT-KO and male SERT-KO rats (Figure 3F). Compared to WT rats, no significant differences were observed in ACh-induced relaxation in aortae from female SERT-KO and male SERT-KO rats within the same gender ($P > 0.05$; ANOVA).

5-HT infusion in WT and SERT-KO rats

Plasma and platelet measurements—5-HT (25 μ g/kg/min, sc) was administered to SERT-KO and WT female and male rats for 7 days. On the final day, plasma and platelet concentrations of 5-HT were measured and the results are shown in Figure 4. The layout of this graph is exactly like that of figure 2 (A = WT, B = SERT KO), allowing for direct comparison of control and 5-HT-infused values. Increased handling and metabolism of 5-HT was supported by increases in 5-HIAA in the platelet poor samples. In the SERT KO rats, all values increased compared to basal blood levels (numbers above bars), save for the female KO (rich) measure of 5-HT in which the variability of measure was high.

Chronic 5-HT uptake in the vasculature of WT and SERT-KO rats: role of SERT

—The amount of 5-HT and its metabolite 5-HIAA were measured in blood vessels from female and male rats from both strains after 5-HT infusion for 7 days (Table 4). No significant differences were observed in blood vessels between female and male within the same strain. An increase in 5-HT and/or 5-HIAA content in aorta, vena cava, carotid artery and jugular vein of female WT infused with 5-HT was observed when compared to the same blood vessel in females with no treatment (Table 4 vs. Table 2). Veins of WT rats took up a substantial amount of 5-HT. By contrast, in the female SERT KO vs WT rats, a decreased

content of 5-HT or 5-HIAA was observed in vena cava, carotid artery and jugular vein, but not aorta. In the males, 5-HT and/or 5-HIAA content was decreased in arteries but not in veins of male SERT-KO rats compared to same blood vessels of male WT rats during this one-week infusion.

Isometric contraction in aortae from 5-HT-infused WT and KO rats—Aorta were removed after a week of 5-HT infusion and taken through isometric contractility experiments as described above. Pharmacological parameters for these responses are reported in Table 5. Control concentration response curves (those from non-infused rats) and their parameters were presented in figure 3 and table 3, respectively. 5-HT-induced contraction was observed in aorta from WT animals infused with 5-HT, but with an ~3-fold reduction in potency than observed in aorta from non-infused rats (Figure 5A). Notably, 5-HT did not contract aortae from SERT-KO rats (female and male) infused with 5-HT (Figure 5B). The maximal contraction induced by 5-HT was smaller in aortae from 5-HT infused female *vs* male WT rats ($P < 0.05$). Compared to untreated WT rats within the same gender, the maximal contraction to 5-HT was significantly smaller in aortae from 5-HT infused female WT (ANOVA, $P < 0.05$) and was increased in 5-HT-infused male WT (ANOVA, $P < 0.05$) rats.

By contrast, phenylephrine was similarly potent in aortae from 5-HT infused female and male WT, as determined by the pD_2 values (Table 5). Maximal phenylephrine-induced contraction was reduced in aorta from 5-HT infused female WT *vs* 5-HT infused male WT (figure 5C). However, maximal contraction to phenylephrine in aortae from 5-HT infused rats was not reduced when compared to phenylephrine-induced contraction from untreated rats (figure 3, table 2). Similarly, the potency of PE in aortae from untreated SERT KO and infused with 5-HT was not different (figure 5D, **compare to** figure 3D). These data suggest general contractility/smooth muscle function was not impaired in the SERT KO and WT rats infused with 5-HT. Similarly, the parameters of acetylcholine-induced relaxation in phenylephrine-contracted aortae were unaffected by 5-HT infusion when considering strain (KO, WT) and gender (male, female; Table 5 and Figures 5E, 5F). As was observed under basal conditions, acetylcholine-induced relaxation was slightly more pronounced in aortic rings from 5-HT infused female rats compared to 5-HT infused male rats within the WT but not SERT KO group.

Discussion

SERT inhibitors have now been on the market over two decades, and there are individuals (in particular females) who have taken these inhibitors just as long. Use of the SERT KO rat provided an opportunity to investigate long-term cardiovascular effects of SERT inhibition. In this paper, we presently focus on vascular uptake and contraction to 5-HT as modified by SERT. Overall, our data suggest that SERT is critical to uptake and contraction to 5-HT in vasculature of the female, and may be modestly more important in female *vs* male.

Uptake and distribution of 5-HT

5-HT concentration was reduced in the platelet rich fraction of blood from the WT female compared to male. In parallel, the free 5-HT in females tended to be higher than the males. These data suggest an overall difference in the mobility/placement of 5-HT within the body of the male *vs* the female. The platelet is the primary cardiovascular source of 5-HT. Platelets possess SERT and it is through SERT that platelets become a reservoir/storage for 5-HT, picking up 5-HT in the gastrointestinal system; platelets do not synthesize 5-HT²¹. The importance of platelet SERT in enabling blood to carry 5-HT is supported by our findings that basal platelet rich and poor plasma concentrations of 5-HT are nearly 1000-fold

reduced in the SERT KO vs WT, regardless of gender. Upon infusion of 5-HT, levels of platelet poor and rich plasma 5-HIAA were elevated significantly, and this was observed both in the WT and SERT KO rats (female and male). Platelet rich fractions of the SERT KO contain more 5-HT than in basal conditions, suggesting that platelets possess alternative, non-SERT dependent mechanisms by which to take up 5-HT.

Contractility

We previously demonstrated that SERT modifies contraction to 5-HT in arteries from the male rat. SERT removes 5-HT from extracellular space, thereby removing 5-HT from its cognate receptors¹⁶ and reducing the sensitivity of the vessel to 5-HT. In the present study, we demonstrate similar findings for aortae of the female. Two points support this finding. First, use of the SERT inhibitor fluvoxamine caused a leftward shift, or increase in potency, for 5-HT in the normal WT aorta. This suggests 5-HT is actively taken up by SERT, removing it from participating in contraction. Fluvoxamine has recently been shown as capable of inhibiting currents carried by the voltage gated K⁺ channel Kv1.5²², raising the possibility that a leftward shift in responsiveness to 5-HT could be because of inhibition of this important channel. However, this action of fluvoxamine is not consistent with our findings that fluvoxamine did not equivalently shift 5-HT curves in arteries from KO and WT, in which no data exist to suggest Kv1.5 function is different. PE-induced contraction was also not leftward shifted in the SERT KO vs WT, but here again we have no information as to whether adrenergic and serotonergic receptors use Kv channels mechanistically in similar or different ways. If Kv channel function or expression is reduced in the SERT KO rat, then this would be consistent with such an action of fluvoxamine, but these measures have not been made. It is most likely that arterial contraction to 5-HT is enhanced because of reduced uptake of 5-HT.

Second, 5-HT was more potent in aortae from the female SERT KO compared to WT. However, the efficacy of 5-HT was dramatically reduced in the SERT KO of both male and female compared to WT. Given that there is less 5-HT in the blood, one might speculate that this would upregulate vascular 5-HT receptors and result in an increase in potency of 5-HT (as observed) but also an increase in efficacy. This was not what we observed. Reasons for the depression in 5-HT-induced maximal contraction are not known, but do not include a general reduction in contractility, because PE-induced maximum contraction was not modified between SERT KO and WT of both genders. Similarly, endothelial cell function, as measured by acetylcholine-induced relaxation, was minimally enhanced in the WT females compared to WT males, and this was not observed in the SERT KO rats. This finding, though modest, suggests that SERT may modify endothelial cell function in the females.

The profound reduction in 5-HT-induced contraction in the arteries from SERT KO rats infused with 5-HT suggests that SERT is somehow connected to 5-HT_{2A} receptor function, as we know the 5-HT_{2A} receptor is the primary contractile receptor in the rat aorta²³. This is supported by the additional observation that infusion of 5-HT, creating a higher blood 5-HT/5-HIAA environment, resulted in a complete loss of 5-HT-induced contraction. This is most likely because of complete desensitization of the 5-HT_{2A} receptor; we did not test another serotonergic agonist to examine this idea. It is possible that removal of 5-HT by SERT is important for keeping the 5-HT_{2A} receptor sensitive to 5-HT, preventing desensitization. It will take significant experimentation to determine why this loss of 5-HT-induced contraction occurs. By contrast, PE-induced contraction was largely intact in the WT and SERT KO infused with 5-HT, though PE-induced maximum contraction was reduced in the female WT compared to male WT (Figure 5). Gender-related differences in contractility have been observed, including contraction to oxidized LDL in the coronary artery²⁴, mesenteric

artery²⁵, 5-HT-induced contraction in the coronary artery²⁶, and 5-HT-induced contraction in mouse aorta²⁷.

Tissue uptake

The importance of SERT to tissue uptake of 5-HT is supported by the findings that different vascular tissues from the SERT KO rat – arteries (aorta, carotid artery) and veins (vena cava, jugular vein)—show diminished levels of 5-HT or 5-HIAA basally. It is interesting that basal 5-HT can still be measured in the tissues of the SERT KO, and this is novel finding for vasculature of the females. We speculate that this is internal synthesis of 5-HT within the vasculature. Tryptophan hydroxylase 1, the rate limiting enzyme of peripheral 5-HT synthesis, is present and active in arteries and veins^{15, 18}. Endogenous synthesis of 5-HT is one mechanism by which 5-HT concentration may be measurable in the SERT KO¹⁸. Alternatively, other sources for 5-HT uptake may exist. The norepinephrine and dopamine transporter can use 5-HT as a substrate, as can organic cationic and extraneuronal transporters^{28, 29}. While the exact contributions of these different transport systems is unknown, they offer a possible explanation as to how 5-HT could be something other than zero in the tissue of the KO rat.

Vasculature from the female actively took up 5-HT, and this was diminished in the vessels from the SERT KO rat. This is largely similar to the male^{15, 16}, but for one finding. The veins of the male SERT KO rat continued to take up 5-HT in a normal manner. This finding confirms results published previously, that uptake of 5-HT was independent of SERT in veins from the male¹⁵. Thus, gender differences in uptake of 5-HT exist. While veins from the male take up 5-HT in a SERT-independent manner, blood vessels (veins and arteries) from the female are more dependent on SERT for uptake of 5-HT and this is a novel aspect of this work.

Several studies have reported on gender-related differences regarding cardiovascular risks. Women often have lower orthostatic tolerance than same-aged male counterparts³⁰. In experimental models of hypertension, male rats develop hypertension earlier and more severely than female rats^{31, 32}, and differences in the human population exist³³. In terms of SERT inhibition, use of inhibitors has been associated with both elevations and decreases in blood pressure^{34–38}, and these studies were not stratified with respect to gender-related responses. Thus, while we can make no conclusions as to how potential differences in vascular SERT function contributes to SERT inhibitor-induced changes in blood pressure, this is of interest. Our telemetry studies suggest there are no differences in basal blood pressure or heart rate between genders, but these experiments were done without an exogenous 5-HT challenge.

In summary, we demonstrate that blood and vasculature from the female rat have a robust ability to handle and take up 5-HT, uptake that is highly dependent on SERT and potentially more consistently dependent on SERT than in the male, especially when faced with a challenge of 5-HT. The vasculature must thus be considered a site for 5-HT uptake, as well as a site in which antidepressants exert potential physiological effects on the cardiovascular system.

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Figure 1.

Western analysis of SERT protein expression in aortae from female wild type (WT) and serotonin transporter knockout (SERT KO) rats. Each lane represents an individual animal. Blots were reprobbed with smooth muscle α -actin as a loading control. SD M = Sprague Dawley Male as a positive control.

Figure 2.

Basal levels of plasma (poor) and platelet (rich) content of 5-HT and 5-HIAA in female and male rats. Numbers above bars are values that are not visible. Bars represent means $\pm$ SEM for N=4–5 animals in each group. * statistical significance from male. Panel A = results for WT rat, Panel B = results for SERT KO rat. 5-HT = 5-hydroxytryptamine, 5-HIAA = 5-hydroxyindoleacetic acid. Boxes emphasize the differences in the y-axes maximum values.

Figure 3.

Basal vascular responses. **A,B:** 5-HT-induced contraction of aortae from female and male WT and SERT KO rats. **C,D:** Phenylephrine-induced contraction of aortae from female and male WT and SERT KO rats. **E,F:** Acetylcholine-induced relaxation of aortae from female and male WT and SERT KO rats. The points represent means $\pm$ SEM of the response expressed as milligrams (A-D) or as a percentage of relaxation of phenylephrine-induced contraction (E,F) for number of animals in parentheses.

Figure 4.

Plasma (poor) and platelet (rich) content of 5-HT and 5-HIAA in female and male rats after a one-week infusion of 5-HT. Bars represent means $\pm$ SEM for N=4–5 animals in each group. Panel A = results for WT rat, Panel B = results for SERT KO rat. 5-HT = 5-hydroxytryptamine, 5-HIAA = 5-hydroxyindoleacetic acid.

Figure 5.

Vascular responses after one week infusion of 5-HT. **A, B:** 5-HT-induced contraction of aortae from female and male WT and SERT KO rats. **C, D:** Phenylephrine-induced contraction of aortae from female and male WT and SERT KO rats. **E, F:** Acetylcholine-induced relaxation of aortae from female and male WT and SERT KO rats. The points represent means $\pm$ SEM of the response expressed as milligrams (A-D) or as a percentage of relaxation of phenylephrine-induced contraction (E, F) for number of animals in parentheses. * = differences from male responses.

Table 1

Physiological parameters of rats.

Body Weight (grams)	WT	SERT KO
Female	202 ± 8 [†]	166 ± 6 ^{*†}
Male	352 ± 11	278 ± 7 [*]
Blood pressure (mm Hg)		
Female	113.3 ± 1.5 (8)	106.6 ± 1.5 (7) [*]
Male	105.5 ± 1.1 (8)	100.5 ± 1.5 (8) [*]
Heart Rate (bpm)		
Female	368.8 ± 1.6 (8)	387.2 ± 4.0 (7) [*]
Male	345.8 ± 5.8 (8)	353.6 ± 5.9 (8)
Activity (arbitrary unit)		
Female	2.59 ± 0.5 (4)	2.28 ± 0.3 (4)
Male	2.62 ± 0.3 (4)	1.98 ± 0.3 (4)

Data are reported as means ± SEM of (n) animals.

^{*} indicates statistical difference (P< 0.05) from WT values.[†] indicates significant difference (P< 0.05) from male values.

WT = wild type, SERT-KO = serotonin transporter knockout

Table 2

5-HT and 5-HIAA content in vasculature from female WT and SERT-KO rats exposed to vehicle or 5-HT acutely.

	WT		SERT-KO	
	5-HT (ng/mg protein)	5-HIAA (ng/mg protein)	5-HT (ng/mg protein)	5-HIAA (ng/mg protein)
Female – Vehicle (15 minutes)				
Aorta	1.65 ± 0.52 (8)	0.41 ± 0.15 (8)	1.04 ± 0.21 (7)	0.012 ± 0.01 (8) [†]
Vena Cava	5.86 ± 1.47 (6) [¶]	0.29 ± 0.052 (6)	4.99 ± 0.59 (6) [¶]	0.06 ± 0.01 (6) ^{†¶}
Carotid artery	0.44 ± 0.14 (5)	0.91 ± 0.32 (5)	0.53 ± 0.21 (5)	0.01 ± 0.01 (5) [†]
Jugular vein	6.93 ± 1.29 (6) [¶]	0.32 ± 0.064 (6)	4.31 ± 1.06 (6) [¶]	0.06 ± 0.01 (6) ^{†¶}
Female – 5-HT (1 µM, 15 minutes)				
Aorta	1.81 ± 0.33 (8)	2.76 ± 0.39 (8) *	1.34 ± 0.10 (8)	0.89 ± 0.03 (8) ^{*†}
Vena Cava	7.98 ± 1.96 (6) [¶]	2.61 ± 0.32 (6) *	4.69 ± 1.00 (6) [¶]	1.01 ± 0.14 (6) ^{*†}
Carotid artery	0.68 ± 0.06 (5)	3.84 ± 0.30 (5) *	2.26 ± 0.81 (5)	0.59 ± 0.07 (5) ^{*†}
Jugular vein	9.76 ± 1.30 (6) [¶]	2.17 ± 0.16 (6) *	4.33 ± 0.52 (6)	1.45 ± 0.12(6) ^{*†¶}

Data are reported as ng/mg of protein ± SEM of (n) animals.

Statistical significant differences (P < 0.05):

* vs vehicle exposed tissue;

[†] vs. WT rats;

[¶] vs. same-sized artery

5-HT: 5-hydroxytryptamine; 5-HIAA = 5-hydroxyindole acetic acid; WT = wild type; SERT KO = serotonin transporter knockout

Table 3

Pharmacological parameters of agonist-induced changes in contractility in aortae from untreated rats.

	WT		SERT-KO	
	pD ₂ (-log EC ₅₀ [M])	E _{max} (5-HT, PE: milligrams ACh: % relaxation)	pD ₂ (-log EC ₅₀ [M])	E _{max} (5-HT, PE: milligrams ACh: % relaxation)
5-HT				
Female	5.8 ± 0.09 (4)	1642 ± 295 (4)	6.40 ± 0.11 (4) [†]	719 ± 197 (4) [†]
Male	5.4 ± 0.15 (4)	1659 ± 61 (4)	6.30 ± 0.07 (3) [†]	500 ± 131 (3) [†]
PE				
Female	7.2 ± 0.05 (4)	1987 ± 210 (4)	7.20 ± 0.15 (4)	1970 ± 249 (4)
Male	7.1 ± 0.06 (4)	2198 ± 40 (4)	7.20 ± 0.10 (4)	1787 ± 243 (4)
ACh				
Female	7.20 ± 0.11 (4)	110.3 ± 7.6 (4)	7.16 ± 0.21 (4)	89.2 ± 4.9 (4)
Male	7.00 ± 0.09 (4)	101.7 ± 5.7 (4)	7.06 ± 0.20 (4)	89.3 ± 2.0 (4)

Data are reported as means ± SEM of (n) animals.

[†] vs. WT rats

5-HT = 5-hydroxytryptamine, PE = phenylephrine, ACh = acetylcholine

Table 4

5-HT and 5-HIAA content in peripheral blood vessels from male and female WT and SERT-KO rats chronically treated with 5-HT.

	WT		SERT-KO	
	5-HT (ng/mg protein)	5-HIAA (ng/mg protein)	5-HT (ng/mg protein)	5-HIAA (ng/mg protein)
Female				
Aorta	4.74 ± 1.66 (4)*	2.41 ± 0.42 (4)*	2.47 ± 0.97 (4)	1.45 ± 0.32 (4)*
Vena Cava	57.13 ± 11.24 (4)*	4.96 ± 0.48 (4)*	18.44 ± 4.87 (4)* [†]	3.21 ± 1.55 (4)*
Carotid artery	3.48 ± 1.92 (4)	6.31 ± 0.89 (4)*	3.66 ± 2.83 (4)	3.50 ± 2.66 (4) [†]
Jugular vein	82.61 ± 10.52 (4)*	8.63 ± 1.90 (4)*	40.48 ± 22.50 (4)	3.18 ± 1.02 (4)* [†]
Male				
Aorta	6.03 ± 1.68 (5)	2.89 ± 0.58 (5)	1.40 ± 0.47 (4) [†]	1.13 ± 0.10 (4) [†]
Vena Cava	47.76 ± 18.54 (5)	5.94 ± 1.76 (5)	43.01 ± 15.85 (5)	5.22 ± 1.27 (5)
Carotid artery	4.37 ± 1.00 (5)	6.86 ± 2.13 (5)	1.70 ± 0.39 (4) [†]	2.96 ± 1.86 (4) [†]
Jugular vein	71.1 ± 30.96 (5)	5.01 ± 0.98 (5)	30.25 ± 15.12 (4)	4.03 ± 0.98 (4)

Data are reported as ng/mg protein for number of animals in parentheses. Statistical significant differences ($P < 0.05$):

* vs. vehicle exposed tissues (Table 2) within the same blood vessel and the same gender.

[†] vs. WT within the same gender.

5-HT: 5-hydroxytryptamine; 5-HIAA = 5-hydroxyindole acetic acid; WT = wild type; SERT KO = serotonin transporter knockout

Table 5

Pharmacological parameters of agonist-induced changes in contractility in aortae from rats infused with 5-HT.

	WT		SERT-KO	
	pD ₂ (-log EC ₅₀ [M])	E _{max} (5-HT, PE: milligrams ACh: % relaxation)	pD ₂ (-log EC ₅₀ [M])	E _{max} (5-HT, PE: milligrams ACh: % relaxation)
5-HT				
Female	5.66±0.06 (4)	1199 ± 239 (4)	6.44 ± 0.08 (4)	166 ± 53 (4)
Male	5.56±0.01 (5)	1879 ± 185 (5)	6.54 ± 0.11 (4)	166 ± 11 (4)
PE				
Female	6.97 ± 0.08 (4)	1922 ± 228 (4)	6.94 ± 0.04 (4)	1905 ± 362 (4)
Male	7.13 ± 0.06 (5)	3021 ± 159 (4) [#]	6.96 ± 0.08 (4)	1676 ± 158 (4) [†]
ACh				
Female	7.41 ± 0.04 (4)	103.1 ± 1.1 (4)	7.25 ± 0.14 (4)	101.4 ± 11.8 (4)
Male	7.18 ± 0.13 (5)	91.2 ± 5.1 (5)	7.16 ± 0.15 (4)	100.7 ± 5.6 (4)

Data are reported as means ± SEM of (n) animals.

[†] vs. WT within the same gender.[#] vs. female within the same group

5-HT = 5-hydroxytryptamine, PE = phenylephrine, ACh = acetylcholine