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A Serotonergic System in Veins: Serotonin Transporter-Independent Uptake

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Abstract

We hypothesized that the 5-hydroxytryptamine (5-HT; serotonin) system is present and functional in veins. In vena cava (VC), the presence of the 5-HT synthesis rate-limiting enzyme tryptophan hydroxylase-1 mRNA and accumulation of the 5-HT synthesis intermediate 5-hydroxytryptophan after incubation with tryptophan supported the ability of veins to synthesize 5-HT. The presence of 5-HT and its metabolite 5-hydroxyindole acetic acid was measured by high-performance liquid chromatography in VC and jugular vein (JV), and it was compared with similarly sized arteries aorta (RA) and carotid (CA), respectively. In rats treated with the monoamine oxidase-A (MAO-A) inhibitor pargyline to prevent 5-HT metabolism, basal 5-HT levels were higher in veins than in arteries. 5-HT uptake was observed after exposure to exogenous 5-HT in all vessels. The presence of MAO-A and the 5-HT transporter (SERT) in VC was observed by immunohistochemistry and Western analysis. However, 5-HT uptake was not inhibited by the SERT inhibitors fluoxetine and/or fluvoxamine in VC and JV, as opposed to the inhibition in RA and CA. Moreover, studies performed in VC from mutant rats lacking SERT showed no differences in 5-HT uptake compared with VC from wild type. These data suggest the SERT is not functional under physiological conditions in veins. The differences in 5-HT handling between veins and arteries may represent alternative avenues for targeting the 5-HT system in the peripheral circulation for controlling vascular tone.

5-Hydroxytryptamine (5-HT; serotonin) was first described as a substance that causes contraction of smooth muscle (Rapport et al., 1948; Erspamer and Asero, 1952). The function of 5-HT as a neurotransmitter is well established, as drugs that affect 5-HT concentration [e.g., Prozac (fluoxetine hydrochloride)] are widely used to treat conditions such as depression, anxiety, and obesity. However, its role in the cardiovascular system is far from being elucidated. For the last decade, accumulating evidence supports the involvement of 5-HT in the control of pulmonary circulation under normal and hypertensive conditions. However, a role for 5-HT in systemic vasculature is a matter of debate (for review, see Watts, 2005).

In the periphery, platelets represent a large 5-HT storage site, and they may function as a buffer, keeping the free circulating 5-HT in low levels (Nilsson et al., 1985; Vanhoutte, 1991; Brenner et al., 2007). Indeed, platelet 5-HT uptake is decreased with age and in hypertension accompanied by an increase in free 5-HT circulating levels (Amstein et al., 1991; Brenner et al., 2007).

5-HT is abundantly synthesized in the enterochromaffin cells of the intestine, representing more than 95% of total body 5-HT. 5-HT is also synthesized in the raphe nuclei of the brain, pineal gland, and in endothelial cells lining the lung. Potential sites of 5-HT synthesis in the systemic vasculature have not yet been identified. 5-HT is synthesized from the essential amino acid tryptophan in a two-step pathway. The hydroxylation of tryptophan forming 5-hydroxytryptophan (5-HTP) by the rate-limiting enzyme tryptophan hydroxylase (TPH) is the first step, followed by the conversion of 5-HTP into 5-HT by the enzyme amino acid decarboxylase.

Some of the effects exerted by 5-HT, such as pulmonary arterial smooth muscle proliferation, are mediated through receptor-independent, 5-HT transporter (SERT)-dependent signaling mechanisms (Marcos et al., 2003). 5-HT, as a protonated molecule, under physiological conditions, is not capable of diffusing across the membrane lipid bilayer. SERT, a bidirectional transporter, is for this reason the major protein responsible for uptake and release of 5-HT (for review, see Ni et al., 2006). SERT is a target of antidepressant drugs such as fluoxetine, fluvoxamine, citalopram, and paroxetine, and the anorexigen (+)-fenfluramine.

In addition to SERT, 5-HT concentration is regulated by the mitochondrial enzyme monoamine oxidase (MAO) and by 5-HT storage. 5-HT is deaminated via MAO to form 5-hydroxyindole acetaldehyde, which in turn is oxidized by aldehyde dehydrogenase to produce 5-hydroxyindole acetic acid (5-HIAA). Tissues or cells that contribute significantly to 5-HT metabolism include the lung, intestine, and endothelial cells of the vascular system, but any cell that can take up 5-HT and possesses MAO has the potential to metabolize 5-HT. MAO activity exists in peripheral arteries (Ni et al., 2004), suggesting that the peripheral vasculature has the ability to metabolize 5-HT and that it may be an important site for the serotonergic system. Our laboratory first demonstrated that peripheral arteries have a functional SERT, and therefore, they are also able to control intracellular and extracellular concentrations of 5-HT (Ni et al., 2004).

In comparison with arteries, less attention is given to the role of veins in the regulation of vascular tone. However, two thirds of the circulating blood volume is in the veins at any given time, indicating that veins have the potential to associate with platelets and 5-HT to a greater extent.

Preliminary data from our laboratory revealed that veins were able to take up 5-HT in a significantly larger amount than arteries (Linder et al., 2007). These novel findings suggest that veins, in addition to platelets, may function as a sink for 5-HT in the cardiovascular system. We presently hypothesize that the serotonergic system is present and functional in

veins as it is in arteries. To test our hypothesis, 5-HT synthesis, metabolism, and uptake were investigated in veins and compared with arteries.

Materials and Methods

Animal Use

Male Sprague-Dawley rats (250–300 g; Charles River Breeding Laboratories, Portage, MI), male mutant rats lacking the SERT (SERT-KO) (Homberg et al., 2006), and their counterparts Wistar wild type (WT) (8–10 weeks old) were used in these studies. In some experiments, animals received injections with the MAO-A inhibitor pargyline (100 mg/kg i.p.) 30 min before euthanasia with pentobarbital (60 mg/kg i.p.). All animal use procedures were performed in accordance with the Institutional Animal Use and Care Committee at Michigan State University.

Reverse Transcription-Polymerase Chain Reaction

Total RNA from ~10-mg sections of vena cava and intestinal mucosa was isolated using the MELT Total RNA Isolation System (Ambion, Austin, TX), and it was quantified on a spectrophotometer (Nano-Drop, Wilmington, DE). One microgram of DNase-treated total RNA from each sample was reverse-transcribed using SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA) as described previously (Ni et al., 2004). Primers for rat TPH-1 sequence (GeneID 24848, mRNA sequence XM_341862): forward 5'-GCCTGCTTTCTTCCATCAGT-3'; reverse 5'-AGACATCCTGGAAGCTTGTGA-3'), designed using Primer3 software (Rozen and Skaletsky, 2000), were synthesized by the Macromolecular Structures and Synthesis Facility at Michigan State University (East Lansing, MI). Primers for rat β -2-microglobulin (B2m) were purchased from SuperArray (Frederick, MD). Results from pilot experiments screening the expression of several housekeeping genes indicated B2m as having the most stable expression in our tissue types. This gene has previously been used for real-time comparative gene expression studies (Wacker and Godard, 2005). Quantification of TPH-1 and B2m amplification was performed using the respective primers (0.1 μ M) and the SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol on the 7500 Real-Time PCR System (Applied Biosystems). For each reaction, the cycle threshold value was determined as the cycle number at which the fluorescence value reached the threshold level. The threshold level was set above the background fluorescence in the exponential phase of the real-time amplification curves.

TPH Activity Assay

TPH-1 activity was assayed for 15 min at 37°C as reported previously by measuring the formation of 5-HTP in the enzyme reaction (Johansen et al., 1995). The standard assay mixture contained 50 mM Tris-HCl, pH 7.4, 1 mM dithiothreitol, 0.05 mg/ml catalase, 200 μ M tryptophan, 100 μ M ferrous ammonium sulfate, 100 μ M BH₄, and 60 μ g of tissue extract. Reactions were arrested after 60 min at 37°C with perchloric acid, and the 5-HTP formed in the reaction was determined by isocratic high-pressure liquid chromatography (HPLC), with fluorescent detection using a model LS5 spectrometer (PerkinElmer Life and Analytical Sciences, Boston, MA).

5-HT Uptake Assay

At room temperature, dissected and washed vessels were placed in physiological salt solution (130 mM NaCl, 4.70 mM KCl, 1.18 mM KH_2PO_4 , 1.17 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.60 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 14.90 mM NaHCO_3 , 5.50 mM dextrose, and 0.03 CaNa_2EDTA , pH 7.2) in 1.5-ml plastic centrifuge tubes containing either vehicle or inhibitor for 30 min. 5-HT (1 μM) or vehicle (deionized water) was then added for 15 min. Tissues were dipped several times in drug-free physiological salt solution to avoid extracellular 5-HT contamination, and then they were placed in 75 μl of 0.05 mM sodium phosphate and 0.03 mM citric acid buffer, pH 2.5, containing 15% methanol. Samples were frozen (-80°C) until assay. Samples were thawed, sonicated for 3 s. 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 coupled with electrochemical detection. The supernatant (20 μl) was injected onto a C18 reverse phase analytical column (ESA Inc., Chelmsford, MA) protected by a precolumn cartridge filter. This column was coupled to a single coulometric electrode-conditioning cell positioned before autosampler (injection) in series with dual electrode analytical cells (ESA Inc.) positioned after the analytical column. The conditioning electrode potential was set at 0.45 V, whereas the coulometric analytical electrodes were set at 0.0 and 0.4 V. Amounts of 5-HIAA and 5-HT were determined by comparing peak areas in samples with those obtained from standards that were performed to obtain a standard curve, and they are reported as a concentration relative to protein content. Protein content was determined by the Lowry method (Lowry et al., 1951). The lower limit of sensitivity for detection of 5-HIAA and 5-HT was 0.5 pg/ μl sample.

Immunohistochemistry

Vena cava and aorta were cleaned of fat, and then they were fixed in 10% formalin for 24 h. Tissues were paraffin-embedded, and 5- μm sections were collected onto glass slides. Sections were dried overnight at 37°C . Slides were dewaxed in Histochoice Clearing Agent (AMRESCO Inc., Solon, OH; two washes for 3 min each), and then they were washed in isopropanol (six washes, 3 min each). Slides were rinsed in deionized water. Slides were immersed in Antigen Unmasking Reagent (Vector Laboratories, Burlingame, CA), and then they were microwaved for 5 min total and subsequently air-dried before use. Endogenous peroxidases were blocked with 0.3% H_2O_2 in phosphate-buffered saline (PBS) for 30 min followed by blocking for nonspecific binding in PBS containing 1.5% blocking serum for 60 min. In a humidified chamber, samples were incubated overnight with antibody [2 $\mu\text{g}/\text{ml}$: SERT antibody against carboxyl terminus, C-20 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA); or 5 $\mu\text{g}/\text{ml}$: MAO-A antibody H-70 (epitope corresponding to amino acids 458–527 of MAO-A of human origin; Santa Cruz Biotechnology, Inc.), with 1.5% blocking serum in PBS] or antibody neutralized with 5-fold excess of competing peptide (mass/mass). Development of slides proceeded according to the manufacturer's kit using 3,3'-diaminobenzidine as the developing substrate (Vector Laboratories), and slides were counterstained with hematoxylin (Vector Laboratories).

Protein Isolation

Vena cava and aorta were cleaned, pulverized in liquid nitrogen, and solubilized in lysis buffer (125 mM Tris HCl, pH 6.8, 4% SDS, and 20% glycerol) with protease inhibitors (0.5 mM phenylmethylsulfonyl fluoride, 10 µg/ml aprotinin, and 10 µg/ml leupeptin), and the tyrosine phosphatase inhibitor sodium orthovanadate (1 mM). Homogenates were centrifuged (11,000g for 10 min at 4°C), and supernatant total protein was measured (bicinchoninic acid kit; Sigma-Aldrich, St. Louis, MO).

Western Blotting

Equal amounts of total vascular protein (50 µg) from tissue samples were separated on 10% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride membrane for Western analyses using an antibody against MAO-A (H-70, 1:1000; Santa Cruz Biotechnology, Inc.) or the SERT antibody against the amino terminus, N-14 (Santa Cruz Biotechnology, Inc.). Membranes were incubated overnight with primary antibody (4°C), and then they were washed three times with Tris-buffered saline + 0.1% Tween 20 and once with Tris-buffered saline. Blots were incubated with IRDye 680 goat anti-rabbit IgG (1:2000) for testing MAO-A and goat horseradish peroxidase-linked anti-rabbit secondary antibody (1:2000; Cell Signaling Technology Inc., Danvers, MA) for testing SERT for 1 h at 4°C with rocking. Blots were visualized using an Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE) for MAO-A and by enhanced chemiluminescence reagents (GE Healthcare, Little Chalfont, Buckinghamshire, UK) for SERT. Smooth muscle α -actin (1:400; Calbiochem, San Diego, CA) was used as a marker to ensure equal loading of protein.

Data Analysis

When comparing two groups, a Student's *t* test was used. When comparing three or more groups, a one-way analysis of variance followed by Newman-Keuls post hoc was used. In all cases, a *P* value of 0.05 was considered significant. All results are presented as mean $\pm$ S.E.M.

Results

5-HT Is Synthesized in Vena Cava

RNA from vena cava was isolated for measurements of TPH mRNA expression by reverse transcription-PCR. The predominant site of 5-HT synthesis and therefore TPH-1 localization is the enterochromaffin cells of the intestinal mucosa. For this reason, intestinal mucosa samples were used as control for TPH-1 mRNA expression. The value at which measurable product was first observed in reverse transcription-PCR (cycle threshold values) was 27.5 ± 0.4 cycles for TPH-1 and 16.8 ± 0.2 cycles for the housekeeping gene B2m in vena cava ($n = 4$) and 28.5 cycles for TPH-1 and 17.7 cycles for B2m in the gut ($n = 2$). Figure 1A shows the amplification plots of TPH-1 and B2m in vena cava.

Cytosolic homogenates from vena cava were extracted to evaluate TPH activity through measurements of 5-HTP, the immediate product of TPH. Figure 1B shows that detectable amounts of 5-HTP were obtained in samples treated with the 5-HT synthesis precursor

tryptophan and cofactor BH₄. In the absence of tryptophan and BH₄, 5-HTP was not detected. These data support the capability of veins to synthesize 5-HT.

5-HT and Its Metabolite 5-HIAA Are Present in Veins and Arteries

5-HT and 5-HIAA were detected by HPLC analysis in vena cava (Fig. 2A), jugular vein (Fig. 2B), aorta (Fig. 2C), and carotid artery (Fig. 2D). 5-HT content was greater in veins (Fig. 2, A and B) than in arteries (Fig. 2, C and D). The ratio of 5-HT to 5-HIAA was greater in veins than in arteries.

5-HT Is Taken Up in Veins

Vessels were next exposed to exogenous 5-HT (1 μ M) for 15 min. There was no significant change in 5-HT concentration in vena cava and jugular vein incubated with 5-HT (Fig. 3, A and C). However, there was an increase in 5-HIAA concentration (Fig. 3, B and D) in these tissues compared with vehicle-exposed vessels. In aorta and carotid arteries, an increase in 5-HT (Fig. 3, A and C) and 5-HIAA (Fig. 3, B and D) was observed after treatment with 1 μ M 5-HT for 15 min compared with arteries treated with vehicle. Despite a lack of increase in 5-HT content in veins after exposure to exogenous 5-HT, 5-HT content was maintained higher in veins than in arteries.

To prevent 5-HT metabolism, rats were treated with the MAO inhibitor pargyline (100 mg/kg i.p.) 30 min before euthanasia, and 5-HT and 5-HIAA content was measured. In vena cava (Fig. 4A) and jugular vein (Fig. 4C) from pargyline-treated rats, basal 5-HT content was higher than in their same-sized arteries, aorta and carotid, respectively. After exposure to exogenous 5-HT, an increase in 5-HT concentration was observed in veins and arteries, compared with control/vehicle. Compared with untreated rats (Fig. 3), 5-HIAA content in pargyline-treated rats after exposure to exogenous 5-HT was significantly decreased in vena cava, jugular vein, aorta, and carotid artery (Fig. 4, B and D). These data indicate that 5-HT can be taken up and metabolized by veins.

The Enzyme MAO-A Is Present in Veins

The presence of MAO-A in vena cava was confirmed by immunohistochemistry (Fig. 5A) and by Western analysis (Fig. 5B). Figure 5A (left) shows immunostaining for the antibody against MAO-A in vena cava (arrows) that was absent when the antibody was omitted (Fig. 5A, right). Figure 5B shows a band that migrated at ~60 kDa in mass in vena cava and aorta that corresponds to the molecular mass of MAO-A.

SERT Protein Is Present in Vena Cava

The presence and localization of the SERT was observed by immunohistochemistry (Fig. 6A) and Western analysis (Fig. 6B). The arrows in Fig. 6A (left) indicate sites of localization of the SERT with the primary antibody against the C terminus of the SERT (C-20), which was competed off by the antibody competing peptide (Fig. 6A, middle) and absent when the primary antibody was omitted (Fig. 6A, right). Figure 6B shows the expression of a protein of ~74 kDa correspondent to the molecular mass of the SERT. The SERT band was weaker and more diffuse in vena cava than in aorta, although an equivalent amount of total protein

(50 µg) was loaded in each lane. We next investigated whether this transporter was responsible for 5-HT uptake in veins.

5-HT Uptake in Vena Cava Is Independent of the SERT

The contribution of SERT to 5-HT uptake in veins was first evaluated in vessels from pargyline-treated rats to allow measurements of 5-HT alone without the interference of its metabolism to 5-HIAA. A 30-min incubation with the SERT inhibitor fluvoxamine (1 µM) before the challenge with exogenous 5-HT (1 µM) for 15 min had no effect in 5-HT uptake in vena cava (Fig. 7A) and jugular vein (Fig. 7B). By contrast, 5-HT uptake was significantly inhibited by fluvoxamine in aorta (Fig. 7C) and carotid (Fig. 7D) arteries from pargyline-treated rats. The SERT inhibitor fluoxetine (1 µM) was also tested in vena cava and aorta, and similar results were obtained (data not shown).

To support the findings that SERT was not involved in 5-HT uptake in veins, we used tissues from a SERT-KO rat. These rats are bred in a Wistar background. In the SERT KO rats, 5-HT content in the platelet was nearly abolished (1.0 ± 0.5 ng/ml) compared with WT (310 ± 96 ng/ml), confirming lack of a functional SERT. The following experiments were performed in vena cava and aorta obtained from nonpargyline-treated WT and SERT-KO rats. In vena cava from SERT-KO rats, an increase in 5-HIAA was observed after exposure to exogenous 5-HT (1 µM) for 15 min compared with vena cava exposed to vehicle. The concentration of 5-HT and 5-HIAA in vena cava from SERT-KO rats obtained by exposure to either vehicle or 5-HT were not different from those obtained in tissues from WT (Fig. 8). These data support the findings obtained in vena cava from Sprague-Dawley rats (Figs. 6 and 7A), suggesting that regardless of the expression of SERT in vena cava, SERT is not functional under our experimental procedures.

Discussion

The present work presents the novel findings that peripheral veins synthesize, take up, and metabolize 5-HT.

5-HT in Veins

The effective driving force for venous return to the heart, mean circulatory filling pressure, can be determined in vivo by venous capacitance and blood volume (Yamamoto et al., 1980). It is interesting to note that in some models of hypertension, an increase in mean circulatory filling pressure is not associated with an increase in total blood volume, suggesting that mean circulatory filling pressure may only be driven by changes in venous tone under these circumstances (Fink et al., 2000).

5-HT modifies venous tone through direct interaction with venous 5-HT receptors (Cushing et al., 1994; Watts and Cohen, 1999; Watts, 2005; Linder et al., 2007) and indirectly by modifying sympathetic control of venous motor tone (Cohen et al., 1999). Thus, it is important to understand how 5-HT is handled by venous tissue, as modification in handling could ultimately affect the actions of 5-HT in veins and therefore blood pressure.

Several structural and functional differences exist between veins and arteries (Szasz et al., 2007; Thakali et al., 2007), making the comparison between the two vessels not a simple issue. In the present study, we compare vena cava with aorta and jugular vein with carotid artery. We chose similarly sized vessels for the comparison. Furthermore, we normalized the data by protein content. Using this comparison system, we observed that the content of 5-HT in these veins was higher than in same-sized arteries. Veins accommodate two thirds of the circulating blood volume at any time; thus, they are longer exposed to free circulating 5-HT. These data suggest that veins, compared with arteries, could be a reservoir of 5-HT in the periphery.

5-HT Synthesis in Veins

Cohen et al. (1999) observed that a TPH inhibitor decreased 5-HT content in saphenous vein, indicating 5-HT synthesis by nonperipheral veins (Cohen et al., 1999). Because of the likely possibility that the 5-HT was synthesized in low levels by vessels, addition of substrates would be necessary, and they were added. Under these conditions, 5-HTP, the intermediary in 5-HT synthesis, was detected in vena cava. This suggests the ability of peripheral veins to synthesize 5-HT.

The view on the peripheral vasculature has been that it is a target for 5-HT to induce its physiological responses by interacting with classic 5-HT receptors. The findings of the present study of an increase in 5-HTP content and TPH mRNA expression in vena cava introduce the new concept of 5-HT synthesis in peripheral vasculature.

5-HT Uptake in Veins

5-HT synthesis alone is probably not the only source of 5-HT contributing to the high 5-HT content measured in veins. Another source of 5-HT is the external environment. Veins from pargyline-treated rats exposed to exogenous 5-HT took up 5-HT, and 5-HT uptake by peripheral arteries was confirmed.

Once inside the cell, 5-HT function is not terminated. 5-HT uptake by SERT mediates pulmonary arterial smooth muscle proliferation to 5-HT (Lee et al., 2001; Marcos et al., 2003). In addition, a finding in the platelet supports the idea that intracellular 5-HT exerts physiological effects. Walther et al. (2003) demonstrated that 5-HT in platelets, taken up by SERT, acts as a substrate for an enzyme class of transglutaminases to covalently modify proteins such as RhoA (transamidation or “serotonylation”). Intracellular 5-HT can also control nitric oxide production (Chanrion et al., 2007). Thus, intracellular 5-HT has functional effects, which could be regulated by SERT by changing intracellular 5-HT concentrations. It is currently unknown whether intracellular 5-HT can alter function in cell types of the peripheral vascular system.

In platelets, all the 5-HT content comes from the extracellular media via an uptake mechanism dependent on SERT, because platelets do not synthesize 5-HT. Consistent with this idea is that 5-HT was not detectable in platelets of the SERT-knockout rat, confirming previous observation by Dr. Cuppen's group (Homberg et al., 2006). We now know that the presence of SERT is not confined to brain sites and circulating platelets; it is also present in the intestine (Bian et al., 2007), vasculature (Ni et al., 2004), heart (Mekontso-Dessap et al.,

2006), and immune system (Gordon and Barnes, 2003). SERT protein was weakly expressed in vena cava. However, 5-HT uptake in veins was not inhibited by the classic SERT inhibitor fluvoxamine, as opposed to arteries. Based on this surprising finding, we made use of an important tool to study the SERT in rats, the SERT-knockout rat. These rats were generated by *N*-ethyl-*N*-nitrosurea-driven, targeted selected mutagenesis, and they lack a functional SERT in the brain and in platelets without major changes in nonserotonergic systems (Homberg et al., 2006, 2007). We confirmed that 5-HT uptake in veins is independent of a functional SERT by showing that vena cava from rats lacking SERT could take up 5-HT in the same manner as vena cava from control rats. These data give further support to the conclusion that despite the presence of SERT in veins, SERT is not functional, at least under the conditions studied.

What SERT is doing in the vena cava if it is not functional remains the question. In aorta, at least, 5-HT uptake is partially dependent on SERT, confirming previous observations (Ni et al., 2004; Linder et al., 2008). In vena cava, it was surprising to observe a lack of functional SERT. According to the present findings, it is unlikely that 5-HT uptake via SERT would occur under acute exposure to 5-HT. However, the possibility of SERT gaining function in veins exposed to chronic increases in 5-HT, such as in hypertension, cannot be excluded. It is possible that in situations in which uptake via the other mechanisms is impaired, 5-HT uptake via SERT would take place. Additional monoamine transporters include the norepinephrine transporter (NET) and dopamine transporter that share many similarities with SERT in terms of function, mechanism, and regulation. Dopamine can be taken up by NET (Gu et al., 1994). It is possible that monoamine transporters have the ability to act promiscuously; thus, dopamine transporter and NET might take up 5-HT in veins. If this is true, norepinephrine and dopamine could also be transported by SERT. Other candidates responsible for non-SERT uptake include the organic cation transporters. Substrates for organic cation transporters can be as diverse as 5-HT, dopamine, norepinephrine, epinephrine, histamine, clonidine, and cimetidine. From a pharmacological perspective, organic cation transporters are difficult to distinguish from one another, but they can be distinguished from SERT by inhibition by corticosterone, *O*-methylisoprenaline, and levamisole (Horvath et al., 2003; Martel and Azevedo, 2003). Extending our studies to use of monoamine transporter knockout animals will help clarify the mechanisms by which veins take up 5-HT.

5-HT Metabolism

Pargyline treatment significantly inhibited 5-HIAA content in all vessels exposed to exogenous 5-HT, indicative of MAO inhibition and therefore MAO function in these vessels. Pargyline treatment increases the amount of 5-HT in saphenous vein (Cohen et al., 1999), indicating the presence of MAO in this vessel. The findings of the present study introduce the novel data supporting the presence and function of MAO-A in peripheral veins, and they confirm 5-HT metabolism by peripheral arteries.

This observation indicates that peripheral vessels (arteries and veins) might play an important role in the clearance of plasma 5-HT through uptake and metabolism. This is important, as increased circulating 5-HT is associated with hypertension (Amstein et al.,

1991; Brenner et al., 2007), which could also theoretically be the result of dysfunction of serotonergic system in peripheral blood vessels.

Altogether, the results of the present study suggest the presence of the serotonergic system in veins, and they highlight the differences in 5-HT handling between peripheral arteries and veins. Several cardiovascular diseases, including hypertension, involve alterations in the synthesis of vasoactive hormones. Understanding the pharmacological and physiological function and regulation of these systems is of extreme value to understanding the pathophysiology and treatment of these diseases. Selective serotonin reuptake inhibitors are widely used for treatment of central disorders due to the ability of SERT inhibition. Because more blood is found in the venous system, veins may be exposed for longer periods to free circulating 5-HT than arteries. The unique findings in this study, showing increased basal intracellular 5-HT concentration in veins compared with arteries, led us to hypothesize that veins may function as a sink for 5-HT in the cardiovascular system and that they may represent a novel target for treatment of vascular diseases involving 5-HT.

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ABBREVIATIONS

5-HT	5-hydroxytryptamine (serotonin)
5-HTP	5-hydroxytryptophan
TPH	tryptophan hydroxylase
SERT	serotonin transporter
MAO	monoamine oxidase
5-HIAA	5-hydroxyindole acetic acid
KO	knockout
WT	wild type
B2m	β -2-microglobulin
PCR	polymerase chain reaction
HPLC	high-performance liquid chromatography
PBS	phosphate-buffered saline
NET	norepinephrine transporter
VC	vena cava
JV	jugular vein

RA	rat aorta
CA	carotid artery
BH₄	tetrahydrobiopterin

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Fig. 1.

Tryptophan hydroxylase is present in vena cava. A, real-time PCR amplification curves of TPH-1 mRNA expression in vena cava using TPH-1 (tph1) primer compared with the housekeeping gene B2m. Representative of four different experiments. B, 5-HTP content in vena cava homogenate incubated with vehicle or BH₄ + tryptophan. The bars represent mean $\pm$ S.E.M. of the concentration as picograms per milligram of extract per minute for $n = 4$.

Fig. 2.

Basal levels of 5-HT and 5-HIAA in vena cava (VC; A), jugular vein (JV; B), aorta (RA; C), and carotid artery (CA; D). VC, JV, RA, and CA were isolated from Sprague-Dawley rats, and then they were cleaned and incubated for 15 min in physiological salt solution for measurements of basal 5-HT and 5-HIAA content by HPLC. Bars represent mean $\pm$ S.E.M. of the concentration as nanograms per milligram of protein for $n = 7$. *, $P < 0.05$ versus same-sized vein.

Fig. 3.

5-HT and 5-HIAA content in veins and arteries after exposure to exogenous 5-HT or vehicle. 5-HT (A and C) and 5-HIAA (B and D) contents were measured by HPLC in VC (A and B), JV (C and D), RA (A and B), and CA (C and D) obtained from Sprague-Dawley rats. The tissues were exposed to vehicle (deionized water) as described in Fig. 2 or to extracellular 5-HT (1 μ M) for 15 min. Bars represent mean $\pm$ S.E.M. of the concentration as nanograms per milligram of protein for $n = 7$. *, $P < 0.05$ versus same-sized artery; #, $P < 0.05$ versus vehicle.

Fig. 4.

5-HT and 5-HIAA measurements in veins and arteries from pargyline-treated rats after exposure to vehicle or exogenous 5-HT. 5-HT (A and C) and 5-HIAA (B and D) contents were measured by HPLC in VC (A and B), JV (C and D), RA (A and B), CA (C and D) obtained from pargyline-treated Sprague-Dawley rats. The tissues were exposed to vehicle (deionized water) or extracellular 5-HT (1 μ M) for 15 min. Bars represent mean $\pm$ S.E.M. of the concentration as nanograms per milligram of protein for $n = 5$. *, $P < 0.05$ versus same-sized artery; #, $P < 0.05$ versus vehicle; @, $P < 0.05$ versus same tissue from nonpargyline-treated rats.

Fig. 5. MAO-A localization and expression in vena cava and aorta. A, immunohistochemical staining of the MAO-A in vena cava paraffin sections from Sprague-Dawley rats treated with the anti-MAO-A antibody H-70 (5 µg/ml) on the left. Arrows indicate the localization of staining that was absent in parallel sections incubated with no primary (secondary alone) on the right. Representative of four separate experiments, each with a different rat. L, lumen. Scale bar, 100 µm. B, Western blot of MAO-A and smooth muscle α-actin in homogenates from vena cava and aorta.

Fig. 6. SERT localization and expression in vena cava and aorta. A, immunohistochemical staining of the SERT in vena cava paraffin sections from Sprague-Dawley rats treated with the anti-SERT antibody C-20 (2 µg/ml) on the left. Parallel sections were quenched with a 5 times excess competing peptide (CP; middle) or no primary (right). Arrows indicate the localization of staining that was displaced by the competing peptide. Representative of four separate experiments, each from a different rat. L, lumen. Scale bar, 100 µm. B, Western blot of SERT and smooth muscle α-actin in homogenates from vena cava and aorta.

Fig. 7.

5-HT uptake in the presence of fluvoxamine. VC (A), JV (B), RA (C), and CA (D) were obtained from pargy-line-treated Sprague-Dawley rats. The tissues were first exposed to vehicle or fluvoxamine (1 μ M) for 30 min. Some tissues were exposed to 5-HT (1 μ M) for 15 min after treatment with vehicle (5-HT) or fluvoxamine (fluvoxamine + 5-HT), and then 5-HT content was measured by HPLC. Vehicle group did not receive 5-HT or fluvoxamine. Bars represent means $\pm$ S.E.M. of 5-HT concentration as nanograms per milligram of protein for $n = 6$. *, $P < 0.05$ versus vehicle; #, $P < 0.05$ versus 5-HT.

Fig. 8. 5-HT and 5-HIAA content in vena cava from nonpargyline-treated Wistar rats that possess SERT (WT) (A) and lack SERT (KO) (B). The tissues were exposed to vehicle (deionized water) or extracellular 5-HT (1 μ M) for 15 min. Bars represent mean $\pm$ S.E.M. of the concentration as nanograms per milligram of protein for five to six experiments. *, $P < 0.05$ versus vehicle.