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Title Page: 5-HT in veins: not all in vain

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Running Title Page: 5-HT in veins: not all in vain

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List of abbreviations:

- 5-HT: 5-hydroxytryptamine; serotonin
- 5-CT: 5-carboxamidotryptamine
- BRL 54443: 5-Hydroxy-3-(1-methylpiperidin-4-yl)-1H-indole
- BRL 56905: (±)-3-amino-6-carboxamido-1,2,3,4-tetrahydrocarbazole
- CP93129: 3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyrid-5-one

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● GR127935: 2´-methyl-4´-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carboxylic acid [4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]amide

●LY215840: *cis*- *n*- (2- hydroxycyclopentyl) - 6- methyl - 1 - (1- methylethyl) ergoline - 8-carboxamide

● LY 344864: 4-Fluoro-N-[3-(1-methyl-4-piperidinyl)-1H-indol-5-yl]-benzamide

● MPPA-4F: *o*-methoxyphenylpiperazide derivative 4F

● PNU-109291: (S)-(-)-1-{2-[4-(4-Methoxy-phenyl)-piperazin-1-yl]-ethyl}-isochroman-6-carboxilic acid methylamine

● Ro 4-4602: DL-Serine 2-(2,3,4-trihydroxybenzyl)hydrazide

● SKF 99101H: (3-(2-dimethylaminoethyl)-4-chloro-5-proxyindole hemifumarate

Abstract

The circulatory system consists of veins and arteries. Compared to arteries, veins have been neglected in cardiovascular research. Though veins are significantly less muscular than similarly sized arteries, the contribution of veins to cardiovascular homeostasis cannot be left unnoted, since veins accommodate 70% of the circulating blood. Circulating blood platelets contain the majority of systemic 5-HT (5-hydroxytryptamine; serotonin). Similar to venous function, the physiological role of 5-HT in the cardiovascular system is not well understood. Here, we present not only a review on 5-HT and veins, but ways in which these two topics might intersect in a physiologically relevant manner. We show here the novel findings that veins exhibit higher amounts of intracellular 5-HT than arteries. Moreover, we also show evidence that similar to arteries, veins have the ability to uptake 5-HT. In this review we introduce the venous system as a reservoir for 5-HT in the periphery, suggesting that veins, in addition to arteries, may represent an important target for drugs that interfere with the serotonergic system. In addition, the serotonergic system, from synthesis to metabolism, 5-HT receptor activation and venous diseases will also be discussed.

Introduction

Serotonin (5-hydroxytryptamine or 5-HT) has long been the subject of study in many areas of biomedical sciences. Its isolation and characterization was made possible due to the hard work of two independent laboratories more than 60 years ago. In 1937, in Italy, Erspamer and Vially observed that a substance derived from the enterochromaffin cells of the gut caused smooth muscle contraction, especially the rat uterus. This substance was called enteramine. In 1948 in the US, Rapport, Green and Page isolated a compound from beef serum that was able to cause vascular contraction, which they called serotonin. Some years later, enteramine and serotonin were found to be the same substance: 5-hydroxytryptamine (5-HT). The recognition of 5-HT as a neurotransmitter after it was found in mammalian brain, brought it into the field of neuroscience. These findings were followed by the proposed role of 5-HT in mental illness (for review, Whitaker-Azmitia, 1999). The involvement of 5-HT in the central nervous system has long been established and, therefore, it is beyond the scope of this review.

Despite initially being described as a vasoconstrictor, the role of 5-HT in the cardiovascular system is far from being elucidated. A role for 5-HT in the pulmonary circulation has become established in the last past decade. However, sites of 5-HT synthesis in the non-pulmonary peripheral vasculature have not yet been identified. Consequently, our current understanding is that the systemic vasculature is exposed to 5-HT only through the release of 5-HT by the platelet. In the peripheral circulation, whereas the function of 5-HT in the blood is to promote platelet aggregation and blood

clotting, the role of 5-HT in the peripheral vasculature has not yet been clarified and is, to be more precise, controversial (Watts, 2005).

The circulatory system consists of arteries and veins. However, studies in veins occupy only a marginal part of the total research in the cardiovascular field when compared to arteries. Paradoxically, venous diseases have been recognized since old times as mentioned by Hippocrates (460-377 B.C.), and they currently affect 1-3% of the population.

A number of cardiovascular diseases 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. The pharmacology of 5-HT in the peripheral vascular system, with focus on the venous system, will be the main thrust of this review. Within this article, the pharmacological manipulation of the serotonergic system from its synthesis to degradation to mechanism of action will be discussed. An introduction of the venous system as an important target for drugs that interfere with the serotonergic system will also be presented.

The following topics will be discussed:

- 5-HT Receptors in the Vascular System
- Neuronal Regulation of Vascular Tone by 5-HT
- Contractile Synergism
- Role of Veins in the Cardiovascular System
- 5-HT Receptor-Independent Effects
- 5-HT Synthesis, Storage, Metabolism, Uptake and Release

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- Venous Diseases
- 5-HT in Veins

5-HT Receptors in the Vascular System:

Traditionally, 5-HT exerts its physiological effects *via* receptor activation. The history of 5-HT receptors dates back to the 1950's, when Gaddum and Picarelli first described two kinds of 5-HT receptors. The authors concluded that the actions of 5-HT in the guinea-pig ileum were mediated by the "M" or muscarinic-sensitive type and "D" or dibenziline (phenoxybenzamine)-sensitive type receptors localized on smooth muscles and neurons, respectively. This classification became largely accepted until the 1970's, when some of the responses elicited by 5-HT in vascular and non-vascular tissues did not fit such criteria. In the 70's and 80's, radioligand binding studies provided the basis for the present 5-HT receptors nomenclature (for review, Saxena and Ferrari, 1992). The present classification establishes that the physiological actions of 5-HT are mediated by seven families of 5-HT receptors ($5\text{-}HT_1$-$5\text{-}HT_7$), with at least 15 different subtypes (Hoyer et al., 1994).

Most of 5-HT receptors belong to the family of seven transmembrane domain receptors coupled to G-proteins. The only exception is the $5\text{-}HT_3$ receptor, which is a ligand-gated ion channel. With so many receptors, the complexity of 5-HT biological responses is not surprising. For example, whereas in porcine vena cava 5-HT induces relaxation (Trevethick et al., 1984), in rabbit vena cava it elicits weak contraction (Hellegouarch et al., 1985). In rat vena cava, we observed that 5-HT induces

approximately 70% of the magnitude of contraction induced by the adrenergic agonist norepinephrine (Figure 1A).

The 5-HT receptors predominantly responsible for modifying tone in arteries and veins are the 5-HT_1, 5-HT_2 and 5-HT_7 receptors. The 5-HT_{2A} receptor is primarily responsible for mediating contraction to 5-HT in large arteries. Veins, on the other hand, contract to 5-HT mainly by 5-HT_{1B} receptor activation (for review, Watts and Cohen, 1999). Figure 1 compares the contraction induced by 5-HT in rat vena cava (Fig 1A, 1C) and rat aorta (Fig 1B, 1D) revealing differences between arteries and veins.

5-HT_1 Receptors: 5-HT_{1B}, 5-HT_{1D} and 5-HT_{1F}

From the 5-HT_1 receptor family, the subtypes 5-HT_{1B}, 5-HT_{1D} and 5-HT_{1F} have been found in blood vessels. Some compounds such as sumatriptan and 2´-methyl-4´-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carboxylic acid [4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]amide (GR127935) do not discriminate between 5-HT_{1B} and 5-HT_{1D} receptors. Fortunately, agonists and antagonists more selective for these receptors have revealed that the contraction induced by activation of 5-HT_1 receptor in the vasculature is primarily mediated by 5-HT_{1B} receptors (Bhattacharya et al., 2004).

In veins, 5-HT_1 receptor activation generally leads to contraction while arteries respond quite differently. For instance, the 5-HT_{1B} agonist 3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyrid-5-one (CP93129) failed to induce contraction in rat aorta, although the 5-HT_{1B} receptor was expressed (Banes and Watts, 2003). On the other hand, we observed that CP93129 and sumatriptan induced contraction of rat mesenteric artery from hypertensive, but not from normotensive rats (Banes and Watts, 2001).

Interestingly, the presence of 5-HT_{1F} mRNA has been detected in several blood vessels, including human cerebral and coronary arteries (Watts and Cohen, 1999). However, selective 5-HT_{1F} agonists such as 4-Fluoro-N-[3-(1-methyl-4-piperidinyl)-1H-indol-5-yl]-benzamide (LY344864) failed to induce contraction in these arteries (Bouchelet et al., 2000). LY344864 did not induce contraction of mesenteric artery (Watts 2002) or rabbit saphenous vein (Cohen and Schenk, 2000). On the other hand, 5-HT_{1F} was not found in rat aorta and in rat vena cava (Ullmer et al., 1995). These data suggest that the 5-HT_{1F} receptor does not mediate contractions in vascular tissues.

5-HT_2: 5-HT_{2A} and 5-HT_{2B}

The expression of the 5-HT_{2A} receptor is significantly higher in rat aorta than in rat vena cava (Kato et al., 1999). As mentioned above, the 5-HT_{2A} receptor subtype is the primary receptor activated by 5-HT to induce contraction in arteries such as aorta (McKune and Watts, 2001). The 5-HT_{2A} receptor agonist alpha-methyl-5-HT caused contraction of rat tail artery (Froldi et al., 2003). Both 5-HT_{2A} and 5-HT_{2B} receptors are expressed and mediate contraction in rat renal artery (Watts and Thompson, 2004). On the other hand, the 5-HT_{2B} receptor is the primary contractile receptor in the aorta, whereas the 5-HT_{2A} receptor remains the primary contractile receptor in the mesenteric resistance arteries of hypertensive rats (Watts, 2002).

5-HT_{2B} receptors are reported to mediate endothelium-dependent relaxation in porcine vena cava and pulmonary artery, and rabbit and rat jugular vein (for review, Watts and Cohen, 1999). However, unpublished data from our laboratory failed to show

vasodilation induced by 5-HT in rat aorta, rat mesenteric artery, rat mesenteric resistance artery and mouse aorta with intact endothelium.

5-HT$_7$

This receptor subtype is positively linked to adenylyl cyclase and is present in the smooth muscle of several blood vessels, including rat aorta and rat vena cava (Ullmer et al., 1995). However, in unpublished data from our laboratory, we have been unable to observe relaxation induced by 5-HT in isolated blood vessels including aorta. These data suggest that 5-HT$_7$ receptor activation in these vessels does not mediate relaxation. There is evidence of 5-HT$_7$ receptor mediating relaxation of canine coronary artery. The 5-HT$_7$ receptor antagonist *cis*-*n*-(2-hydroxycyclopentyl)-6-methyl-1-(1-methylethyl)ergoline-8-carboxamide (LY215840) inhibits 5-HT-induced relaxation of canine coronary artery (Watts and Cohen, 1999). To our knowledge, 5-HT-induced relaxation of rat vena cava has not been reported.

Neuronal Regulation of Vascular Tone by 5-HT

5-HT can also exert vascular effects by acting on receptors that are not located directly on the smooth muscle or endothelial cell. Pre-synaptic 5-HT receptors on the postganglionic sympathetic nerves, which regulate neurotransmitter release from noradrenergic neurons in blood vessels of various species are heterogeneous. In dog saphenous vein, the 5-HT$_{1B/1D}$ receptor agonists (±)-3-amino-6-carboxamido-1,2,3,4-tetrahydrocarbazole (BRL 56905), 3-(2-dimethylaminoethyl)-4-chloro-5-proxyindole hemifumarate (SKF 99101H) and sumatriptan inhibited norepinephrine release

(Medhurst et al., 1997). On the other hand, Cohen *et al.* reported that in the rabbit saphenous vein, 5-HT enhances the release of norepinephrine by stimulating prejunctional 5-HT_{1A} receptors on the noradrenergic nerves (Cohen et al., 1999). These data indicate that 5-HT may also regulate vascular smooth muscle tone by modulating the sympathetic neurotransmission in blood vessels in a complex way.

Contractile Synergism

Synergism comes from the Greek "synergos" that means working together. Pharmacologically, the effect of two drugs working together results in an effect greater than the sum of the individual agents. Physiological concentrations of 5-HT that are not able to cause contraction by themselves enhance the contraction to norepinephrine in rat arteries (Watts, 2000). 5-HT can also interact synergistically with other vasconstrictors including angiotensin II, endothelin-1, histamine and prostaglandin $F_{2\alpha}$ (Watts, 2000). The reverse - potentiation of 5-HT effects by other hormones - is also true. Neuropeptide Y potentiates 5-HT-induced contraction in porcine coronary artery and 5-HT sensitivity is increased by phenylephrine in rabbit femoral artery (Tsurumaki et al., 2006). In rabbit saphenous vein, histamine and the thromboxane A_2 agonist increased the potency and efficacy of the 5-$HT_{1B/1D}$ agonist sumatriptan, an effect that was not observed in basilar artery (Bhattacharya et al., 2003).

From this glimpse at 5-HT receptor pharmacology in arteries and veins, we can observe the complex effects resulting from 5-HT receptor activation. 5-HT activates distinct receptor subtypes in arteries and veins, which may underlie the different

contributions of these vessels to vascular homeostasis. Compared to the arterial system, the role of the venous system in the control of vascular homeostasis is significantly less studied and elucidated and, therefore, deserves our attention.

Role of Veins in the Cardiovascular System

The circulatory system is comprised of arteries and veins, which are critical for providing tissues with nutrients and oxygen and removing waste byproducts of tissue metabolism (for review, Thakali et al., 2007).

Factors that determine blood pressure include total peripheral resistance, determined by arterial tone, and cardiac output, the volume of blood pumped out of the heart per minute. This means that blood pressure can be controlled by blood volume and by vasoconstriction. The mechanisms by which arterial smooth muscle tone and structure affect blood pressure have been extensively studied, whereas venous contribution has been relatively neglected. A majority (60-70%) of the circulating blood volume is in the veins at any given time, indicating that veins can modulate cardiovascular homeostasis.

The tunica media of a blood vessel is responsible for the control of vasomotor tone by contracting and relaxing to different stimuli. Compared to arteries, veins express considerably less α-actin, the key protein for contraction. Given the thinner smooth muscle layer and decreased α-actin expression in veins compared to arteries, one could postulate that veins do not contribute to cardiovascular homeostasis. However, based on all the previous information, there is strong evidence that veins do contract to agonists, including 5-HT. Moreover 5-HT can modulate the sympathetic system and/or

interact synergistically with other systems in veins. Altogether, these data highlight the importance of studying the serotonergic system in veins.

Venous tone can be estimated *in vivo* by measuring mean circulatory filling pressure, which is determined by venous capacitance and blood volume. Venous capacitance is a measure of venous compliance, or the state of venous contractility. In some models of hypertension, total blood volume is not increased, despite an increase in mean circulatory filling pressure, suggesting that mean circulatory filling pressure is driven exclusively by changes in venous tone. Upon a decrease in venous return, as it occurs following a change in posture from supine to the upright position, cardiac output and blood pressure decrease. The low blood pressure reduces afferent nerve firing from the arterial baroreceptors, and this increases sympathetic firing to resistance, as well as capacitance vessels. Increased venous sympathetic nerve activity causes the constriction of the venous smooth muscle, which reduces venous capacity to increase venous return. Venomotor tone, for this reason, can be modulated by drugs that interfere with the sympathetic nerve activity. Body venous tone is also altered in various physiological and pathophysiological conditions such as diabetes mellitus, hypertension, mental stress, aging and autonomic dysfunction, to name a few (for review, Pang, 2001). Altogether, these data support the concept that veins actively participate in the regulation of vascular tone.

5-HT receptor-independent effects

Intriguingly, some of the effects exerted by 5-HT, such as pulmonary arterial smooth muscle proliferation, are mediated through receptor-independent signaling

mechanisms (Marcos et al., 2003). A receptor-independent response to 5-HT involving 5-HT transamidation to small GTPases has been reported in platelets (Walther et al., 2003a). These data indicate that once inside the cell, the effects of 5-HT are not extinguished. However, 5-HT synthesis in platelets and blood vessels has not been reported to date, indicating that the intracellular 5-HT is coming from the extracellular media. What, then, is the mechanism for 5-HT uptake by the vascular cell? An excellent candidate is the 5-HT transporter (SERT) as inhibition of SERT inhibits pulmonary arterial cell proliferation (Marcos et al., 2003). In the next section of this review, 5-HT biochemistry will be discussed with emphasis in 5-HT uptake *via* SERT.

5-HT: from synthesis to metabolism

The predominant site of 5-HT synthesis, storage, and release is the enterochromaffin cells of the intestinal mucosa. Over 95% of total body 5-HT is synthesized in the enterochromaffin cells of the intestine. The remaining 5-HT is mostly synthesized in the raphe nuclei of the brain, pineal gland and in neuroendothendothelial cells lining the lung (for review, Ni et al., 2005).

Synthesis:

5-HT is synthesized in a two-step enzymatic pathway. The hydroxylation of the aromatic amino acid tryptophan forming 5-hydroxytryptophan (5-HTP) by the rate-limiting enzyme tryptophan hydroxylase (TPH) is the first step in the synthesis of serotonin. 5-HTP is then converted into 5-HT by the enzyme amino acid decarboxylase (AADC).

TPH: The discovery that 5-HT is synthesized independently in the peripheral tissues and in the brain by two different TPH isoforms came from the studies performed by Bader and colleagues. The authors were interested in understanding the physiological impact of loss of 5-HT synthesis in TPH knock out mice. Interestingly, the TPH knock out mice presented a 5-HT deficiency in the periphery, but almost normal levels in the brain. We now know that the rate limiting step of 5-HT synthesis in peripheral tissues and in the brain is controlled by two different isoforms of TPH, TPH-1 and TPH2, respectively (for review, Walther and Bader, 2003).

In idiopathic pulmonary arterial hypertension, the endothelium expresses increased TPH-1 gene (Eddahibi et al., 2006). TPH-1 and peripheral 5-HT play an essential role in the development of hypoxia-induced elevation in pulmonary pressures and pulmonary vascular remodeling (Morecroft et al., 2007). Inhibition of 5-HT synthesis by blocking TPH with p-chlorophenylalanine methyl ester (PCPA) reduces the hyperplasia of the pulmonary arterial smooth muscle (Eddahibi et al., 2006).

In saphenous vein, Cohen et al. (1999) observed that 5-HT concentration is reduced by the TPH inhibitor PCPA (Cohen et al., 1999), suggesting 5-HT synthesis in the peripheral vasculature. However, it is important to note that the saphenous vein is richly innervated, and neuronal 5-HT synthesis may account for the measured 5-HT. Preliminary data from our laboratory show TPH-1 gene expression in rat aorta (Cycle threshold values for TPH-1 and the housekeeping gene β-2-microglobulin, respectively: 28.77 ± 0.33 cycles and 17.24 ± 0.09 cycles). The relatively poor innervation of rat aorta supports the presence of the enzyme in smooth muscle or endothelium cells. However, TPH activity in blood vessels requires further investigation.

AADC: The effects of AADC can be inhibited by compounds such as 3-hydroxybenzylhydrazine (NSD 1015), DL-Serine 2-(2,3,4-trihydroxybenzyl)hydrazide (Ro 4-4602) and carbidopa. Carbidopa had no significant effects on responses to 5-HT but ablated the ability of 5-HTP to increase renal vascular resistance and decrease renal blood flow in rats (Ding et al., 1989), suggesting the importance of 5-HT synthesis by AADC to these responses. We have previously observed that treatment of animals with the AADC inhibitor NSD 1015 did not reveal the 5-HT precursor in rat aorta (Ni et al., 2004). These results suggest that it is unlikely that arteries synthesize 5-HT. However, the possibility that 5-HTP may leave the cell, becoming unavailable for measurements, can not be excluded. This possibility is further supported by preliminary data from our laboratory indicating both 5-HTP uptake and the presence of AADC in rat aorta.

Whereas the enzymatic inhibitors would lead to decreased levels of 5-HT, increased administration of the 5-HT precursors tryptophan and 5-HTP can increase 5-HT synthesis. Increased contraction to acetylcholine was observed in mouse duodenum after tryptophan administration due to 5-HT synthesis. However, whereas 5-HTP induces contraction in cerebral vessels, it failed to induce contraction of femoral arteries.

We present here evidence that the peripheral blood vessels may function as possible sites of 5-HT synthesis, and this information is new with respect to veins. Further studies are ultimately needed to fill the gap in this area.

Storage:

Biogenic amines such as 5-HT can be stored in intracellular organelles present in nerve endings, mast cells, adrenal medullary cells and platelets. Other than playing a crucial role in 5-HT synthesis, enterochromaffin cells are also a storage site for 5-HT. In the periphery, platelets are the major 5-HT storage site. Platelets themselves do not synthesize 5-HT, but they can take up 5-HT from the gut and lung through SERT and function as a buffer, keeping the free circulating 5-HT at low levels. Excluding platelets, the free circulating levels of 5-HT in plasma (15-120 nM) are lower than the levels of 5-HT in whole blood (µM range). As the carrier and storage site of 5-HT, the platelets store 5-HT in dense, electron-opaque granules. Storage of 5-HT in platelet granules requires active uptake of 5-HT from the cytoplasm by vesicular monoamine transporter 2 (VMAT-2).

Different forms of 5-HT storage may exist, as suggested by the findings in enterochromaffin cells showing that a small part of 5-HT remains outside the secretory granules. 5-HT could also be covalently bound to proteins to be stored in platelets (Walther et al., 2003a).

We reported the presence of 5-HT in aorta (Ni et al., 2004). 5-HT content in saphenous vein has been previously measured (Cohen et al., 1999). Preliminary data from our laboratory reveals the presence of basal levels of 5-HT in veins in higher amounts than that observed in arteries (Figure 2). However, whether and how 5-HT is stored in aorta and in veins is yet to be proven.

Metabolism:

Metabolism of 5-HT primarily occurs by deamination *via* mitochondrial monoamine oxidase (MAO) to form 5-hydroxyindole acetaldehyde, which in turn is oxidized by aldehyde dehydrogenase to produce 5-hydroxyindole acetic acid (5-HIAA). MAOs are degradative enzymes of monamines such as norepinephrine, 5-HT and dopamine. There are two subtypes of MAO, MAO-A and MAO-B, based on their selectivity to substrate and inhibitors. Whereas MAO-A preferentially metabolizes norepinephrine and 5-HT and is inhibited by clorgyline, MAO-B acts on dopamine and is inhibited by L-deprenyl. Pargyline and iproniazid inhibit both MAO-A and MAO-B. Tissues or cells that contribute significantly to 5-HT metabolism include the lung, intestine and endothelial cells of the vascular system. Over 90% of endogenous 5-HT is cleared in the pulmonary circulation of the lung, but any cell that can take up 5-HT and possesses MAO has the potential to metabolize 5-HT.

MAO activity is measurable in rat aorta. For example, we observed increased levels of 5-HT simultaneously with blunted levels of 5-HIAA in aorta, carotid and superior mesenteric arteries from pargyline-treated rats (Ni et al., 2004). Cohen et al. (1999) found that pargyline treatment increased the amount of 5-HT measured in saphenous vein, indicating the presence of this enzyme in this tissue (Cohen et al., 1999). Altogether, these data suggest that the peripheral vasculature has the ability to metabolize 5-HT and may, therefore, be an important site for the serotonergic system.

Uptake and Release of 5-HT:

5-HT is a protonated molecule that, under physiological conditions, is not capable of crossing the membrane lipid bilayer. The 5-HT transporter (SERT), a bidirectional transporter, is the major protein responsible for uptake and release of 5-HT. SERT, cloned in rat, human and mouse, is an integral protein that decreases the function of 5-HT at its cognate receptors by removing 5-HT from the site of action and bringing it into the cell for metabolism and storage (for review, Ni et al., 2006).

SERT is widely distributed in the central nervous system and a target of antidepressant drugs, such as fluoxetine, fluvoxamine, citalopram, and paroxetine, and the anorexigen (+)-fenfluramine. Whereas fluoxetine inhibits SERT, (+)-fenfluramine is a SERT substrate and potent 5-HT releaser. SERT is also found in the peripheral sympathetic nervous system as well as in platelets, gastrointestinal system and lung.

SERT abnormalities are strongly associated with pulmonary hypertension. The classic concept that 5-HT function is terminated once inside the cell has been challenged by the findings that intracellular 5-HT mediates pulmonary arterial smooth muscle proliferation after uptake by SERT (Marcos et al., 2003).

After observing 5-HT immunostaining in rat aorta, we investigated the serotonergic system in peripheral arteries. Arteries exposed to extracellular 5-HT were capable of concentrating 5-HT. Moreover, this increase in arterial 5-HT levels was inhibited by the SERT inhibitor fluoxetine. A functional SERT in the rat peripheral arteries exists and SERT inhibition potentiates 5-HT-induced contraction (Ni et al., 2004). These findings opened a new avenue towards the understanding of the effects of local 5-HT in the peripheral vasculature. Most interestingly, we observed that whenever

exposed to exogenous 5-HT, vena cava is more likely to uptake larger amounts of 5-HT than aorta (Figure 2). However, whether SERT is involved in 5-HT uptake in veins remains unsolved.

In light of the obvious differences between veins and arteries, it becomes clear that studies involving the venous system are of extreme importance to understand whether these differences may influence the physiology and the pathophysiology of the cardiovascular system.

Venous Diseases:

Venous diseases include deep venous thrombosis and varicose veins, among others. Injury to the endothelium may play an important role in the origin of deep venous thrombosis. The venous endothelium is anti-thrombotic under normal circumstances. On the other hand, under conditions favoring thrombosis, the endothelium becomes pro-thrombotic. Among the factors produced by the endothelium in pro-thrombotic stage are the von Willebrand factor, and fibronectin (Yamamoto et al., 1997). Deep venous thrombosis is strongly associated with an imbalanced activation of the coagulation system. Platelets play a key role not only in hemostasis but also in thrombogenesis. The beginning of the formation of an intravascular thrombus involves the adhesion of blood platelets to the damaged blood vessel, having the von Willebrand factor as the trigger for platelets to adhere to the subendothelium (for review, Jackson et al., 2003). 5-HT enhances von Willebrand factor secretion from endothelial cells *in vitro* (Palmer et al., 1994). However, it is not clear whether 5-HT can induce von Willebrand factor

secretion in platelets. 5-HT increases procoagulant activity and reduce fibrinolytic activity of endothelial cells via 5-HT_{2A} receptor activation, contributing to thrombus formation (Kawano et al., 2001). Whether the high amount of 5-HT found in veins contribute to the coagulation system remains to be investigated.

Varicose vein disease is a complex venous pathology affecting mainly the lower extremities. The development of varicose veins is associated with venous stasis, and resulting hypoxia. The resulting ischemic condition can trigger the endothelial cells to release growth factors and mediators of inflammation, as well as platelet aggregating factors with the potential involvement of 5-HT. We postulate that under the longer periods of exposition to poor oxygenated blood during stasis, veins may become more susceptible to the effects of the higher intracellular amounts of 5-HT.

5-HT in veins

The intriguing fact that the levels of 5-HT in veins are higher than in arteries led us to hypothesize that veins may function as a 5-HT reservoir in the periphery. Whether there is 5-HT synthesis, uptake via SERT and storage in veins remains to be established. We hypothesize that the serotonergic system, from synthesis to metabolism, is present and active in veins, as shown in Figure 3.

Conclusion

A number of 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. The physiological and pathological significance of 5-HT in the peripheral cardiovascular system has not been clarified. Whereas the receptor-mediated effects of 5-HT in the peripheral cardiovascular system have been investigated, only little attention has been paid to the receptor-independent mechanisms. This is not surprising, due to the fact that only recently the first report showing the presence of a functional SERT in peripheral arteries was published, raising the question as to the function of SERT in regulating peripheral effects of 5-HT and the impact of intracellular 5-HT. We present here evidence of increased basal intracellular 5-HT concentration and increased 5-HT uptake in veins when compared to arteries. These novel findings led us to hypothesize that veins may function as a sink for 5-HT in the cardiovascular system.

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3) The authors would like to apologize for not citing many important references that have contributed to this review due to space limitations.

Legends for Figures

Figure 1: Contractile responses to 5-HT. Concentration-effect curves to 5-HT (1 nM to 30 μM) were performed in rat vena cava (A; C), and rat aorta (B; D). The points represent the percentage of norepinephrine (NE; 10 μM)- or phenylephrine (PE; 10 μM)-induced response ± SEM of N different experiments for rat vena cava (A) and rat aorta (B). Representative tracings of the concentration-effect curve to 5-HT in mg of contraction obtained in rat vena cava (C) and rat aorta (D).

Figure 2: Quantification of 5-HT in rat aorta and vena cava incubated with vehicle (□) or 1 μM of exogenous 5-HT (■) for 15 minutes. Blood vessel segments were isolated from pargyline-treated rats [to inhibit MAO and increase the measurable amount of 5-HT in rat aorta, as previously reported (Ni et al., 2004)] and placed in physiological salt solution at room temperature. The vessels were exposed to vehicle or 5-HT, followed by 5-HT measurements through high performance liquid chromatography. The result was normalized to protein content and expressed as ng/mg protein. Bars represent means ± SEM for at least 4 different experiments. * $P < 0.05$ compared to vehicle-incubated tissues. # $P < 0.05$ compared to rat aorta in the same treatment group.

Figure 3: Schematic proposal of the serotonergic system in veins. We propose that the serotonergic system, from synthesis to metabolism, is present and active in the smooth muscle and endothelial cells of veins. 5-hydroxytryptamine; TPH: tryptophan hydroxylase; 5-HTP: 5-hydroxytryptophan; AADC: amino acid decarboxylase; MAO-A:

monoamine oxidase-A; 5-HIAA: 5-hydroxyindoleacetic acid; VMAT: vesicular monoamine transporter; TG: transglutaminase.

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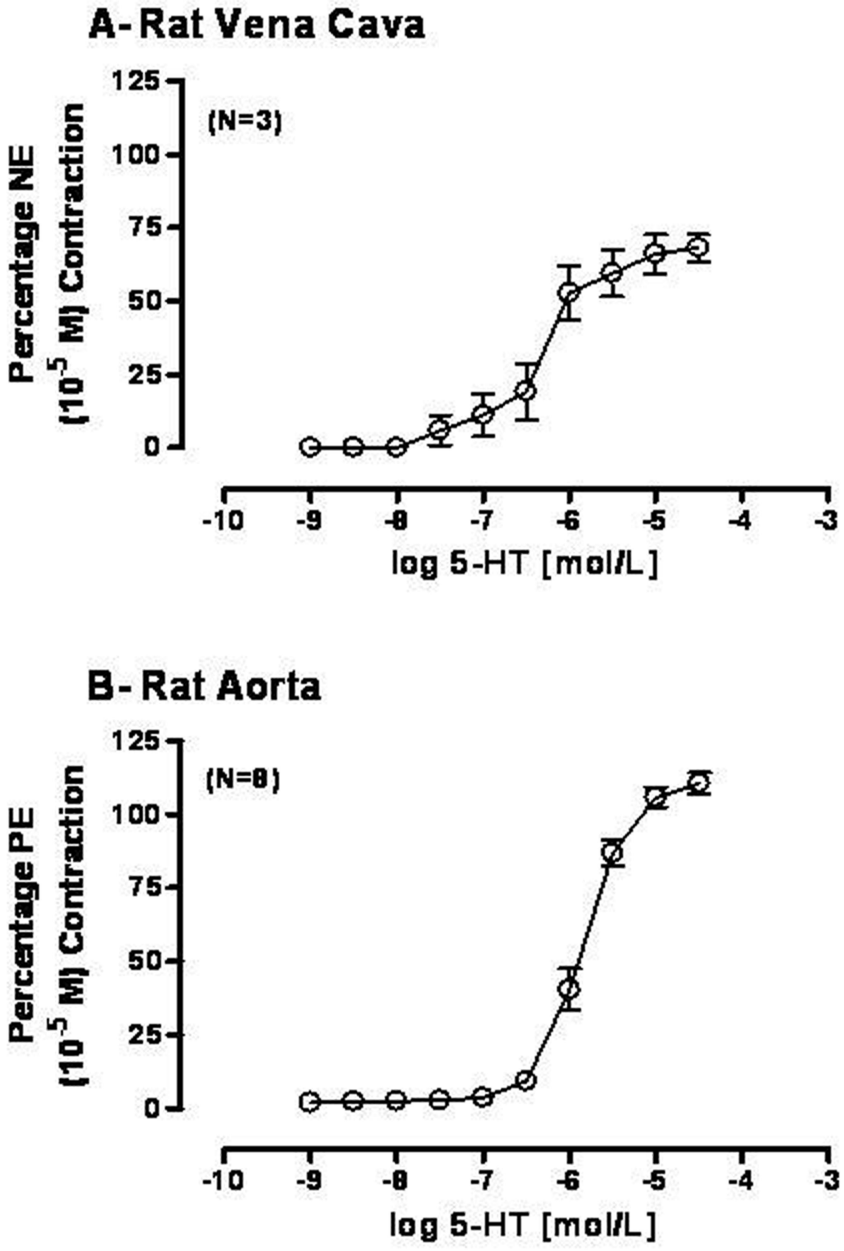

Figure 1

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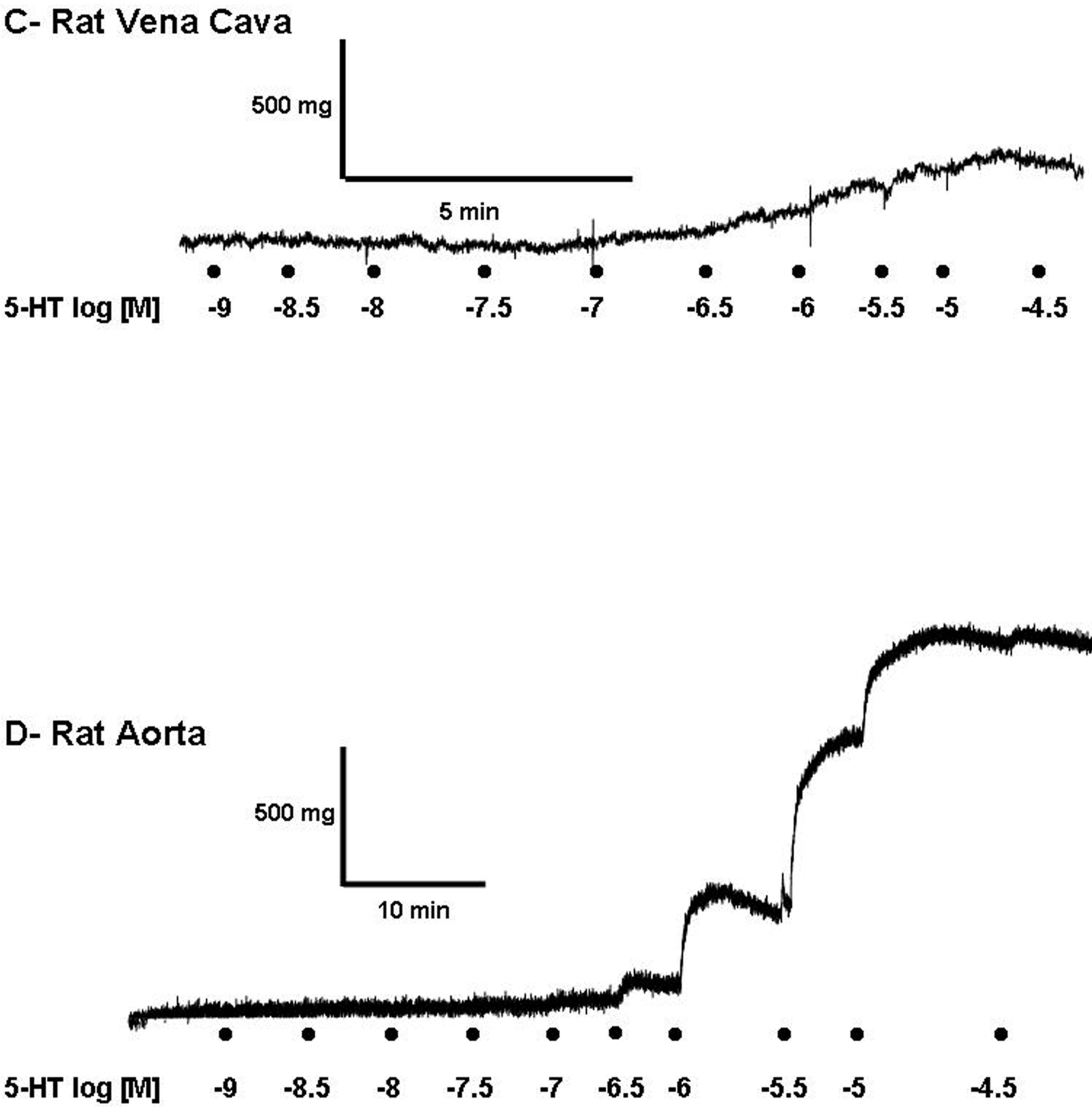

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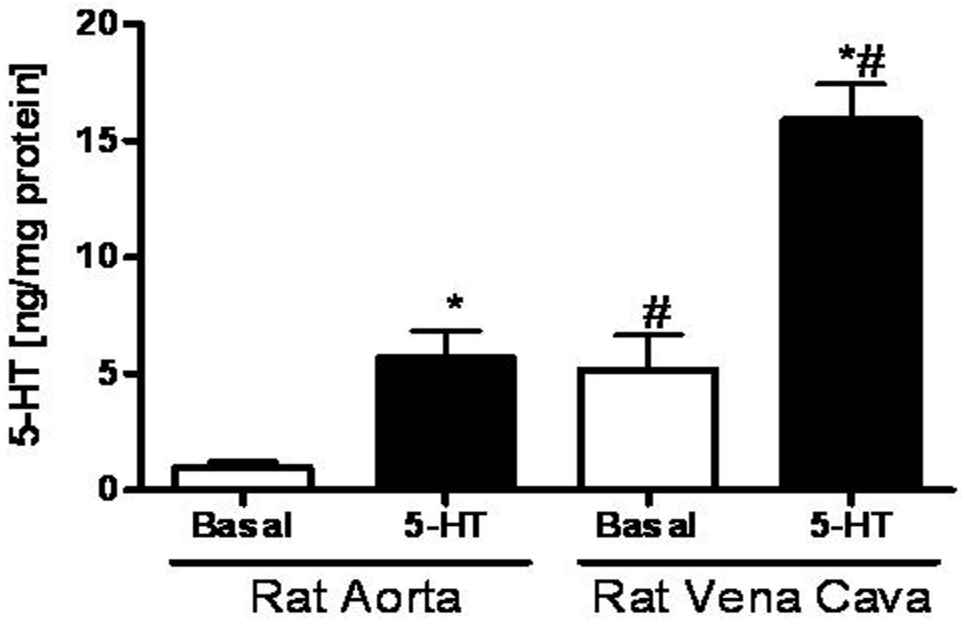

Figure 2

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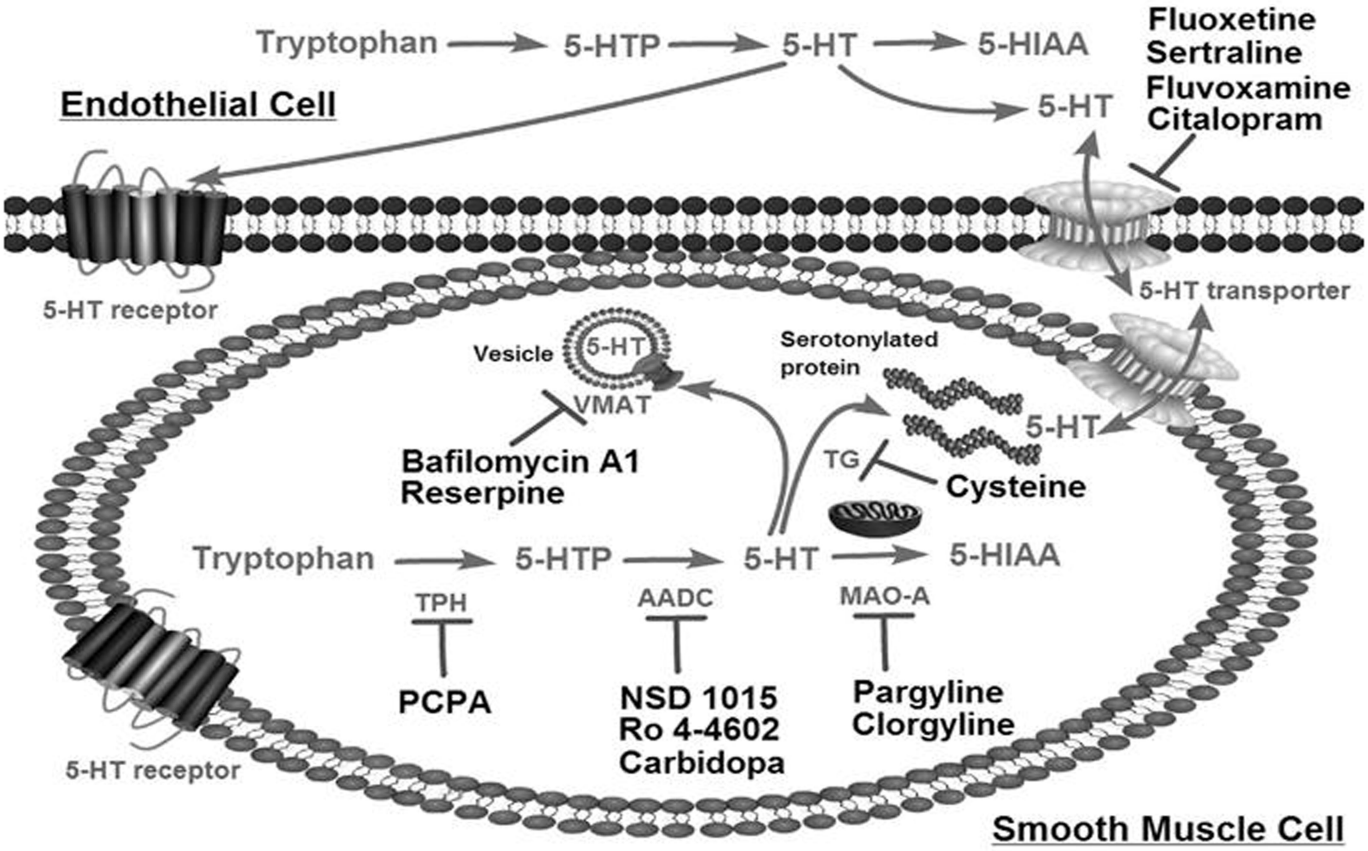

Figure 3