Serotonin
A Different Player in Hypertension-Associated Thrombosis

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Serotonin (5-hydroxytryptamine; 5-HT) was discovered in the lining of the gastrointestinal tract in 1935; a detailed history on its discovery and characterization was published several years later. 5-HT is a derivative of the amino acid L-tryptophan, which is synthesized by tryptophan hydroxylase (TPH) with its 2 isoforms. TPH1 is responsible for the production of 5-HT in peripheral tissues and TPH2 leads to the synthesis of 5-HT in the central nervous system. 5-HT is metabolized in liver and eventually excreted as 5-hydroxyindoleacetic acid. Platelets are likely the biological storage of circulating 5-HT. Platelets do not synthesize 5-HT but acquire it, mainly from the blood, after being secreted by the enterochromaffin cells of the intestine. The free 5-HT level in the blood is tightly regulated by the specific 5-HT transporter, SERT (SLC6A4), which is expressed on the platelet surface. SERT removes 5-HT from the blood via a saturable reuptake mechanism. Once in the cytoplasm, 5-HT is sequestered by the vesicular monoamine transporter type 2 into intracellular dense granules. 5-HT concentration in resting platelets is millimolar versus in the blood where it is in the low nanomolar range. Although 5-HT is better known as a neurotransmitter with key roles in a variety of psychiatric diseases, it also has extracerebral roles: multifunctional signaling molecule, growth factor, endocrine hormone, or paracrine messenger, from fetal to adult life.

5-HT in Platelet Aggregation
Platelets are derived from the fragmented cytoplasm of megakaryocytes and enter the circulation in an inactive form. The initial activation of platelets stabilizes them in hemostasis. Further platelet activation enlists more platelets at a fibrin-stabilized hemostatic area to form a thrombus after associating with the endothelium or each other. The role of circulating, free 5-HT in platelet adhesion, aggregation, and thrombus formation has not been resolved completely, but clinical and biochemical findings infer a complex process. An elevation in free 5-HT levels in plasma accelerates the exocytosis of dense and α-granules; in turn, these secrete 5-HT and other procoagulant molecules that will mediate hemostasis. Supporting these hypotheses is the fact that platelets of 5-HT-infused mice, in the absence of cardiovascular problem, show an enhanced aggregation profile; however, when the 5-HT-infused mice were injected with a selective 5-HT reuptake inhibitor (SSRI) or a 5-HT antagonist, the effect of elevated free 5-HT levels in plasma was reversed and the platelet aggregation profile normalized. The importance of the plasma 5-HT level and platelet SERT in the platelet aggregation phenomenon is supported by findings in platelets of mice lacking the gene for TPH1 or the gene for SERT, where granular secretion rates as well as the risk of thrombosis are significantly reduced. After the interaction with von Willebrand factor and 5-HT receptors, 5-HT-mediated platelet aggregation occurs via 5-HT2A- or SERT-dependent pathways.

In a receptor-dependent pathway, 5-HT initiates G-protein-dependent signaling pathway to mobilize calcium from intracellular stores. Free, calcium activates trans-glutaminase, which transamidates cytoplasmic 5-HT to the GTP-GDP hydrolysis domains of the small GTPases. In the active, GTP-bound form, small GTPases promote the exocytosis of the granule. At this point, the contribution of intracellular 5-HT to the SERT-dependent platelet aggregation pathway seems important. Once the free/unbound 5-HT is taken up by SERT in platelet cytosol, it is stored in dense granules. However, on the saturation of dense granules, free/unbound 5-HT in the platelet cytosol is transamidated on the GTP-GDP hydrolysis domain of the small GTPases converting them to their active GTP-bound form to enhance α-granule secretion. Concurrently, the association between Rab4-GTP and SERT tethers the transporter to an intracellular compartment to prevent further rises in cytoplasmic 5-HT. Additionally, elevated plasma 5-HT activates p21 activating kinase, which phosphorylates the intermediate filament vimentin. We reported that under physiological conditions, SERT binds to vimentin during internalization from the plasma membrane. However, following the phosphorylation of vimentin, the level of SERT on phosphovimentin, as well as the internalization rate of SERT from the plasma membrane to intracellular compartments, are elevated. Based on these findings, we propose that plasma 5-HT at high levels leads to abnormalities in the platelet trafficking of SERT, which reduces the density of SERT molecules on the plasma membrane. These events are correlated with the platelet aggregation process. Mechanistically, these finding investigate the link between elevated plasma 5-HT and loss of surface SERT by focusing on the intracellular tethering of SERT by Rab4 and the 5-HT-mediated phosphorylation of vimentin that promotes...
SERT internalization in platelets (Figure 1). In supporting our findings on the importance of SERT and SSRI in platelet aggregation process, studies with a different approach showed that stimulation of 5-HT$_{2A}$ decreases the adhesive property of platelet by shedding of GPIb from the platelet surface. However, platelet SERT clears 5-HT from plasma which becomes no longer available to stimulate 5-HT$_{2A}$ stimulation; in the presence of SSRI, platelet loses adhesive properties.

In a recent study, we reported that at elevated level, plasma 5-HT was associated with a change in the density and composition of N-glycans on the platelet surface, and this abnormality was allied with an enhanced rate of platelet aggregation. Earlier studies with the platelets of fawn-hooded rats showed a connection between plasma 5-HT level and platelet functions. The genetic disorder in the platelet function of fawn-hooded rat seems similar to the storage pool disease. The reduced level of ATP, ADP, and 5-HT in the platelets and the glycoprotein structure on the surface of platelets of fawn-hooded rats reduce their aggregation rates and contribute to the platelet disorder.

5-HT infusion in mice elevates plasma 5-HT levels and alters the terminal N-glycan content of platelet surface proteins; this enhances platelet aggregation, establishing the surface glycan as a key mediator in platelet activation. Others reported that sialic acid at the terminal position of N-glycans promoted the cell–cell adhesion by acting as a ligand for receptors, such as P- and E-selectins. Therefore, this feature of sialic acids could also be expected for the aggregation characteristics of platelets. However, before our studies, neither the involvement of 5-HT in N-glycan switching nor the role of N-glycans on platelet aggregation was reported. Comparing the 5-HT-infused TPH1-KO and SERT-KO mice models with wildtype counterparts, we found that the plasma membrane of platelets isolated from 5-HT-infused mice had predominantly N-glycolyl-neuraminic acid (Neu5Gc) containing N-glycans. Plasma 5-HT at a high level elevates the Neu5Gc level on the platelet surface, not only promoting its biosynthesis through the catalytic activity of CMP-N-acetyl-neuraminic acid hydroxylase, but also promoting the translocation rates of the vesicles carrying Neu5Gc containing N-glycans to the plasma membrane (Figure 2). 5-HT-infused SERT KO mice (deficient in intracellular 5-HT) showed nearly 2-fold higher CMAH activity than the control mice. Because Neu5Gc is formed from Neu5Ac via the catalytic action of CMAH, the predominance of Neu5Gc on platelets of 5-HT-infused mice suggests that 5-HT signaling enhances the catalytic function of CMAH in a 5-HT$_{2A}$ receptor-dependent pathway and elevates the density of Neu5Gc on the platelet surface.

Figure 1. A. High 5-hydroxytryptamine (5-HT) leads to abnormalities in the platelet trafficking of SERT, which reduces the density of SERT molecules on the plasma membrane. B. Based on our findings for the known actions of 5-HT in platelets, we propose that high levels of uptake leads to saturation of dense granules and 5-HT appears in the platelet cytoplasm. Vesicular monoamine transporter (VMAT) is disabled and cannot remove 5-HT in dense granules anymore. This leads to the serotonylation of small GTPases, such as Rab4, Rho, and Rac, via Ca$^{2+}$-activated TGase. In GTP form, Rab4 binds and prevents the translocation of SERT to the plasma membrane. C. These processes are involved in platelet activation and aggregation in a two-step process: in the first step, elevated 5-HT controls the platelet 5-HT uptake rates via altering the membrane trafficking of plasma membrane SERT, which in turn elevates the plasma 5-HT level further; then in the second step, the elevated plasma 5-HT level activates 5-HT$_{2A}$, which accelerates the exocytosis of α-granules. Secretion of prothrombotic molecules from α-granules to the plasma with high levels of 5-HT propagates the thrombus formation. However, even at the highest levels of plasma 5-HT, there are always a number of SERT molecules on the plasma membrane that still continue to clear plasma 5-HT, but with a lower rate, most probably until the plasma 5-HT level returns to the physiological level.
findings concurred with fluorescence-activated cell sorting analysis of platelets stained with Neu5Gc antibodies.

In summary, the involvement of 5-HT in platelet aggregation mechanism seems to be through multiple pathways.

5-HT in Hypertension-Associated Platelet Aggregation

The plasma 5-HT level is elevated in various conditions, including hypertension and thrombosis. However, in the absence of cardiovascular disease, in vivo administration of 5-HT does not increase systolic blood pressure, suggesting that the elevation in plasma 5-HT level could be a consequence rather than a cause of some forms of hypertension. The action of plasma 5-HT on blood pressure can be varied with its acute or chronic elevation and the location in the circulation system. For example, the blood pressure of deoxycorticosterone acetate-salt hypertensive rats was decreased over the course of 1 month of 5-HT infusion. If the prothrombotic action of elevated 5-HT level in plasma were ignored, its blood pressure lowering capacity would be a novel approach to the hypertension studies.

Hypertension is characterized by an increased vascular resistance that can be caused by vasoconstriction. Several studies report an elevation in plasma 5-HT concentration in hypertensive subjects that concurrently have diabetes mellitus and coronary artery disease, as well as in a variety of other cardiovascular pathologies, including cerebrovascular disease and arterial thrombosis. Because these conditions share platelet activation as a disease mechanism, attention has focused on identifying the complex mechanisms by which 5-HT may promote platelet activation.

Cocaine-Induced Mouse Model of Hypertension

Cocaine is a powerful sympathomimetic agent that causes acute elevations in arterial pressures; the net effect of cocaine on SBP and diastolic blood pressures in humans is an increase of 20 and 10 mmHg, respectively. In peripheral tissues, cocaine produces a sympathomimetic response by inhibiting the reuptake of 5-HT and catecholamines, leading to a transient bradycardia followed by tachycardia, hypertension, and acute thrombosis in coronary arteries.

Figure 2. The stimulation of cells with 5-hydroxytryptamine (5-HT) activates, via 5-HT2A signaling, the production of Neu5Gc via the catalytic function of CMP-N-acetyl-neuraminic acid hydroxylase (CMAH). 5-HT signaling is mediated by the G protein-coupled 5-HT2A, which facilitates the formation of inositol 1,4,5-triphosphate (IP3), resulting in a rise of cytoplasmic Ca2+ in platelets. Based on our reported and unpublished data, we propose that elevated intracellular Ca2+ activates CMAH, which elevates the number of Neu5Gc containing N-glycans on the plasma membrane of platelets. Our studies showed that 5-HT signaling is important either on trafficking of Neu5Gc (represented in the diagram in blue) containing vesicles to the plasma membrane or enhancing the catalytic ability of CMAH or both in inducing platelet activation and aggregation.
Through a variety of mechanisms, cocaine increases the risk of thrombosis.\textsuperscript{51} Even in the absence of systemic platelet activation, endothelial dysfunction, or cardiovascular complications, cocaine is associated with acute thrombosis of coronary arteries.\textsuperscript{51,52} Autopsy studies have demonstrated the presence of coronary atherosclerosis in young cocaine users along with associated thrombus formation;\textsuperscript{53} thus, cocaine use is associated with premature coronary atherosclerosis and thrombosis. Platelets isolated from cocaine-injected mice seem hyperreactive and form thrombi as a result of elevated exocytosis of \( \alpha \)-granules and of plasminogen-activator inhibitor inhibitor level but a decreased antithrombin-III level.\textsuperscript{51,53,55}

The role of 5-HT in cocaine-mediated thrombus formation is poorly understood. Cocaine acts as a ligand on SERT and reduces the 5-HT reuptake rates of the cells. However, when mice were injected with cocaine, their plasma 5-HT was elevated to the level found in SSRI-injected mice;\textsuperscript{46} however, contrary to the effects of SSRI, platelets became hyperreactive.\textsuperscript{54} Platelets of cocaine-injected TPH1-KO mice compared with platelets isolated from TPH1-KO mice had higher aggregation rates by 10%; surprisingly, in cocaine-injected DAT-KI mice (cocaine-insensitive dopamine transporter-knocked), platelets were already prothrombotic with aggregation rates 20% higher than the rates observed in the control group.\textsuperscript{46} DAT-KI mice are introduced genetically with cocaine-insensitive DAT, which made them 70-fold less sensitive to cocaine but fully functional for dopamine uptake.\textsuperscript{56}

Our findings suggest participation of the sympathetic nervous system in cocaine-mediated platelet aggregation via the synergistic actions of 5-HT and nonserotominergic amines, such as catecholamines.\textsuperscript{56}

### Ang II–Induced Mouse Model of Hypertension

Ang II, part of the renin–angiotensin–aldosterone system, contributes to hypertension.\textsuperscript{57} Whether Ang II–mediated hypertension is associated with a prothrombotic state remains controversial. Although some studies report a prothrombotic effect of Ang II,\textsuperscript{57,58} others report a protective effect of Ang II that is independent of its pressor effects\textsuperscript{60–62} and may rely on elevated prostacyclin or nitric oxide to inhibit platelet aggregation.\textsuperscript{52,63} Thus, we studied the correlation between elevated serum Ang II and the adhesive properties of platelets.\textsuperscript{16} The hypertensive model was established on adult C57BL/6J male mice infused isotonic saline or Ang II via osmotic minipumps.\textsuperscript{16} Baseline SBP was measured on 6 consecutive days before insertion of the mini-pumps. Then, SBP in each mouse was averaged from ≥6 trials each day for 1 week after saline- or Ang- II infusion. Ang II infused for 1 week increased SBP from 101±8.6 to 180±12 mm Hg (average increase of 78%).

### 5-HT and Platelet Aggregation in Ang II and Cocaine Models

After confirmation of an SBP increase after cocaine injection or Ang II infusion, plasma 5-HT concentration in saline, Ang-infused, and cocaine-injected mice was determined.\textsuperscript{16,46} Neither 30 minutes cocaine-injection nor 7 days Ang II infusion caused a change in circulating platelet counts (not shown). However, accentuated collagen-induced aggregation was observed in platelets from cocaine-injected mice; this was on average 50% higher compared with that observed in platelets from saline- or Ang II–infused mice.\textsuperscript{46,46} These findings were confirmed by showing a significant elevation in P-selectin (marker of platelet activation) by flow cytometry. Thus, exposure to cocaine coincides with a heightened platelet response to collagen and enhanced platelet activation (Table). The 5-HT level was 2.8-fold higher in cocaine-injected mice than in Ang II–infused mice, but in both groups, Ang II and cocaine, the levels of SBP and 5-HT levels were elevated. Hypertension was associated with 1 week Ang II infusion or 30 minutes cocaine injection; plasma 5-HT levels were relatively elevated, but only the platelets of cocaine-injected mice were hyperreactive.

### Known Effects of Drugs Interacting With SERT Activity on Platelet Function in the Setting of Systemic Hypertension

Plasma 5-HT levels are elevated in a variety of pathologies, including hypertension, thrombotic disease, and carcinoid syndrome. The direct contribution of 5-HT to thrombosis is difficult to assess in complex diseases, such as hypertension. However, in carcinoid syndrome, carcinoid tumors overproduce 5-HT, and the increased circulating level of 5-HT (and other hormones) is correlated with the formation of disseminated intravascular coagulation. Although the evidence linking 5-HT to hypercoagulable states is mainly correlative, Sarpgorelate–ANPLAG\textsuperscript{®} is marketed in Japan, China, and Korea as an antiplatelet agent.\textsuperscript{64–66}

These implicate that 5-HT in human diseases associated with abnormal coagulation. One of the best examples in connecting 5-HT and hypertension is serotonin syndrome which generally occurs if the physiological 5-HT level is elevated by using 2 of the following drugs at the same time, triptans, SSRI, selective serotonin/norepinephrine reuptake, ecstasy, lysergic acid diethylamide, monoamine oxidase inhibitors, meperidine, and dextromethorphan. The combination of these drugs causes the elevation of 5-HT level significantly higher than the physiological level, which creates a prothrombotic environment for platelet. Despite the availability of a wide range of effective BP-lowering agents, a substantial proportion of patients with hypertension fail to achieve target BP levels. The majority of patients with hypertension need ≥2 drugs
to achieve BP control, and so it is important to identify new medication targets.

The cardiovascular effects of 5-HT are not uniform: bradycardia or tachycardia, hypotension or hypertension, vasodilatation or vasoconstriction. These responses are mediated by 5-HT\textsubscript{1A}, 5-HT\textsubscript{2A}, 5-HT\textsubscript{3A}, 5-HT\textsubscript{1D}, and 5-HT\textsubscript{2C} receptors, as well as by a tyramine-like action in the central nervous system, autonomic ganglia, postganglionic nerve endings, vascular smooth muscle, endothelium, and cardiac tissue. For example, BP response to administration of 5-HT is triphasic: initial short-lasting vasodopressor phase (reflex bradycardia caused by 5-HT\textsubscript{3A} receptors on vagal afferents), a middle vasopressor phase (5-HT\textsubscript{2C} receptors), and a late, longer-lasting, vasodopressor phase (direct vasorelaxation by activation of 5-HT\textsubscript{7} receptors located on vascular smooth muscle, inhibition of the vasopressor sympathetic outflow by sympathoinhibitory 5-HT\textsubscript{1A} receptors, and release of endothelial nitric oxide by 5-HT\textsubscript{2B} and 5-HT\textsubscript{2C} receptors). Furthermore, central administration of 5-HT can cause both hypotension (5-HT\textsubscript{1A} receptors) and hypertension (5-HT\textsubscript{2C} receptors).

Because of this complexity, until now, only 2 drugs have been found to have blood pressure lowering effect. Both work on alpha\textsubscript{1}-adrenoceptors, as well as 5-HT receptors. Ketanserin affects baroreflex function by blocking 5-HT\textsubscript{2A} receptors and alpha\textsubscript{1}-adrenoceptors through central and peripheral mechanisms.\textsuperscript{56,67} On the other side, Urapidil has an \( \alpha\)-blocking effect and also a central sympatholytic effect mediated via stimulation of 5HT\textsubscript{1A} receptors in the central nervous system.\textsuperscript{58,69}

Further studies on the role of 5-HT in hypertension, new compounds with high affinity, and selectivity for the different 5-HT receptor subtypes together with SERT may be used as a therapy in the hypertension armamentarium.

Concluding Remarks

5-HT in peripheral tissues, as well as the central nervous system, has a rich history in pharmacology, and this has translated into a widely exploited therapeutic target. New insights into the regulation of SERT and 5-HT\textsubscript{2A} functions in platelet biology may open new dimensions in the targeting of the additive effect of plasma 5-HT on platelet aggregation. We have focused here on the known mechanisms in which 5-HT affects platelet biology in cardiovascular diseases.

Elevations of plasma 5-HT levels have been reported in a variety of cardiovascular pathologies, including hypertension, atherosclerosis, coronary artery disease, angina, and arterial thrombosis.\textsuperscript{6,11–14,39,40} Because these conditions share platelet activation as a disease component, attention has focused on identifying the complex mechanisms by which 5-HT may promote platelet activation.

Considering the role of 5-HT in platelet aggregation, a loss of platelet SERT coupled with elevated plasma 5-HT may play a significant role in cardiovascular diseases, diabetes mellitus, metabolic syndrome, atherosclerosis, and peripheral arterial disease; this is thought to reflect a common prothrombotic state. Numerous factors have been identified that confer this increased susceptibility to thrombosis, including a loss of endothelial-derived nitric oxide, vascular smooth muscle cell hypertrophy, hyperinsulinemia, and other metabolic abnormalities, obesity, and inflammation.\textsuperscript{1,2,21,37} The development of possible antithrombotic therapies for patients with cardiovascular disease has focused on reducing these risk factors rather than on targeting endogenous mechanisms responsible for the prothrombotic state. In this regard, therapies designed to promote the expression of SERT on the platelet surface and thereby reduce plasma levels of 5-HT may represent a novel approach to alleviating thrombotic events, as well as controlling blood pressure.

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Disclosures

The authors declare no competing financial interests.

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