Abstract

Platelets transport and store serotonin at a high concentration in dense granules and release it upon activation. Abnormal serotonin concentrations in the blood plasma or increased platelet serotonin release promote the development of thrombosis, sepsis, allergic asthma, myocardial infarction, and stroke. Consequently, experimental data suggest possible benefits of serotonin receptor blockade or inhibition of platelet serotonin uptake in the indicated human diseases. Here, we highlight the current state of basic biological research regarding the role of platelet serotonin in normal and pathophysiological conditions focusing on thrombotic and inflammatory diseases. We also describe the possible clinical applicability of targeting thrombo-immune-modulatory effects of platelet serotonin to treat common health problems.

Keywords: platelets, serotonin, inflammation, thrombosis, selective serotonin reuptake inhibitors

1. Introduction

Serotonin (5-HT) is a well-known neurotransmitter, which regulates neural activity and a variety of neuropsychological processes [1]. As it has been shown to be involved in the regulation of systemic and cellular functions, alterations in serotonin concentration in the body are associated with many different diseases, such as irritable bowel syndrome, restless legs syndrome, sudden infant death syndrome, autism, headache, insomnia, anxiety, depression, anorexia, schizophrenia, Parkinson’s and Alzheimer’s disease, pulmonary hypertension, and...
myocardial infarction. 5-HT was first described in 1930 by Vittrorio Erspamer who isolated it from enterochromafin cells of the gut [1]. Only a small amount of 5-HT is synthesized in brain (5%), whereas 95% is produced by the enterochromafin cells of the gastrointestinal (GI) tract. 5-HT is synthesized from the essential amino acid l-tryptophan (TRP) to 5-hydroxytryptophan (5-HTP), by the enzyme l-tryptophan hydroxylase (TPH)-1 in the brain and
TPH-2 in the periphery [2, 3]. The activity of these TPH enzymes is the rate-limiting step in the production of 5-HT in both organs. After its synthesis in the gastrointestinal tract, 5-HT is released into the bloodstream. 5-HT can bind and activate several isoforms of 5-HT receptors expressed throughout the body (Figure 1). 5-HT receptors were identified on different blood cells and in the vessel wall including lymphocytes, endothelial, and smooth muscle cells, respectively, which can respond to 5-HT under certain physiological conditions. These receptors constitute a family of seven different receptor sub-classes: 5-HT_{1} (A-F, P, S), 5-HT_{2} (A-D), 5-HT_{3}, 5-HT_{4}, 5-HT_{5}, 5-HT_{6}, and 5-HT_{7} [3, 4]. All these receptors belong to the GPCR superfamily with the exception of 5-HT_{3}, which is a member of nicotinic acetylcholine receptor superfamily and is a ligand-gated ion channel.

5-HT can also be taken up from plasma into several cells—such as platelets—via the 5-HT transporter (5-HTT, SERT). After uptake, 5-HT can be then stored in vesicles and granules through the action of vesicular monoamine transporter (VMAT)-1/2 which is expressed in neurons, neuroendocrine cells, and platelets. The largest quantity of serotonin is believed to be stored in platelets, from where it can be released upon platelet activation, for example, during thrombus formation or inflammatory reactions. Interestingly, chemical precursors of 5-HT can pass across the blood-brain barrier, but 5-HT cannot, thereby effectively isolating the brain 5-HT pool from the periphery and vice versa. In the brain, 5-HT regulates several complex networks, such as mood, perception, reward, anger, memory, appetite, attention, and sexuality. There are two major routes of 5-HT metabolism, which convert 5-HT to melatonin and 5-HIAA. 5-HT is metabolized by neurons and endothelial cells by monoamine oxidases (MAOs) and the products of this breakdown are then excreted by the kidney [3, 5–7]. Peripheral 5-HT regulates heart development and rate, valvulopathy, pain, nociception, embryonic development, vasoconstriction/vasodilatation, blood flow, hemostasis, and many other important processes. Platelets are not able to synthesize serotonin, but take it up from plasma via 5-HTT, store it in dense granules (via VMAT-1), and release it into the blood during their activation. Platelet serotonin has not only well-established autocrine functions during platelet activation and thrombus growth but also paracrine functions in the vasculature including modulation of endothelial, smooth muscle, and immune cell function.

2. Autocrine-regulatory mechanisms of platelet serotonin

Platelets store 5-HT in their dense granules at millimolar range and secrete it after activation [8]. Dense granule and 5-HT release support the recruitment of circulating platelets to preformed thrombi, thereby leading to thrombus growth. This process is mediated through the interaction between 5-HT and its receptor 5HT_{2A} expressed on circulating platelets. Activated 5-HT_{2A} receptor transduces the signal to G_{q}-phospholipase C (PLC) β-signaling cascade. Enhanced PLCβ activity results in intracellular Ca^{2+} mobilization from the store through inositol 3-phosphate (IP3) receptor and mediates 1,2-diacylglycerol (DAG)-dependent protein kinase C (PKC) activation, thereby amplifying platelet reactivity (Figure 2).

In addition to the mobilization of cytosolic Ca^{2+} [9, 10], receptor-ligand interactions are also known to regulate 5-HT uptake kinetics. In human platelets, the rise of cytoplasmic Ca^{2+} in
the absence of exocytosis reduces 5-HT transport into the cytoplasm, thereby decreasing the release of 5-HT [9]. Interestingly, rabbit platelets activated in the presence of the extracellular Ca²⁺ chelator ethylene tetraacetic acid also displayed a decrease in 5-HT transport activity [11, 12]. Consistently, human platelets treated with the membrane permeant Ca²⁺ chelator BAPTA-AM also had reduced 5-HT transport in the presence of extracellular Ca²⁺ [9]. Activation of the Orai1 Ca²⁺ channel induces a robust Ca²⁺ influx called store-operated Ca²⁺ entry (SOCE), which is triggered through the release of Ca²⁺ from intracellular stores.
This process is controlled by functional coupling of activated stromal interaction molecule 1 (STIM1) to Orai1 [13]. Interestingly, strongly reduced SOCE was found in 5Htt−/− platelets [14]. This suggests that secreted platelet 5-HT contributes to the regulation of SOCE through binding to 5-HT2A which activates Gq-PLCβ-mediated Ca2+ store release, thereby further activating STIM1/Orai1 complex. Interestingly, SOCE-induced signal can strongly inhibit 5-HT uptake in human platelets via 5-HTT [9, 11]. This could be an important step to keep 5-HT outside of platelets, thereby increasing extracellular 5-HT concentration and permanently activating 5-HT2A on the platelet surface. Therefore, 5-HT cannot enter the platelet cytosol during SOCE. Interestingly, 5-HTT contains several consensus sites for PKC. It has been shown that PKC activity is required for the internalization of the transporter suggesting a link between 5-HT uptake and intracellular Ca2+ level [15–18]. Altogether, Ca2+ signaling, Ca2+ store release, and Ca2+ influx through SOCE play an important regulatory role for 5-HT cycling in human and mouse platelets.

After Ca2+ store release and PKC activation, integrins exposed and activated on the platelet surface support aggregation and thrombus formation. In β3 integrin-deficient platelets, 5-HT uptake was strongly reduced, indicating a functional crosstalk between 5-HTT and β3 integrin [19]. Integrin activation defect in response to glycoprotein VI (GPVI) or C-type lectin-like receptor 2 (CLEC-2) stimulation was found in 5Htt−/− mouse platelets, which was fully rescued in the presence of extracellular 5-HT [14]. The physical interaction between 5-HTT and β3 seems to be dispensable for β3 integrin activation. The observed integrin activation defect is due to the lack of the secreted platelet 5-HT which further amplifies “inside-out” activation of integrins through Ca2+-dependent and independent pathways mediated by Ca2+- and DAG-regulated guanine exchange factor-1 (CalDAG-GEFI) and PKC, respectively.

Although 5-HT is mainly stored in dense granules, intracellular-free 5-HT in the cytoplasm has been proposed to activate diverse biological processes called serotonylation. It has been shown that small-guanosine triphosphate-binding protein (GTP)-ases covalently bind 5-HT, thereby changing the structure and activity of GTPase, leading to α-granule exocytosis from platelets. This process requires tissue transglutaminase and factor XIIIa, both activated by mobilized Ca2+. Transglutaminase may mediate the transamidation of small GTPases, like cytoplasmic Ras homolog gene family member A (RhoA) and a small GTP-binding protein Rab4. Serotonylation in turn blocks the inactivation of both molecules. A complex composed of Ca2+ and calmodulin (CaM) may also activate guanine exchange factors (GEFs), which induce the exchange of guanosine di- (GDP) to triphosphate (GTP) on RhoA and Rab4 and thus stimulates activation of the respective protein. These two active molecules play an important role in cytoskeleton rearrangement, exocytosis of α-, and dense granule contents. Some bioactive molecules stored in platelet granules, such as fibrinogen and factor V, are also known to be serotonylated [8]. Upon platelet activation, these proteins are exposed at the platelet surface and are used to mark a subpopulation of highly activated, pro-coagulant platelets, the so-called collagen and thrombin-activated (COAT) platelets. Coated platelets express high levels of phosphatidylserine and strongly support prothrombinase activity [8, 20].

Besides the dopamine transporter (DAT), the noradrenaline transporter (NET), and the organic transporter (OCT), 5-HTT is an important 5-HT transporter to regulate 5-HT uptake from the blood plasma and reuptake of the released platelet 5-HT in certain physiological
5-HTT is encoded by the SLC6A4 gene containing 14 exons. The protein structure of 5-HTT contains 12 transmembrane domains. In humans, the splice variants of 5-HTT and their mutations are associated with several pathologies, such as anxiety, suicide, depression, substance abuse, autism, and neurogenic disorders [21–24]. 5-HTT is abundantly expressed not only on neurons, endothelial cells, mast cells, immune cells, in intestine, and vasculature, but also in platelets [25, 26]. It is well established that in platelets 5-HTT plays an important role in the uptake of 5-HT from the circulation. Monoamine transporters are thought to be able to compensate for one another where they are co-expressed. For example, 5-HT may be taken up in venous vessels independently of 5-HTT expression [25, 27]. Interestingly, and in sharp contrast to venous vessels, genetic ablation of 5Htt in mice completely abolished 5-HT uptake in platelets, since no detectable secreted 5-HT was observed upon platelet activation, indicating an essential role of 5-HTT for 5-HT uptake into platelets, which cannot be compensated by other transporters [14]. Altogether, these results highlight the cell-type-specific regulation of 5-HT uptake in mammalian cells.

5-HTT can be targeted by several antidepressants, such as selective serotonin reuptake inhibitors (SSRIs) (cf., Section 5), which are widely used in the treatment of psychiatric diseases to increase 5-HTT concentrations in the synaptic space. The blockade of 5-HTT with the SSRI citalopram reduces the aggregation response to collagen in human platelets [28] due to reduced phosphorylation of a tyrosine-protein kinase Syk in the GPVI signalosome. Syk can also bind and phosphorylate 5-HTT suggesting an Syk-mediated functional crosstalk between 5-HTT and GPVI complex. Interestingly, 5Htt−/− mouse platelets could not show any abnormalities in the tyrosine phosphorylation cascade of the GPVI signalosome, as Syk phosphorylation was normal after GPVI stimuli. Consequently, Syk and 5-HTT interaction seems to be dispensable for the initial activation of GPVI complex, but enhanced Syk activity may regulate the 5-HT uptake in platelets [29].

3. Paracrine-regulatory mechanisms of platelet serotonin

During degranulation, activated platelets secrete a significant amount of 5-HT from dense granules which is clinically relevant to induce acute thrombotic events [30, 31] by promoting vasoconstriction and cellular activation of neighboring platelets and lymphocytes through their 5-HT receptors.

5-HT receptors expressed on endothelial, smooth muscle, and immune cells respond to platelet-derived 5-HT (Figure 3). 5-HT has growth-promoting effects on endothelial cells, which may facilitate tissue healing after vascular damages [32]. However, 5-HT may also exert dual effects either stimulating constriction or dilatation of microvasculature. In the liver, 5-HT appears to mainly promote constriction of hepatic sinusoid vessels, since mice lacking peripheral 5-HT display elevated sinusoidal perfusion under physiological and pathological conditions [33]. By contrast, platelet-derived 5-HT coordinates the formation of gaps between endothelial cells in the joint microvasculature, which in arthritic conditions may contribute to inflammation [34]. How these processes are regulated is still not clear but presumably may involve differential signaling pathways through specific 5-HT receptors expressed on vascular endothelial and smooth muscle cells.
Platelet-derived 5-HT can regulate the function of T- and B-cells, natural killer cells, monocytes, and neutrophils under certain conditions [35–38]. In the spleen, 5-HT increases monocyte differentiation into dendritic cells and early naive T-cell activation via the 5-HT$_{2A}$ receptor [38, 39]. Furthermore, it also has been shown that lymphocytic cytokine levels in mice are reduced after treatment with SSRI [40]. In a mouse model of viral hepatitis, the release of 5-HT by platelets was responsible for tissues damage caused by CD8 (+) T-cells, microcirculatory events, and reduced clearance of infiltrated viruses [33]. Moreover, specific antagonism of 5-HT receptors in mice attenuated asthmatic attacks and sepsis [37, 41].

5-HT released from dense granules upon activation by the inflamed endothelium also contributes to the recruitment of immune cells to the vascular wall [37]. Indeed, platelet-derived 5-HT promotes leukocyte migration, possibly via activation of endothelial cells, thereby enhancing P-selectin exposure and IL-8 release [37], which trigger neutrophil rolling, adhesion, and extravasation. Moreover, locally increased levels of platelet-released 5-HT had paracrine effects on endothelial cells, thereby inducing microvasculature leakage through the activation of transglutaminase and the phosphorylation of vimentin [42]. By contrast, in solid tumors platelet-released 5-HT has been described as a major regulator of the tumor vascular homeostasis that continuously prevent bleeding. Interestingly, tumor-infiltrating leukocytes have been identified as the cause of tumor bleeding [43, 44]. Altogether, these studies suggest that under specific conditions, platelet-released 5-HT promotes clot formation and modulates immune cell functions.
In humans, 5-HT levels appear elevated in infection and autoimmune diseases, suggesting that SSRI could be applicable for vascular and immune system modulation. Since platelets are the major 5-HT store in the blood, pharmacological blockage of 5-HT uptake in platelets increases the level of 5-HT in the blood plasma transiently. Unexpectedly, 5Htt<sup>−/−</sup> mice display reduced 5-HT levels in plasma [14]. In 5Htt<sup>−/−</sup> mice, elevated urinary 5-HIAA levels were detected suggesting a faster 5-HT metabolism in the peripheral blood. Consequently, platelet 5-HT uptake and storage play an important regulatory role for controlling systemic 5-HT metabolic cycles. Future studies are needed to specify the exact mechanisms of platelet-derived 5-HT on vascular and immune system modulation in normal physiology and diseases.

4. Pathophysiological consequences of abnormal platelet serotonin release

5-HT plasma concentration was analyzed in several pathological contexts. It became widely recognized that 5-HT is an independent risk factor for platelet aggregation and for thrombus formation in animal models (cf., Section 6) and human patients [19, 45–49]. Plasma 5-HT can support platelet aggregation and thrombus growth through 5-HT<sub>2A</sub>-dependent or independent signaling pathways. Pharmacological blockade of 5-HT<sub>2A</sub> receptor increases the 5-HT uptake rates in animal models of hypertension, as well as ex vivo platelet aggregation. Vikenes et al. detected a 10-fold increase of plasma 5-HT in patients undergoing angiography after admission for myocardial infarction [50]. In these patients, high plasma 5-HT was associated with cardiac events. In another study, more than 10-fold rise in 5-HT has been noticed in coronary vessels of patients following angioplasty. Importantly, in these patients the level of 5-HT in the systemic plasma was normal [51]. Together, these studies suggest that in vivo the interplay between circulating 5-HT and platelet function could be a predictive factor.

5-HT levels are drastically increased during myocardial ischemia, and blockade of the 5-HT<sub>2</sub> receptor improves the outcome after myocardial infarction in different mouse models [52, 53]. 5-HT also enhances the survival of cardiomyocytes via the 5-HT<sub>2B</sub> receptor. In hepatic ischemia models, platelets promote tissue repair [54], and proliferation of hepatocytes was shown to be partly mediated by platelet 5-HT after liver ischemia [55]. 5-HT also contributes to intratumoral homeostasis by dysbalancing permeability factors [44]. 5-HT-induced growth of human hepatocellular carcinoma cells and specific blockade of the 5-HT<sub>2</sub> receptor decreased recruitment of circulating tumor cells [56, 57]. It has been suggested that the inhibition of platelet granule contents might be effective to induce intratumoral bleeding, thereby decreasing tumor viability and growth. Additionally, plasma 5-HT levels are increased in patients with colorectal, liver, and intestinal cancers [58, 59].

Allergic airway inflammation provokes a local release of 5-HT in mouse models and human patients [41]. Interestingly, after challenge with an allergen, 5-HT increased 10-fold in broncho-alveolar lavage of predisposed patients, inducing asthmatic attacks. In line with these studies, 5-HT is known as a key regulator of pulmonary vascular resistance and vessel wall integrity [60, 61].
5. Clinical applications: effects of selective serotonin reuptake inhibitors on platelet functions

Selective serotonin reuptake inhibitors are commonly used drugs for the treatment of patients with severe depressive and anxiety disorders [62]. SSRIs were developed to selectively inhibit the uptake of 5-HT through the 5-HTT transporter in the brain, while having minimal side effects on DAT and NET proteins which can also transport 5-HT [63]. The action of SSRIs relies on the modulation of the allosteric region of the transporter, thereby leading to a conformational change and blocking of the uptake of 5-HT [63]. The uptake of 5-HT into neurons is very important for the clearance of the synaptic cleft, preventing firing rates and overstimulation of receptors [64]. This uptake and the later release are blocked upon treatment with SSRIs, such as fluvoxamine, fluoxetine, nortryptiline, citopram, and escitalopram [65]. The different SSRIs vary in kinetics being competitive and non-competitive inhibitors. Two distinct binding sites on 5-HTT have been identified, a low-affinity allosteric site, mediating the dissociation of SSRIs from their high-affinity site, which induces the blockade of 5-HT uptake [64].

There is evidence that targeting 5-HT receptors or using serotonin-like molecules is effective in the treatment of non-neuronal diseases. The use of tricyclic antidepressants, but not SSRIs, is associated with an increased risk of myocardial infarction. SSRIs have shown no cardiac toxicity, even in patients with heart disease. Several epidemiologic studies reported lower cardiovascular morbidity and mortality in patients treated with SSRI [66–68].

Depression is a significant risk factor for ischemic heart and cerebrovascular disease as well as mortality following myocardial infarction. The potential effects of SSRIs upon the cardiovascular system may therefore play an important role. These drugs had potential benefit in hypertensive patients after myocardial infarction and hypertensive responses to depression were reduced in patients who had been prescribed SSRIs [30]. In blood samples of depressive patients taking fluoxetine, the platelet aggregation response to submaximal collagen stimulation was decreased [69]. In this study, a significant decrease in 5-HT concentration was observed in platelet-rich plasma associated with the use of fluoxetine but not with the tricyclic antidepressant amitryptiline. It is intriguing whether lowered platelet 5-HT content translates into less 5-HT release during platelet activation in patients with thrombotic diseases. Enhanced platelet reactivity was observed in patients suffering from depression and chronic heart disease due to the upregulated β-thromboglobulin (β-TG) and platelet factor 4 (PF4) levels [70]. Lowered PF4 and β-TG levels have been observed upon treatment with SSRI paroxetine [71], suggesting that reduced platelet aggregation in vivo may impact coronary artery-related mortality. SSRI treatment also decreases platelet reactivity in patients with heart failure. Other SSRIs, sertraline, and N-desmethylsertraline were also shown to dampen platelet responses [72].

SSRIs have been shown to increase the risk of bleeding in patients with liver cirrhosis and liver failure. Importantly, SSRIs may also directly increase gastric acidity with ulcerogenic effect resulting in GI bleeding. The risk of SSRI-associated GI bleeding is increased with the concurrent use of nonsteroidal anti-inflammatory drugs, anticoagulants, and antiplatelet agents,
and is decreased by concurrent proton pump inhibitors [73, 74]. In conclusion, SSRIs appear to be protective against cardiovascular diseases and may enhance the risk for GI bleeding. However, to date this evidence is not yet conclusive.

6. Experimental studies on the role of platelet serotonin in arterial thrombosis and stroke

Over the past decades, the functions of peripheral 5-HT have received increasing attention. It has been shown that peripheral 5-HT plays a major role in a variety of important processes, including hemostasis and immune defense. This has been addressed by using Tph1−/− mice, which lack peripheral 5-HT in the circulation, due to the lack of the enzyme that converts hydroxylases tryptophan to 5-HT in the gut [75]. In humans, abolished or decreased level of TPH1 is associated with impulsive behavior, aggression, irritable bowel syndrome, anxiety, and other pathologies [76–79]. Genetic ablation of TPH1 function in mice not only leads to several disorders, such as mild anemia, cardiomyopathy, and diabetes, but also to other defects in hemostasis, erythropoiesis, pulmonary hypertension, and lung regeneration. The lack of 5-HT in this mouse model is associated with decreased neutrophil recruitment to inflammatory sites, diabetics, and mild anemia [37, 80].

Recent studies using wild-type mice infused with 5-HT or Tph1−/− mice have demonstrated that peripheral 5-HT is required for platelet aggregation [14]. Additionally, in vivo 5-HT infusion generates hyperreactive platelets with reduced bleeding time and shortened occlusion time of the carotid arteries in wild-type mice. 5Htt−/− mice have prolonged bleeding time, reflecting the increased bleeding risk described to occur using long-term SSRI treatment in human patients. In comparison to this relatively mild hemostatic defect, 5Htt−/− mice were not able to form occlusive thrombi in response to mechanical injury of the abdominal aorta as compared to wild-type animals [14].

Platelets contribute to the progression of infarct growth after transient brain ischemia by thrombo-inflammation with platelet-immune cell interactions. SSRI treatment of stroke patients has been described to enhance brain function recovery, indicating a therapeutic benefit of the direct blockade of 5-HTT function. Neuroblast proliferation and cell migration have been shown to be enhanced and associated with increased microvessel density during SSRI treatment, explaining the possible role of 5-HTT in tissue repair after ischemic insults [81–83]. 5Htt−/− mice have been studied in the tMCAO (transient intraluminal filament model of middle cerebral artery occlusion) model of ischemic stroke. Unexpectedly, these mice developed similar brain infarcts to wild-type controls and the 5-HTT neurological outcome was indistinguishable [14]. In line with this study, SSRI treatment could not reduce infarct size or cerebral edema in mice [82], suggesting that this treatment cannot protect neurons or other cells in the ischemic brain. Altogether, these results indicate that SSRI treatment may have a long-term effect in the ischemic brain tissue which positively influences post-stroke recovery. Further investigation is necessary to understand the specific role of peripheral and brain 5-HT in thrombo-inflammation during stroke and infarct progression.
7. Conclusions

5-HT is an ancient molecule that is better known for its functions in the brain than in the periphery. However, literature describing the contribution of peripheral 5-HT, including platelet 5-HT, is rapidly growing. It became evident that platelet 5-HT has a complex role involving many bidirectional interactions with tissue microenvironment to regulate platelet and immune cell functions. SSRI treatment in animal models appears to improve thrombotic and inflammatory diseases. Further fundamental and preclinical studies are needed for a better understanding of platelet 5-HT functions in humans. In conclusion, targeting thrombo-immune-modulatory functions of platelet serotonin may provide new important therapeutic approaches.

Author details

Elmina Mammadova-Bach¹, Maximilian Mauler², Attila Braun¹ and Daniel Duerschmied²*

*Address all correspondence to: daniel.duerschmied@universitaets-herzzentrum.de

1 Department of Experimental Biomedicine, University Hospital and Rudolf Virchow Center, Wuerzburg, Germany

2 Department of Cardiology and Angiology I, Heart Center, Faculty of Medicine, University of Freiburg, Germany

References

[1] Whitaker-Azmitia PM. The discovery of serotonin and its role in neuroscience. Neuropsychopharmacology. 1999 Aug;21(2 Suppl):2S-8S

[2] Hickman AB, Klein DC, Dyda F. Melatonin biosynthesis: The structure of serotonin N-acetyltransferase at 2.5 A resolution suggests a catalytic mechanism. Molecular Cell. 1999 Jan;3(1):23-32

[3] Berumen LC, Rodriguez A, Miledi R, Garcia-Alcocer G. Serotonin receptors in hippocampus. Scientific World Journal. 2012;2012:823493

[4] Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, et al. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). Pharmacological Reviews. 1994 Jun;46(2):157-203

[5] Nowak JZ, Szymanska B, Zawilska JB, Bialek B. Hydroxyindole-O-methyltransferase activity in ocular and brain structures of rabbit and hen. Journal of Pineal Research. 1993 Aug;15(1):35-42
[6] Keszthelyi D, Troost FJ, Masclee AA. Understanding the role of tryptophan and serotonin metabolism in gastrointestinal function. Neurogastroenterology and Motility: The Official Journal of the European Gastrointestinal Motility Society. 2009 Dec;21(12):1239-1249

[7] Mossner R, Lesch KP. Role of serotonin in the immune system and in neuroimmune interactions. Brain, Behavior, and Immunity. 1998 Dec;12(4):249-271

[8] Walther DJ, Peter JU, Winter S, Holtje M, Paulmann N, Grohmann M, et al. Serotonylation of small GTPases is a signal transduction pathway that triggers platelet alpha-granule release. Cell. 2003 Dec 26;115(7):851-862

[9] Turetta L, Bazzan E, Bertagno K, Musacchio E, Deana R. Role of Ca(2+) and protein kinase C in the serotonin (5-HT) transport in human platelets. Cell Calcium. 2002 May;31(5):235-244

[10] Rink TJ, Sage SO. Calcium signaling in human platelets. Annual Review of Physiology. 1990;52:431-449

[11] Nishio H, Nezasa K, Nakata Y. Role of calcium ion in platelet serotonin uptake regulation. European Journal of Pharmacology. 1995 Jan 16;288(2):149-155

[12] Watanabe Y, Kobayashi B. Differential release of calcium, magnesium and serotonin by rabbit and human platelets. Journal of Pharmacobio-Dynamics. 1988 Apr;11(4):268-276

[13] Ramanathan G, Gupta S, Thielmann I, Pleines I, Varga-Szabo D, May F, et al. Defective diacylglycerol-induced Ca2+ entry but normal agonist-induced activation responses in TRPC6-deficient mouse platelets. Journal of Thrombosis and Haemostasis. 2012 Mar;10(3):419-429

[14] Wolf K, Braun A, Haining EJ, Tseng YL, Kraft P, Schuhmann MK, et al. Partially defective store operated calcium entry and hem(ITAM) signaling in platelets of serotonin transporter deficient mice. PloS One. 2016;11(1):e0147664

[15] Ramamoorthy S, Giovanetti E, Qian Y, Blakely RD. Phosphorylation and regulation of antidepressant-sensitive serotonin transporters. The Journal of Biological Chemistry. 1998 Jan 23;273(4):2458-2466

[16] Qian Y, Galli A, Ramamoorthy S, Risso S, DeFelice LJ, Blakely RD. Protein kinase C activation regulates human serotonin transporters in HEK-293 cells via altered cell surface expression. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience. 1997 Jan 01;17(1):45-57

[17] Anderson GM, Horne WC. Activators of protein kinase C decrease serotonin transport in human platelets. Biochimica et Biophysica Acta. 1992 Nov 17;1137(3):331-337

[18] Myers CL, Lazo JS, Pitt BR. Translocation of protein kinase C is associated with inhibition of 5-HT uptake by cultured endothelial cells. The American Journal of Physiology. 1989 Oct;257(4 Pt 1):L253–L258

[19] Carneiro AM, Cook EH, Murphy DL, Blakely RD. Interactions between integrin alphalbetalbeta3 and the serotonin transporter regulate serotonin transport and platelet aggregation in mice and humans. The Journal of Clinical Investigation. 2008 Apr;118(4):1544-1552
[20] Dale GL. Coated-platelets: An emerging component of the procoagulant response. Journal of Thrombosis and Haemostasis: JTH. 2005 Oct;3(10):2185-2192

[21] Heils A, Teufel A, Petri S, Stober G, Riederer P, Bengel D, et al. Allelic variation of human serotonin transporter gene expression. Journal of Neurochemistry. 1996 Jun;66(6):2621-2624

[22] Stober G, Heils A, Lesch KP. Serotonin transporter gene polymorphism and affective disorder. Lancet. 1996 May 11;347(9011):1340-1341

[23] Murphy DL, Lerner A, Rudnick G, Lesch KP. Serotonin transporter: Gene, genetic disorders, and pharmacogenetics. Molecular Interventions. 2004 Apr;4(2):109-123

[24] Murphy DL, Lesch KP. Targeting the murine serotonin transporter: Insights into human neurobiology. Nature Reviews Neuroscience. 2008 Feb;9(2):85-96

[25] Linder AE, Ni W, Szasz T, Burnett R, Diaz J, Geddes TJ, et al. A serotonergic system in veins: Serotonin transporter-independent uptake. The Journal of Pharmacology and Experimental Therapeutics. 2008 Jun;325(3):714-722

[26] Ahern GP. 5-HT and the immune system. Current Opinion in Pharmacology. 2011 Feb;11(1):29-33

[27] Linder AE, Diaz J, Ni W, Szasz T, Burnett R, Watts SW. Vascular reactivity, 5-HT uptake, and blood pressure in the serotonin transporter knockout rat. American Journal of Physiology Heart and Circulatory Physiology. 2008 Apr;294(4):H1745–H1752

[28] Tseng YL, Chiang ML, Huang TF, Su KP, Lane HY, Lai YC. A selective serotonin reuptake inhibitor, citalopram, inhibits collagen-induced platelet aggregation and activation. Thrombosis Research. 2010 Dec;126(6):517-523

[29] Pavanetto M, Zarpellon A, Borgo C, Donella-Deana A, Deana R. Regulation of serotonin transport in human platelets by tyrosine kinase Syk. Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology. 2011;27(2):139-148

[30] Watts SW, Morrison SF, Davis RP, Barman SM. Serotonin and blood pressure regulation. Pharmacological Reviews. 2012 Apr;64(2):359-388

[31] Watts SW. Serotonin-induced contraction in mesenteric resistance arteries: Signaling and changes in deoxycorticosterone acetate-salt hypertension. Hypertension. 2002 Mar 01;39(3):825-829

[32] Pakala R, Willerson JT, Benedict CR. Mitogenic effect of serotonin on vascular endothelial cells. Circulation. 1994 Oct;90(4):1919-1926

[33] Lang PA, Contaldo C, Georgiev P, El-Badry AM, Recher M, Kurrer M, et al. Aggravation of viral hepatitis by platelet-derived serotonin. Nature Medicine. 2008 Jul;14(7):756-761

[34] Cloutier N, Pare A, Farndale RW, Schumacher HR, Nigrovic PA, Lacroix S, et al. Platelets can enhance vascular permeability. Blood. 2012 Aug 09;120(6):1334-1343
[35] Iken K, Chheng S, Fargin A, Goulet AC, Kouassi E. Serotonin upregulates mitogen-stimulated B lymphocyte proliferation through 5-HT1A receptors. Cellular Immunology. 1995 Jun;163(1):1-9

[36] Ito T, Ikeda U, Shimpo M, Yamamoto K, Shimada K. Serotonin increases interleukin-6 synthesis in human vascular smooth muscle cells. Circulation. 2000 Nov 14;102(20):2522-2527

[37] Duerschmied D, Suidan GL, Demers M, Herr N, Carbo C, Brill A, et al. Platelet serotonin promotes the recruitment of neutrophils to sites of acute inflammation in mice. Blood. 2013 Feb 07;121(6):1008-1015

[38] Gershon RK. A disquisition on suppressor T cells. Transplantation Reviews. 1975;26:170-185

[39] Fuchs BA, Campbell KS, Munson AE. Norepinephrine and serotonin content of the murine spleen: Its relationship to lymphocyte beta-adrenergic receptor density and the humoral immune response in vivo and in vitro. Cellular Immunology. 1988 Dec;117(2):339-351

[40] Gobin V, Van Steendam K, Denys D, Deforce D. Selective serotonin reuptake inhibitors as a novel class of immunosuppressants. International Immunopharmacology. 2014 May;20(1):148-156

[41] Durk T, Duerschmied D, Muller T, Grimm M, Reuter S, Vieira RP, et al. Production of serotonin by tryptophan hydroxylase 1 and release via platelets contribute to allergic airway inflammation. American Journal of Respiratory and Critical Care Medicine. 2013 Mar 01;187(5):476-485

[42] Li Y, Hadden C, Cooper A, Ahmed A, Wu H, Lupashin VV, et al. Sepsis-induced elevation in plasma serotonin facilitates endothelial hyperpermeability. Scientific Reports. 2016 Mar 09;6:22747

[43] Ho-Tin-Noe B, Goerge T, Wagner DD. Platelets: Guardians of tumor vasculature. Cancer Research. 2009 Jul 15;69(14):5623-5626

[44] Ho-Tin-Noe B, Goerge T, Cifuni SM, Duerschmied D, Wagner DD. Platelet granule secretion continuously prevents intratumor hemorrhage. Cancer Research. 2008 Aug 15;68(16):6851-6858

[45] Ottervanger JP, Stricker BH, Huls J, Weeda JN. Bleeding attributed to the intake of paroxetine. The American Journal of Psychiatry. 1994 May;151(5):781-782

[46] Ziu E, Mercado CP, Li Y, Singh P, Ahmed BA, Freyaldenhoven S, et al. Down-regulation of the serotonin transporter in hyperreactive platelets counteracts the pro-thrombotic effect of serotonin. Journal of Molecular and Cellular Cardiology. 2012 May;52(5):1112-1121

[47] Mercado CP, Quintero MV, Li Y, Singh P, Byrd AK, Talabnin K, et al. A serotonin-induced N-glycan switch regulates platelet aggregation. Scientific Reports. 2013 Sep 30;3:2795

[48] Berry CN, Lorrain J, Lochot S, Delahaye M, Lale A, Savi P, et al. Antiplatelet and antithrombotic activity of SL65.0472, a mixed 5-HT1B/5-HT2A receptor antagonist. Thrombosis and Haemostasis. 2001 Mar;85(3):521-528
[49] Przyklenk K, Frelinger AL, 3rd, Linden MD, Whittaker P, Li Y, Barnard MR, et al. Targeted inhibition of the serotonin 5HT2A receptor improves coronary patency in an in vivo model of recurrent thrombosis. Journal of Thrombosis and Haemostasis: JTH. 2010 Feb;8(2):331-340

[50] Vikenes K, Farstad M, Nordrehaug JE. Serotonin is associated with coronary artery disease and cardiac events. Circulation. 1999 Aug 03;100(5):483-489

[51] Leosco D, Fineschi M, Pierli C, Fiaschi A, Ferrara N, Bianco S, et al. Intracoronary serotonin release after high-pressure coronary stenting. The American Journal of Cardiology. 1999 Dec 01;84(11):1347-1355

[52] Shimizu Y, Minatoguchi S, Hashimoto K, Uno Y, Arai M, Wang N, et al. The role of serotonin in ischemic cellular damage and the infarct size-reducing effect of sarpogrelate, a 5-hydroxytryptamine-2 receptor blocker, in rabbit hearts. Journal of the American College of Cardiology. 2002 Oct 02;40(7):1347-1355

[53] Simpson PJ, Schelm JA, Jakubowski JA, Smallwood JK. The role of serotonin (5HT2) receptor blockade in myocardial reperfusion injury: Effects of LY53857 in a canine model of myocardial infarction. The Journal of Pharmacology and Experimental Therapeutics. 1991 Sep;258(3):979-985

[54] Lesurtel M, Graf R, Aleil B, Walther DJ, Tian Y, Jochum W, et al. Platelet-derived serotonin mediates liver regeneration. Science. 2006 Apr 07;312(5770):104-107

[55] Nocito A, Georgiev P, Dahm F, Jochum W, Bader M, Graf R, et al. Platelets and platelet-derived serotonin promote tissue repair after normothermic hepatic ischemia in mice. Hepatology. 2007 Feb;45(2):369-376

[56] Soll C, Jang JH, Riener MO, Moritz W, Wild PJ, Graf R, et al. Serotonin promotes tumor growth in human hepatocellular cancer. Hepatology. 2010 Apr;51(4):1244-1254

[57] Skolnik G, Bagge U, Blomqvist G, Djarv L, Ahlman H. The role of calcium channels and serotonin (5-HT2) receptors for tumour cell lodgement in the liver. Clinical & Experimental Metastasis. 1989 Mar-Apr;7(2):169-174

[58] Lee HZ, Wu C. Serotonin-induced protein kinase C activation in cultured rat heart endothelial cells. European Journal of Pharmacology. 2000 Sep 08;403(3):195-202

[59] Dowling P, Hughes DJ, Larkin AM, Meiller J, Henry M, Meleady P, et al. Elevated levels of 14-3-3 proteins, serotonin, gamma enolase and pyruvate kinase identified in clinical samples from patients diagnosed with colorectal cancer. Clinica Chimica Acta; International Journal of Clinical Chemistry. 2015 Feb 20;441:133-141.

[60] Eddahibi S, Humbert M, Fadel E, Raffestin B, Darmon M, Capron F, et al. Serotonin transporter overexpression is responsible for pulmonary artery smooth muscle hyperplasia in primary pulmonary hypertension. The Journal of Clinical Investigation. 2001 Oct;108(8):1141-1150

[61] Lederer DJ, Horn EM, Rosenzweig EB, Karmally W, Jahnes M, Barst RJ, et al. Plasma serotonin levels are normal in pulmonary arterial hypertension. Pulmonary Pharmacology & Therapeutics. 2008;21(1):112-114
[62] Vaswani M, Linda FK, Ramesh S. Role of selective serotonin reuptake inhibitors in psychiatric disorders: A comprehensive review. Progress in Neuro-Psychopharmacology & Biological Psychiatry. 2003 Feb;27(1):85-102

[63] Stahl SM. Mechanism of action of serotonin selective reuptake inhibitors. Serotonin receptors and pathways mediate therapeutic effects and side effects. Journal of Affective Disorders. 1998 Dec;51(3):215-235

[64] Tatsumi M, Groshan K, Blakely RD, Richelson E. Pharmacological profile of antidepressants and related compounds at human monoamine transporters. European Journal of Pharmacology. 1997 Dec 11;340(2-3):249-258

[65] Maurer-Spurej E. Serotonin reuptake inhibitors and cardiovascular diseases: A platelet connection. Cellular and Molecular Life Sciences: CMLS. 2005 Jan;62(2):159-170

[66] Lopez-Munoz F, Alamo C. Monoaminergic neurotransmission: The history of the discovery of antidepressants from 1950s until today. Current Pharmaceutical Design. 2009;15(14):1563-1586

[67] Kessler RC, Aguilar-Gaxiola S, Alonso J, Chatterji S, Lee S, Ormel J, et al. The global burden of mental disorders: an update from the WHO World Mental Health (WMH) surveys. Epidemiologia e Psichiatria Sociale. 2009 Jan-Mar;18(1):23-33

[68] Ramachandraih CT, Subramanyam N, Bar KJ, Baker G, Yeragani VK. Antidepressants: From MAOIs to SSRIs and more. Indian Journal of Psychiatry. 2011 Apr;53(2):180-182

[69] Menys VC, Smith CC, Lewins P, Farmer RD, Noble MI. Platelet 5-hydroxytryptamine is decreased in a preliminary group of depressed patients receiving the 5-hydroxytryptamine re-uptake inhibiting drug fluoxetine. Clinical Science. 1996 Jul;91(1):87-92

[70] Laghrissi-Thode F, Wagner WR, Pollock BG, Johnson PC, Finkel MS. Elevated platelet factor 4 and beta-thromboglobulin plasma levels in depressed patients with ischemic heart disease. Biological Psychiatry. 1997 Aug 15;42(4):290-295

[71] Pollock BG, Laghrissi-Thode F, Wagner WR. Evaluation of platelet activation in depressed patients with ischemic heart disease after paroxetine or nortriptyline treatment. Journal of Clinical Psychopharmacology. 2000 Apr;20(2):137-140

[72] Serebruany VL, Gurbel PA, O'Connor CM. Platelet inhibition by sertraline and N-desmethylsertraline: A possible missing link between depression, coronary events, and mortality benefits of selective serotonin reuptake inhibitors. Pharmacological Research. 2001 May;43(5):453-462

[73] Anglin R, Yuan Y, Moayyedi P, Tse F, Armstrong D, Leontiadis GI. Risk of upper gastrointestinal bleeding with selective serotonin reuptake inhibitors with or without concurrent nonsteroidal anti-inflammatory use: A systematic review and meta-analysis. The American Journal of Gastroenterology. 2014 Jun;109(6):811-819

[74] Paton C, Ferrier IN. SSRIs and gastrointestinal bleeding. British Medical Journal. 2005 Sep 10;331(7516):529-530
[75] Walther DJ, Bader M. Serotonin synthesis in murine embryonic stem cells. Brain Research Molecular Brain Research. 1999 May 07;68(1-2):55-63

[76] New AS, Gelernter J, Yovell Y, Trestman RL, Nielsen DA, Silverman J, et al. Tryptophan hydroxylase genotype is associated with impulsive-aggression measures: A preliminary study. American Journal of Medical Genetics. 1998 Feb 07;81(1):13-17

[77] Jun SE, Kohen R, Cain KC, Jarrett ME, Heitkemper MM. TPH gene polymorphisms are associated with disease perception and quality of life in women with irritable bowel syndrome. Biological Research for Nursing. 2014 Jan;16(1):95-104

[78] Paterson DS, Darnall R. 5-HT2A receptors are concentrated in regions of the human infant medulla involved in respiratory and autonomic control. Autonomic Neuroscience: Basic & Clinical. 2009 May 11;147(1-2):48-55

[79] Fehr C, Schleicher A, Szegedi A, Anghelescu I, Klawe C, Hiemke C, et al. Serotonergic polymorphisms in patients suffering from alcoholism, anxiety disorders and narcolepsy. Progress in Neuro-Psychopharmacology & Biological Psychiatry. 2001 Jul;25(5):965-982

[80] Suidan GL, Duerschmied D, Dillon GM, Vanderhorst V, Hampton TG, Wong SL, et al. Lack of tryptophan hydroxylase-1 in mice results in gait abnormalities. PloS One. 2013;8(3):e59032

[81] Mead GE, Hsieh CF, Lee R, Kutlubaev MA, Claxton A, Hankey GJ, et al. Selective serotonin reuptake inhibitors (SSRIs) for stroke recovery. The Cochrane Database of Systematic Reviews. 2012 Nov 14;11:CD009286

[82] Espinera AR, Ogle ME, Gu X, Wei L. Citalopram enhances neurovascular regeneration and sensorimotor functional recovery after ischemic stroke in mice. Neuroscience. 2013 Sep 05;247:1-11

[83] McFarlane A, Kamath MV, Fallen EL, Malcolm V, Cherian F, Norman G. Effect of sertraline on the recovery rate of cardiac autonomic function in depressed patients after acute myocardial infarction. American Heart Journal. 2001 Oct;142(4):617-623
