5-Hydroxytryptamine and Its Receptors in Systemic Vascular Walls

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5-Hydroxytryptamine (5-HT) in the bloodstream is largely contained in platelets and circulates throughout the entire vascular system. 5-HT released from activated platelets dramatically changes the function of vascular smooth muscle cells (VSMCs) and endothelial cells (ECs). In VSMCs, 5-HT induces proliferation and migration via 5-HT1A receptors. These effects are further enhanced by vasoactive substances such as thromboxane A2 and angiotensin II. 5-HT1A receptor activation in VSMCs also causes both enhancement of prostaglandin I2 production by inducing cyclooxygenase-2 and reduction of nitric oxide (NO) by suppressing inducible NO synthase. Evidence showing that 5-HT in ECs plays a principal role in angiogenesis now exists. Stimulation of 5-HT1, and/or 5-HT2 receptors has been implicated in the angiogenic effect of 5-HT. The extracellular signal-regulated kinase and endothelial NO synthase (eNOS) activation-dependent pathways are involved in the mechanisms. Moreover, 5-HT1 receptor in ECs have been shown to also regulate angiogenesis. Recent reports show sarpogrelate, a selective antagonist of the 5-HT2A receptor, indirectly enhances the function of 5-HT1B receptors in ECs via inhibition of 5-HT2A receptors in VSMCs or platelets. This indirect action of 5-HT1B receptors in ECs may increase NO production derived from eNOS and a vasodilator response. Furthermore, sarpogrelate and other 5-HT2A receptor antagonists have been shown to reduce the constitutive activity of 5-HT2A receptors. It is believed that increasing evidence on the role of 5-HT receptors will contribute to the expansion of the clinical application of existing therapeutic drugs such as sarpogrelate, and to the development of new 5-HT receptor-related drugs for treating cardiovascular diseases.

Key words 5-hydroxytryptamine; vascular smooth muscle cell; vascular endothelial cell; sarpogrelate; angiogenesis

1. INTRODUCTION

5-Hydroxytryptamine (5-HT) was discovered as a substance that caused contraction of smooth muscle in the mid-1900s. Although 5-HT was first discovered in the cardiovascular system, the function of 5-HT as a neurotransmitter in the central nervous system has been extensively explored and drugs that affect 5-HT receptors and 5-HT transporter (SERT), which incorporates 5-HT into presynaptic vesicles, have been developed and are widely used for diseases such as depression, schizophrenia and anxiety. In the last decade, the precise functions and mechanisms of 5-HT in the systemic vasculature and pulmonary circulation have been established.

Enterochromaffin cells in the intestinal mucosa produce approximately 95% of the total 5-HT in the human body. Blood platelets do not synthesize 5-HT, but possess the SERT and acquire a high amount of 5-HT released from the intestine.1) Most of the peripheral 5-HT incorporated into platelets circulates in the bloodstream throughout the entire vascular system. In fact, in the healthy human cardiovascular system, the mean 5-HT concentration was reported to be approximately 10 pmol in blood plasma, 1 µmol in serum and 400 µmol in platelets.2) At the site of local vascular injury, a large amount of 5-HT is released from activated platelets and the plasma 5-HT level rises. The released 5-HT dramatically affects the functions of vascular smooth muscle cells (VSMCs) and endothelial cells (ECs), such as the contraction, proliferation, migration or release of vasoactive mediators. Recent studies show that peripheral arteries are able to synthesize and metabolize 5-HT through tryptophan hydroxylase 1, monoamine oxidase A and SERT.3,4)

5-HT1, 5-HT2, 5-HT3 and 5-HT7 receptor mRNAs are expressed in both VSMCs and vascular ECs.5) Basically, 5-HT is believed to cause vasoconstriction by the activation of 5-HT2A receptors on VSMCs and vasodilation by a nitric oxide (NO)-dependent mechanism via 5-HT1B receptor in ECs. Over the last decade, the further function and signal transduction of 5-HT in these cells have been clarified. Here, we introduce current topics regarding the function and signaling pathways of 5-HT in VSMCs and ECs. Although the roles of 5-HT in the pulmonary artery are unique and important for the pathogenesis of pulmonary hypertension, in this mini review we focus on the roles of 5-HT in systemic vasculature.

2. FUNCTION AND SIGNALING MECHANISMS IN VASCULAR SMOOTH MUSCLE CELLS

Although the expression of mRNA of 5-HT1, 5-HT2, and

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5-HT receptors has been reported in VSMCs, the effects of 5-HT on VSMCs are mostly mediated by 5-HT2A receptor activation. Thus, the role of the 5-HT2A receptor and its signal transduction in VSMCs have been actively characterized.

Both proliferation and migration of VSMCs are involved in the pathogenesis of atherosclerosis. 5-HT has been shown to exert its proliferative effect via 5-HT2 receptors in VSMCs. Pakala et al. have shown that 5-HT induces [3H]thymidine incorporation into VSMCs and upregulates 5-HT receptor mRNA expression, and interestingly docosahexaenoic acid and eicosapentaenoic acid, which are dietary n-3 polyunsaturated fatty acids, reduce the 5-HT-induced proliferative effect via the downregulation of 5-HT2 mRNA expression. 5-HT also causes migration of human aortic VSMCs. Since this migration is abolished by ketanserin, which is a 5-HT2 receptor antagonist, the migration is mediated by 5-HT2A receptors. Furthermore, 5-HT-induced Rho A and extracellular signal-regulated kinase (ERK) activation and the subsequent increase in stress fiber formation are involved in the mechanisms. Since 5-HT-induced proliferation and migration are further enhanced by treatment with vasoactive substances such as thromboxane A2, angiotensin II, thrombin and platelet-derived growth factor (PDGF), the mediators derived from activated platelets are critical to the pathogenesis of atherosclerosis as well.

In VSMCs, 5-HT also increases or decreases several enzyme activities to produce important vasoactive substances such as NO and prostaglandins. Several reports including from our laboratory have shown that protein kinase C (PKC) is an enzyme that is critical to 5-HT2A receptor signal transduction. Ito et al. reported that the activation of 5-HT2A receptors increases interleukin-6 (IL-6) synthesis in human VSMCs, at least partially through a PKC-dependent manner. On the other hand, Yu et al. reported that the 5-HT2A receptor-specific agonist (R)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane suppresses tumor necrosis factor-α (TNF-α)-induced gene expressions of IL-6, intracellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and nitric oxide synthase (NOS) activity in rat VSMCs. The PKC activation that inhibits TNF-α-induced nuclear factor-κB translocation is involved in the mechanisms. The PKC signaling pathway by 5-HT2A receptor activation also inhibits IL-1β-induced NO production and inducible NOS (iNOS) expression.

5-HT2A receptor stimulation also affects the arachidonate cascade in VSMCs. We have reported that 5-HT induces vasodilating prostaglandin I2 (PGI2) and cyclooxygenase-2 (COX-2) expression via Src, PKC and mitogen-activated protein kinase (MAPK) activation. This effect was also observed by treatment of α-methyl-5-HT and was abolished by treatment of sarpagrelate, a selective 5-HT2A receptor antagonist, suggesting that 5-HT2A receptors mediate these effects. Interestingly, the roles of MAPK activation in COX-2 expression in VSMCs are different apparently depending on different stimulators such as PDGF, angiotensin II and sphingosine 1-phosphate. 5-HT stimulation activates ERK, p38 MAPK and c-Jun N-terminal kinase (JNK) within 30 min. By using several inhibitors, 5-HT-induced Src has been found to activate these MAPKs. PKC activation is upstream of JNK, but not ERK and p38 MAPK. JNK may also be activated by ERK or downstream of ERK. Overall, the activations of Src, PKC and MAPK via 5-HT2A stimulation are each essential for the full expression of COX-2.

PGI2 production and NO production in the vascular wall are well recognized to have important pathophysiological roles in cardiovascular diseases. Besides their vasodilating actions, both NO and PGI2 regulate platelet aggregation and adhesion, leukocyte adhesion, and VSMC proliferation and migration. Thus, 5-HT2A receptor activation seems to have both inhibitory and stimulatory effects on vascular reactivity through regulating NO and PGI2 production. The opposite effect on iNOS and COX-2 expression is also observed when VSMCs are treated with angiotensin II and sphingosine 1-phosphate.

3. FUNCTION AND SIGNALING MECHANISMS IN ENDOTHELIAL CELLS

Recently, signaling pathways by 5-HT stimulation in several primary endothelial cell lines have been revealed in detail by Zamani and Qu. They showed that (1) 5-HT phosphorylated all signaling kinases tested, i.e., Src, phosphatidylinositol 3-kinase (PI3K), protein kinase B (AKT); mammalian target of rapamycin (mTOR); 70-kDa ribosomal protein S6 kinase (p70S6K); ERK and p38 MAPK in human umbilical vein ECs (HUVECs), human aortic ECs (HAECs), human microvascular ECs and human pulmonary artery ECs; (2) 5-HT1B, but not 5-HT1A, 5-HT1D, 5-HT3B, and 5-HT7 receptor activation phosphorylated Src/PI3K/AKT/mTOR/p70S6K/ERK, whereas 5-HT3B and 5-HT7 receptor activation may phosphorylate p38 MAPK; and (3) Src activation, an upstream signaling of PI3K/AKT/mTOR, is mediated by Goi activation of 5-HT1B receptors. 5-HT receptor-coupled Gfip protein mediates p38 MAPK activation.

One of the most important roles of 5-HT in ECs is to activate endothelial NOS (eNOS). In fact, 5-HT is a dominant substance that is released by aggregating platelets and it triggers NO production. NO plays a pivotal role in the protective function of the endothelium against coronary disease. McDuffie et al. showed that Gi-coupled 5-HT1B receptors activate eNOS in bovine aortic ECs (BAECs). Iwabayashi et al. also reported that the 5-HT1B receptor activates eNOS via Akt phosphorylation in HAECs. On the other hand, 5-HT induces eNOS expression via 5-HT2B receptors and ERK activation in HUVECs.

Over the last decade, evidence showing that 5-HT receptor activation in ECs plays a principal role in angiogenesis has accumulated. Angiogenesis is composed of multiple steps that include proliferation, migration, morphogenesis and maturation of ECs. Stimulation of 5-HT1 and/or 5-HT2 receptors has been implicated in the angiogenic effect of 5-HT. 5-HT enhances migration of HAECs through stimulation of 5-HT7 receptors. The authors showed that RhoA and subsequent ERK activation-dependent pathways are involved in the mechanism of migration. Asada et al. suggested that the selective inhibition of 5-HT2B receptors in ECs suppresses tumor angiogenesis through a mechanism involving 5-HT-induced ERK and eNOS inhibition in ECs.

Some reports have shown that the 5-HT4 receptor in ECs is a positive regulator of angiogenesis. The 5-HT4 receptor exhibits a high level of agonist-independent constitutive activity. Mosapride, a selective 5-HT4 receptor agonist, has been shown to inhibit proliferation and migration in HUVEC.
On the other hand, Profirovic et al.\textsuperscript{26} reported that RS 39604, a 5-HT\textsubscript{2A} receptor antagonist, significantly inhibited not only capillary tube formation in HUVECs \textit{in vitro}, but also the migration and adhesion of ECs, through 5-HT\textsubscript{2A} receptor-coupled Gz13 and its downstream, RhoA. Angiogenesis is now widely recognized to play a critical role in tumor growth. Further studies will hopefully lead to the development of new 5-HT-related drugs for cancer treatment.

Several reports have clarified the role of 5-HT\textsubscript{2A} receptors in ECs using a pharmacological approach. Kawano et al.\textsuperscript{27} showed that 5-HT induces the expression of tissue factor and plasminogen activator inhibitor-1 in rat aortic ECs, and that MCI-9042, a selective 5-HT\textsubscript{2A} receptor antagonist, suppresses their expressions. Sarpogrelate inhibits 5-HT-increased VCAM-1 expression in HUVEC cultured in high glucose (27.8 mM) conditions.\textsuperscript{28} Sarpogrelate has also been shown to inhibit high glucose (25 mM)-induced ICAM-1 expression and adherence of monocytes in HUVEC.\textsuperscript{29} The precise signal transduction of 5-HT\textsubscript{2A} activation in ECs is still a topic of debate.

4. DIRECT AND INDIRECT ACTIONS OF SARPOGRELATE ON VASCULAR SMOOTH MUSCLE CELLS AND ENDOTHELIAL CELLS

Sarpogrelate is widely used for patients with ischemic diseases associated with thrombosis. Sarpogrelate inhibits 5-HT responses mediated by 5-HT\textsubscript{2A} receptors, such as platelet aggregation, vasoconstriction, and vascular smooth muscle proliferation.\textsuperscript{30} Recent reports show that sarpogrelate indirectly enhances the action of 5-HT\textsubscript{1B} receptors in ECs \textit{via} selective inhibition of 5-HT\textsubscript{2A} receptors in VSMCs or platelets. Selective blockade of the 5-HT\textsubscript{2A} receptor by sarpogrelate seems to increase the amount of 5-HT which can act on 5-HT\textsubscript{1B} receptors in ECs. Fujita et al.\textsuperscript{31} were the first to show that sarpogrelate increases coronary blood flow by augmentation of cardiac NO production through indirect 5-HT\textsubscript{1B} receptor activation, which resulted from the blockade of 5-HT\textsubscript{2A} receptors in ischemic canine hearts. They also confirmed that ischemia induced the cardiac release of 5-HT from the ischemic myocardium, which was augmented by sarpogrelate. The increased 5-HT may then increase the cardiac release of NO \textit{via} 5-HT\textsubscript{1B} receptors. Increasing blood flow by sarpogrelate treatment was also demonstrated in a hindlimb ischemia model created in severely diabetic mice.\textsuperscript{32} Sarpogrelate enhanced the decreased Akt phosphorylation and eNOS expression in the diabetic mice. Again, these reports suggest that selective inhibition of the 5-HT\textsubscript{2A} receptor indirectly enhances the actions of 5-HT\textsubscript{1B} receptors in ECs, possibly resulting in an increase in NO production and a vasodilator response. Considering that sarpogrelate inhibits 5-HT-induced iNOS suppression in VSMCs\textsuperscript{3,10,11} as mentioned above, sarpogrelate may have the potential to increase NO production \textit{via} both eNOS in ECs and iNOS in VSMCs.

5. INVERSE AGONIST ACTIVITY OF 5-HT\textsubscript{2A} RECEPTOR ANTAGONIST

It has been reported that sarpogrelate has an inverse agonist activity on a constitutively active mutant of the human 5-HT\textsubscript{2A} receptor.\textsuperscript{32,33} Originally, the constitutive activity of 5-HT\textsubscript{2A} and 5-HT\textsubscript{2C} receptors was shown to have some physiological function in the central nervous system \textit{in vivo}.\textsuperscript{34} In fact, the constitutive activity of 5-HT\textsubscript{2A} and 5-HT\textsubscript{2C} receptors has physiological relevance for acquisition of an associative-learning response\textsuperscript{35} and for regulation of dopamine release in rat brain.\textsuperscript{36} In contrast to \textit{in vivo} studies, the constitutive activity of 5-HT\textsubscript{2A} receptors on phospholipase C activity \textit{in vitro} has been shown only in the case of either mutation of the receptor or overexpression of G protein to enhance the constitutive activity. Using a constitutively active mutant of the human 5-HT\textsubscript{2A} receptor, Muntaris et al.\textsuperscript{37} showed that sarpogrelate as well as ketanserin and other 5-HT\textsubscript{1} receptor antagonists reduced the basal total inositol phosphate levels. The authors implied that activating mutations of the 5-HT\textsubscript{2A} receptor may be responsible for disease states such as ischemic heart diseases, and that stabilization of the inactive conformation of the 5-HT\textsubscript{2A} receptor may be a key component of the mechanism of action of sarpogrelate. Yet, it is still unclear if the constitutive active form of the 5-HT\textsubscript{2A} receptor has important roles in physiological or pathological function \textit{in vivo}.

6. CONCLUSION

Recently, a number of studies have revealed the functions and mechanisms, including signal transduction, of 5-HT receptors in vascular walls. However, the effects of 5-HT within the cardiovascular system are still not fully understood, for example, the role of 5-HT in hypertension is still unclear.\textsuperscript{37} A part of the reason for the complication is that the serotonergic neurons in the central nervous system also have an important role in cardiovascular functions. Indeed, the activation of 5-HT\textsubscript{1A} receptors has been shown to have a prominent role in the cardiac autonomic response and subsequent tachycardia, arrhythmias and sudden cardiac death due to acute and/or chronic psychosocial stress in 5-HT\textsubscript{1A} receptor knockout (KO) mice.\textsuperscript{38} Furthermore, the physiological and pathological roles of the constitutive activity of 5-HT receptors \textit{in vivo} are not yet fully understood.

It is expected that increasing evidence on the role of 5-HT receptors will contribute to expansion of the clinical application of existing therapeutic drugs such as sarpogrelate, and to the development of new 5-HT receptor-related drugs for the treatment of cardiovascular diseases. Furthermore, we anticipate the development of new drugs for cancer treatment based on the regulation of 5-HT-induced angiogenesis.

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