5-Hydroxytryptamine (5-HT) is involved in regulation of both physiological and pathophysiological conditions in tissues throughout the body. 5-HT induces vascular smooth muscle constriction in most vessels. The vasoconstrictive effects of 5-HT are mediated by 5-HT_{1B} and 5-HT_{2A} receptors located on the membrane of smooth muscle cells, except in the intracranial arteries which constrict only through 5-HT_{1B} receptors. 5-HT also acts as vasodilator because it releases nitric oxide from endothelial cells. This response is dominantly mediated by 5-HT_{1B} receptors but not by 5-HT_{2A} receptors. In this review, we focus on the action of 5-HT via G protein-coupled 5-HT receptors involved in some vascular-related pathophysiological responses. Furthermore, we describe the possibilities of 5-HT receptors as targets for drug therapy against saphenous vein graft diseases (especially in patients with diabetes mellitus), migraine and pulmonary arterial hypertension.

Key words  serotonin; migraine; pulmonary hypertension; saphenous vein graft disease

1. INTRODUCTION

5-Hydroxytryptamine (5-HT, serotonin) was initially isolated from bovine serum as a substance with a vasoconstrictor effect. The name “serotonin” consists of two parts. “Sero-” which means blood serum, and “-tonin” which means modifying the tone of blood vessels. 5-HT plays crucial roles in both physiological and pathophysiological responses everywhere in the body. More than 90% of the 5-HT is synthesized and stored in enterochromaffin cells in the gut, where it regulates gastrointestinal motility. 5-HT secreted from enterochromaffin cells to the bloodstream is incorporated into platelets through the serotonin transporter. A small portion of internal 5-HT is also enclosed within synaptic vesicles of central serotonergic neurons as a neurotransmitter.

Signaling of 5-HT is transmitted via receptors located on the plasma membrane of vascular smooth muscle cells, platelets, neurons and many other cell types. There are seven major families of 5-HT receptors (5-HT_{1A}–5-HT_{7}). Except for the 5-HT_{3} receptor, which is a ligand gated ion channel, most 5-HT receptors are heterotrimeric G protein-coupled, heptahedral proteins. The binding of 5-HT to one of these receptors induces a conformational change that activates the receptor-coupled G protein and modulates the subsequent signaling molecules.

This review describes the action of 5-HT via G protein-coupled 5-HT receptors involved in several vascular-related pathophysiological responses. Furthermore, we discuss the possibility of 5-HT receptors as potential targets for drug therapy against saphenous vein graft diseases, especially in patients with diabetes mellitus, migraines, and/or pulmonary hypertension.

2. SUBTYPES OF G PROTEIN-COUPLED 5-HT RECEPTORS INVOLVED IN LOCAL CONTROL OF VASCULAR TONE

There are at least 15 separate 5-HT receptor subtypes, which are grouped into 7 families based on the subsequent signaling molecules. Among them, the 5-HT_{1A} and 5-HT_{2} families are found in blood vessels and are involved in vascular constriction. Acute vascular constriction by 5-HT can be induced by 5-HT_{1B} and 5-HT_{2A} receptor activation, except in intracranial arteries which are constricted only by 5-HT_{1B} receptor-mediating signals. In general, 5-HT-induced cerebral and peripheral vascular constriction is dominantly mediated via 5-HT_{1B} and 5-HT_{2A} receptors, respectively.

2.1. 5-HT_{1B} Receptors  The family of 5-HT_{1B} receptors couple to Bordetella pertussis toxin-sensitive G proteins (G_{i}/G_{o}) to initiate signal transduction pathways by inhibition of adenylyl cyclase. There are five subtypes of cloned 5-HT_{1B} receptors, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E} and 5-HT_{1F}. 5-HT_{1C} was reclassified as the 5-HT_{3C} because it was found to have more in common with the 5-HT_{3} family receptors after...
the receptor had been cloned.\textsuperscript{13) 5-HT}\textsubscript{1B} receptors are widely distributed throughout the body. In the central nervous system, the 5-HT\textsubscript{1B} receptors are present on serotonergic nerve endings and regulate 5-HT release from the nerve ending itself (autoreceptors). This subtype could be involved in the control of mood, motor function and cognition.\textsuperscript{14,15) 5-HT}\textsubscript{1B} receptors also exist on the smooth muscle membrane of blood vessels and mediate 5-HT-induced vasoconstriction in human coronary artery,\textsuperscript{16) human pulmonary artery,\textsuperscript{17) human cerebral artery,\textsuperscript{18) and various blood vessels in humans and other species.\textsuperscript{2,10,19) 5-HT}\textsubscript{1B} receptors can mediate vasoconstriction of intra- and extracranial arteries, therefore, selective 5-HT\textsubscript{1B/1D} agonists, generally called triptans, are used as antimigraine agents. This matter is discussed in greater detail in the next chapter. 5-HT\textsubscript{1A} receptors are mainly located in the central nervous system.\textsuperscript{20) There is a lack of evidence for the localization of this subtype of receptor in the cardiovascular system, accompanied by a lack of evidence for its direct effects on the heart and the vasculature.

2.2. 5-HT\textsubscript{2A} Receptors The 5-HT\textsubscript{2} receptor group consists of 3 different subtypes, 5-HT\textsubscript{2A}, 5-HT\textsubscript{2B} and 5-HT\textsubscript{2C}.\textsuperscript{29) The 5-HT\textsubscript{2A} receptors activate phospholipase C (PLC) through \(G_\text{q/11}\) and lead to an accumulation of inositol triphosphate (IP\textsubscript{3}) and diacylglycerol, which in turn increases intracellular \(Ca^{2+}\) concentrations and activates protein kinase C, respectively.\textsuperscript{20,21) Ga (q/11) also activates Dbl-family guanine nucleotide exchange factor (RhoGEF), which enhances signals downstream of RhoA.\textsuperscript{22,23) PLC/IP\textsubscript{3} signaling and RhoGEF/RhoA signaling evoke vasoconstriction through the activation of myosin light chain kinase and inhibition of myosin light chain phosphatase, respectively.\textsuperscript{24)}

A previous study using reverse transcription polymerase chain reaction (RT-PCR) found 5-HT\textsubscript{2A} receptor mRNA in smooth muscle cells of human pulmonary artery and aorta, and 5-HT\textsubscript{2A} receptors mediate the vasoconstrictive responses to 5-HT\textsuperscript{25) Ketanserin, a 5-HT\textsubscript{2A} receptor antagonist, induces pulmonary vasodilation and inhibits 5-HT-induced pulmonary artery vasoconstriction in fetal sheep.\textsuperscript{26)}

2.3. 5-HT Mediated Vasodilation 5-HT acts not only as a vasoconstrictor but also as a vasodilator. 5-HT\textsubscript{1B} receptors are located in endothelial cells, and stimulation of this receptor induces vasodilation through the production of nitric oxide (NO).\textsuperscript{21) However, vascular endothelial cells may not have 5-HT\textsubscript{2A} receptors, because 5-HT\textsubscript{2A} receptor mRNA was not found in these cells.\textsuperscript{27) Intra-arterial infusion of 5-HT or the selective 5-HT\textsubscript{1B/1D} receptor agonist L-694247 was reported to produce vasodilation in the hindquarters of anesthetized rats.\textsuperscript{27) These effects may be explained by endothelium-dependent vasodilation. However, the NO synthase inhibitor N(G)-nitro-L-arginine (L-NAME) did not block while the selective \(\beta_2\)-adrenoceptor antagonist ICI 118551 and bilateral adrenalectomy did block the vasodilation induced by the 5-HT infusion. It has been suggested that local 5-HT\textsubscript{1B/1D} activation in the sympathetic nervous system stimulates the release of adrenaline, which then cause relaxation of vascular smooth muscle mediated by \(\beta_2\)-adrenoceptor stimulation.\textsuperscript{21) Additionally, chronic infusion of 5-HT produced a persistent fall in blood pressure in both deoxycorticosterone acetate (DOCA)-salt hypertensive rats and sham normotensive rats, which was contrary to expectations.\textsuperscript{28) It has been suggested that 5-HT\textsubscript{1B} (also 5-HT\textsubscript{2B} and 5-HT\textsubscript{3}) receptors may be involved in the dilation of peripheral arteries.\textsuperscript{29) However, activation of these receptors using a specific agonist did not result in relaxation, at least not in the endothelium-intact superior mesenteric artery.\textsuperscript{30) 5-HT acts as a vasoconstrictor or vasodilator in different vascular beds depending on differences in the 5-HT receptor distribution in each vascular smooth muscle, surrounding vessel tissue, and control system for vascular tone including autonomic nerves. In vivo studies using anesthetized dogs have demonstrated that intrarenal administration of 5-HT could induce a transient decrease and then a sustained increase in renal blood flow.\textsuperscript{30–32) 5-HT thus exerts complex effects on vascular tone-related hemodynamics in a variety of animals.

3. MIGRAINE

Migraine is a chronic, recurrent and disabling primary headache disorder. The one-year period prevalence for migraine differs slightly among countries. It is approximately 11.7% in the United States,\textsuperscript{33) 8.4% in Japan\textsuperscript{34) and 10.6% in Germany.\textsuperscript{35) Interestingly, the prevalence of migraines in women is about three-times higher than in men in all countries.\textsuperscript{32,35) In clinical practice, a headache is diagnosed according to International Classification of Headache Disorders (ICDH-II) criteria\textsuperscript{36) after screening for musculoskeletal disorders and psychiatric comorbidity, and patients with headache are provided with treatment according to clinical guidelines.

The pathophysiology of migraine is still not completely understood. However, extracranial, dural and/or pial arterial dilation are considered pivotal in causing migraine headache.\textsuperscript{37,38) Triptans, which are selective 5-HT\textsubscript{1B/1D} receptor agonists, were developed as cranial vasoconstrictors due to their efficacy on 5-HT\textsubscript{1B} receptors.\textsuperscript{39) The vasoconstriction of both human isolated middle meningeal and temporal arteries induced by sumatriptan, the first commercially available triptan preparation, was blocked by the selective 5-HT\textsubscript{1B} receptor antagonist SB224289.\textsuperscript{40) In contrast, BRL15572, a selective 5-HT\textsubscript{1D} receptor antagonist, had little or no effect on sumatriptan-induced vasoconstriction of these arteries.\textsuperscript{40) Similar results are shown in carotid artery in dogs.\textsuperscript{41) Therefore, the direct vasoconstrictive effect of sumatriptan, and perhaps also the other triptan preparations, is mediated by 5-HT\textsubscript{1B} receptors and not by 5-HT\textsubscript{1D} receptors.

Triptans act not only on the paracranial arteries but also on the trigeminal nerve and its nervous connections. Based on a large number of studies over the past two decades, neuropeptide calcitonin-gene-related peptide (CGRP) has been suggested to play crucial roles in the pathophysiological events of migraine.\textsuperscript{42) Trigeminal nerve excitation induces CGRP and substance P release, which results in neurogenic inflammation with plasma protein extravasation.\textsuperscript{43) Brain vasculature receives innervation from the trigeminal nerve ganglion. CGRP is released from trigeminal ganglion neurons at the cranial artery and meninges, and then induces vasodilation which is found in the migraine attack.\textsuperscript{44) Triptans may inhibit the release of CGRP and substance P by stimulation of 5-HT\textsubscript{1D} receptors at the trigeminal nerve endings, and block the succeeding migraine-inducing pathophysiological changes.\textsuperscript{45) It is now known that triptans have modest affinity for 5-HT\textsubscript{1F} receptors in addition to their affinity for 5-HT\textsubscript{1B/1D} receptors.\textsuperscript{46) 5-HT\textsubscript{1F} receptors are located at trigeminal nerve...
endothelins as well as at 5-HT\textsubscript{1D} receptors. Furthermore, it is speculated that stimulation of 5-HT\textsubscript{1D} receptors may play a significant role, at least in part, in the antimigraine efficacy of triptans. Therefore, selective 5-HT\textsubscript{1B} receptor agonists are being developed as novel antimigraine drugs.\textsuperscript{37,67} Needless to say, triptans could increase cardiovascular risk because these drugs induce peripheral vasoconstriction mediated by 5-HT\textsubscript{1B} receptors.\textsuperscript{40} Indeed, package inserts state that triptans are contraindicated for use in patients with cardiac infarction, coronary spasm, uncontrolled hypertension, cerebral vascular disturbance, or peripheral vascular disorders. Thus, it is expected that selective 5-HT\textsubscript{1B} receptor agonists will become safe antimigraine agents for treating patients with cardiovascular risk factors.

4. PULMONARY ARTERIAL HYPERTENSION

Pulmonary arterial hypertension (PAH) develops both as a complication of other diseases and as a primary disease for which no underlying cause can be found.\textsuperscript{49} The features of PAH include pulmonary artery pressure (PAP) $>25$ mmHg, pulmonary vascular resistance (PVR) $>3$ Wood units, and pulmonary wedge pressure $<15$ mmHg. Elevation of either PAP or PVR causes endothelial dysfunction, resulting in vasoconstriction, remodeling of arterial walls, and formation of arterial microthrombi.\textsuperscript{50}

In the pathogenic mechanisms of PAH, 5-HT could play crucial roles in either hyperplasia (occlusion) or vasoconstriction of pulmonary arteries. A case report showed that plasma 5-HT levels increased in a patient with PAH.\textsuperscript{51} Several studies have indicated that 5-HT promotes proliferation of pulmonary artery smooth muscle cells leading to an increase in PVR. However, this 5-HT-caused mitogenesis of smooth muscle cells may be induced by intracellular uptake of 5-HT through the membrane 5-HT transporter (called SERT) rather than its action on G-protein-coupled receptors.\textsuperscript{52,53} In this review, we focus on the vasoconstrictive effect of 5-HT mediated via G-protein-coupled 5-HT receptors.

It has been reported that the increased vasoconstrictor response to 5-HT in pulmonary hypertension model rats is due to an increase in 5-HT\textsubscript{2A} receptor-mediated contraction.\textsuperscript{54} Administration of sarpogrelate (for 3 weeks, intraperitoneal (i.p.)), a specific 5-HT\textsubscript{2A} receptor antagonist, suppressed the development of monocrotaline-induced PAH with severe pulmonary vascular remodeling and right heart failure in rats.\textsuperscript{55} Thus, 5-HT\textsubscript{2A} receptors are expected to be a therapeutic target of PAH. At present, several 5-HT\textsubscript{2A} selective antagonists are undergoing clinical trials for the treatment of PAH.\textsuperscript{56} On the other hand, the 5-HT\textsubscript{1B} receptor selective antagonist SB224289 significantly reduced the expression of S100A4/Mts, a calcium-binding protein which increases in patients with pulmonary vascular diseases, in cultured human pulmonary artery smooth muscle cells (hPA-SMC).\textsuperscript{57} Moreover, GR55562, a 5-HT\textsubscript{1B/1D} receptor selective antagonist, more potently inhibits the vasoconstriction of the ring segments of human pulmonary arteries than ketanserin.\textsuperscript{57} 5-HT\textsubscript{1B} receptors, like 5-HT\textsubscript{2A} receptors, could therefore be an interesting target for the treatment of PAH. 5-HT\textsubscript{1B} receptor antagonists may also induce a reduction in NO from pulmonary endothelium. Thus, we believe it is difficult to use selective 5-HT\textsubscript{1B} receptor antagonists as anti-PAH drugs.

5. SAPHENOUS VEIN GRAFT DISEASES ASSOCIATED WITH CORONARY ARTERY BYPASS GRAFTING OPERATION

Coronary artery bypass grafting (CABG) is a useful treatment for severe coronary arteriosclerosis which is the leading cause of cardiac death. The saphenous vein (SV) continues to be the most commonly used conduit vessel for CABG because of its ready availability and suppleness.\textsuperscript{59} Unfortunately, grafted SV eventually develop high-grade stenosis or occlusion in nearly half of the patients who have undergone CABG.\textsuperscript{59} These problems are called saphenous vein graft (SVG) diseases and have a critical impact on the life prognosis of the patients. Therefore, finding ways to prevent SVG diseases is a pressing issue for medical researchers.

There are three major pathological changes found in SVG diseases, e.g., thrombosis, intimal hyperplasia and atherosclerosis. The process of thrombogenesis is initiated by platelet activation, following which several mediators, including 5-HT, are released from the activated platelets. Released 5-HT induces platelet aggregation and vasospasm of the grafts.\textsuperscript{60,61} In CABG using a SV graft, it has been established that, before implantation into the coronary arterial circulation, the segment used as a vein graft is distended by high intraluminal pressures with injection of saline or a patient’s heparinized blood to check for leaks and to increase the vascular diameter. Thus, the SV graft did not retain functional endothelium. In fact, we observed that acetylcholine failed to induce vasodilation of the SV segments preconstricted with noradrenaline.\textsuperscript{62} Furthermore, light and immunohistochemical microphotographs of the SV segment demonstrated that the venous wall was mainly composed of smooth muscle cells, and almost all endothelial cells were desquamated.\textsuperscript{62} Thus, the endothelial 5-HT\textsubscript{1B} receptor-mediated vasodilation may not occur and a selective 5-HT\textsubscript{1B} receptor antagonist may act as merely a vaso-dilator in the SV graft. In our previous study, sarpogrelate or the 5-HT\textsubscript{1B} receptor antagonist SB224289 partially inhibited, and the combination of these antagonists almost abolished the 5-HT-induced vasoconstriction in human SVs.\textsuperscript{62} Thus, concurrent use of 5-HT\textsubscript{2A} and 5-HT\textsubscript{1B} antagonists may potentiate postoperative 5-HT-induced spasm of SV grafts in patients who have undergone CABG.

Diabetes mellitus (DM) is a metabolic disorder principally characterized by elevated blood glucose levels accompanied by micro- and macrovascular complications. The rate of cardiovascular events is 2- to 8-fold higher in diabetic subjects than in non-diabetic subjects of a combined age, sex and living environment.\textsuperscript{63,64} Furthermore, increasing risk for cardiovascular death depends on the diabetic duration.\textsuperscript{65} It is well known that DM can be a risk factor for the perioperative complications in CABG.\textsuperscript{66,67} SV grafts harvested from diabetic individuals were significantly more responsive to 5-HT than those harvested from non-diabetic individuals.\textsuperscript{68} Insulin may play a key role in causing the large difference in the response of vascular smooth muscle to 5-HT between diabetic and non-diabetic individuals. Smooth muscle contraction and relaxation are regulated by the phosphorylation and dephosphorylation of myosin light chain (MLC\textsubscript{20}) at Thr\textsuperscript{18} and Ser\textsuperscript{19} by myosin light chain kinase and myosin light chain phosphatase (MLCP). These enzymes are downstream of 5-HT\textsubscript{2A} receptor signaling. Enzymatic activity of MLCP, which is involved in the Ca$^{2+}$
sensitivity of smooth muscle cells, is decreased by phosphorylation of its regulatory subunit MYPT1 at Thr\(^{696}\) or Thr\(^{853}\) through the RhoA/Rho kinase pathway, and phosphorylation of the small inhibitor protein CPI-17 at Thr\(^{38}\) by PKC.\(^{69–72}\) We have recently reported that (1) 5-HT-induced constriction of human endothelium-denuded SVs was greater in DM patients than in non-DM patients, (2) the total protein level of MYPT1 was significantly lower in the DM group than in the non-DM group, and (3) the ratio of P(Thr\(^{696}\) )-MYPT1 to total MYPT1 was significantly higher in the DM group than in the non-DM group.\(^{24}\) These results suggest that the hyperreactivity to 5-HT in the SV smooth muscle of patients with DM is due to not only enhanced phosphorylation of MLCP but also to a deficiency in the protein level of MLCP. Thus, we have revealed for the first time that the deficiency in the protein level of MLCP in the DM group can partially explain the poor patency of SVG grafts harvested from patients with DM.\(^{24}\) Our findings are consistent with a previous report in which insulin inactivated Rho kinase and increased MLC\(_{20}\) dephosphorylation in primary-cultured vascular smooth muscle cells from rat aorta.\(^{73}\) Therefore, at least one reason for enhancement of sensitivity in primary-cultured vascular smooth muscle cells from diabetic patients than in non-DM patients, (2) the total protein level of MYPT1 was significantly lower in the DM group than in the non-DM group, and (3) the ratio of P(Thr\(^{696}\) )-MYPT1 to total MYPT1 was significantly higher in the DM group than in the non-DM group.\(^{24}\) These results suggest that the hyperreactivity to 5-HT in the SV smooth muscle of patients with DM is due to not only enhanced phosphorylation of MLCP but also to a deficiency in the protein level of MLCP. Thus, we have revealed for the first time that the deficiency in the protein level of MLCP in the DM group can partially explain the poor patency of SVG grafts harvested from patients with DM.\(^{24}\) Our findings are consistent with a previous report in which insulin inactivated Rho kinase and increased MLC\(_{20}\) dephosphorylation in primary-cultured vascular smooth muscle cells from rat aorta.\(^{73}\) Therefore, at least one reason for enhancement of the response to 5-HT in isolated SVs from diabetic individuals could be explained by a reduction in insulin-mediated MLCP activity.

Insulin itself may affect 5-HT receptors on vascular smooth muscle cells. It has been reported that insulin evokes internalization of β\(_2\)-adrenceptors, which are members of the G-protein-coupled receptor superfamily, from the membrane surface and counterregulates catecholamine action.\(^{74–76}\) Therefore, we prepared HEK293 cells which have yellow fluorescent protein-conjugated 5-HT\(_{2A}\) receptors (5-HT\(_{2A}\)-YFP) to investigate the signaling crosstalk between insulin receptors and 5-HT receptors. Using these cells, we observed that 5-HT\(_{2A}\) receptors are internalized within 10 min after stimulation with insulin.\(^{77}\) We recently reported that insulin induced vasodilation in a concentration-dependent manner in endothelium-denuded human SVs that had been preconstricted with 5-HT.\(^{78}\) Furthermore, insulin caused internalization of 5-HT\(_{2A}\) receptors also in a concentration-dependent manner. A large number of studies suggest that insulin induces vasodilation via the production of NO in endothelial cells and a reduction of Ca\(^{2+}\) concentrations in vascular smooth muscle cells.\(^{79–83}\) However, we observed insulin-induced vasodilation in endothelium-denuded and 5-HT-preconstricted vessels. Therefore, the internalization of 5-HT\(_{2A}\) receptors from the plasma membrane contributes, at least in part, to the vasodilator effect of insulin. Based on these findings, we firmly believe that specific 5-HT\(_{2A}\) receptor antagonists could be useful preventive agents for SVG diseases in patients with diabetes.

6. CONCLUSION

To date from the discovery of serotonin, many medicinal agents associated with 5-HT receptors have been developed and administered to patients with various diseases, especially cardiovascular and mental disorders. These agents have saved a great number of patients from suffering and/or disease progression. In the future, further clarification of the distributions and roles of each subtype of 5-HT receptor is needed. In addition, more selective agonists or antagonists will be required to prevent the onset of side effects.

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