Serotonin Signaling in Pulmonary Hypertension

Mark de Caestecker

Serotonin (5-HT, 5-hydroxytryptamine) has long been recognized as one of the most potent naturally occurring pulmonary vasoconstrictors. It was first implicated in the pathogenesis of pulmonary arterial hypertension (PAH) after an outbreak of the disease in Switzerland in the 1960’s among patients taking aminorex fumarate, an appetite suppressant that inhibits serotonin uptake by platelets. Since that time further outbreaks of PAH have been identified in Europe and the USA associated with the use of fenfluramine-derivate anorexigens, eventually leading to their withdrawal from the world market in 1997. Although this was, at least in retrospect, a predictable tragedy, it has ironically opened avenues of research into the biology of serotonin signaling in PAH. As fenfluramine-derivatives are substrates for the serotonin transporter (5-HTT, SERT) proteins, this suggests that abnormal SERT expression or functional activity could play a role in the pathogenesis of PAH. There is now a body of evidence supporting this hypothesis that provides hope for the development of effective therapeutic strategies targeting specific components of this signaling pathway in patients with these diseases.

Most of the serotonin produced in the body is secreted by enterochromaffin cells of the intestine into the portal circulation where it is partially metabolized by the liver. However, levels of free circulating serotonin are maintained in the low nanomolar range through energy-dependent SERT-mediated transport into platelets. This led some researchers to hypothesize that fenfluramines might cause PAH by increasing free plasma levels of serotonin. However, this hypothesis is inconsistent with the observation that chronic treatment with fenfluramine-derivatives if anything reduces plasma levels of serotonin. This suggests that other SERT-related effects promote PAH in susceptible patients. This is supported by the observation that patients with idiopathic PAH have increased frequency of the so called L-type polymorphism in the SERT promoter, which is associated with increased SERT expression and activity in platelets and pulmonary artery smooth muscle cells (PASMCs). These studies have recently come under fire as more extensive analyses have failed to confirm an association between the L-genotype and idiopathic or familial PAH. Nonetheless, subgroup analysis suggests that the homozygous LL-SERT genotype is associated with early onset of disease in patients with familial PAH. Furthermore, analysis of cultured PASMCs indicates that SERT expression is regulated independently of the L-genotype but correlates with severity of pulmonary hypertension (PH) in patients with secondary PH associated with other cardiopulmonary diseases. These findings support the concept that increased SERT expression may be a mechanism or indicator of disease progression in patients with different forms of PH.

Experimental data support the hypothesis that alterations in SERT expression could play a role in the pathogenesis of PH. SERT is expressed in pulmonary vascular endothelial and smooth muscle cells. Furthermore, serotonin induces proliferation of cultured PASMCs and adventitial fibroblasts, and this effect is inhibited by incubation with serotonin re-uptake inhibitors (SSRIs). These agents bind with high affinity to SERT proteins and block serotonin uptake, suggesting that the mitogenic effects of serotonin are dependent on SERT. There is also evidence that SERT plays a role in the pathogenesis of experimental PH. Treatment with SSRIs abrogates PH in chronically hypoxic mice and rats with monocrotaline-induced PH. Furthermore, mice carrying null mutations at the SERT locus are protected from the development of PH associated with prolonged hypoxia. The latter findings are complicated, as the same study shows that acute hypoxic pulmonary vasoconstriction is enhanced in SERT mutant mice. These findings are supported by the observation that SSRIs potentiate serotonin-dependent pulmonary vascular contractility in Fawn Hooded rats, and suggest that SERT has opposing effects in acute versus chronic phases of hypoxic PH. To evaluate the effects of SERT overexpression, YAC transgenic mice carrying extra copies of the SERT gene (along with all of its normal cis-acting regulatory elements) have been used to overexpress SERT in its endogenous distribution domains. These mice develop spontaneous PH and more severe pulmonary vascular remodeling in response to chronic hypoxia. These findings support the hypothesis that SERT overexpression plays a role in exacerbating pulmonary vascular disease in patients with PAH, but do not discriminate between pulmonary versus systemic effects of SERT overexpression. This is of importance as there is evidence that PH in Fawn Hooded rats is caused by an inherited defect in platelet serotonin storage. In addition there is a case report of a patient with familial platelet storage disease developing PAH associated with increased circulating levels of serotonin. These observations suggest that defective serotonin transport by platelets could play a role in regulating pulmonary vascular responses and, conversely, raise the question as to whether overexpression of SERT in the pulmonary vasculature plays any role in the pathogenesis of PAH.

The article by Guignabert and colleagues in this issue of Circulation Research provides the first direct evidence that SERT overexpression in the vasculature can promote pulmonary vascular remodeling. In these studies, the authors use a 2.2-kb
fragment of the mouse SM-22 promotor to selectively drive transgenic expression of SERT in pulmonary (and systemic) arterial smooth muscle cells. These mice develop spontaneous PH along with progressively severe pulmonary vascular remodeling and proliferation. They also show increased susceptibility to PH induced by chronic hypoxia and treatment with an active (in mice) monocrotaline-derivative. These findings are consistent with observations in SERT null and SERT YAC transgenic mice, and suggest that overexpression of SERT in the vascular smooth muscle cells may account for SERT-dependent effects in the pulmonary vasculature described in these earlier studies. The precise mechanism by which SERT mediates these effects remains unclear. However, the authors do show that SM-22 5-HTT transgenic mice have decreased expression of two voltage-gated potassium channels, Kv1.5 and Kv2.1, in the lung. These findings are supported by recent data demonstrating SSRI (fluoxetine) sensitive downregulation of Kv channel activity by serotonin in rat PASMCs. As downregulation of Kv channels protects against apoptosis in pulmonary vascular smooth muscle cells, these findings could account for the increased pulmonary vascular remodeling seen in these mice.

These studies leave unanswered questions regarding the signaling pathways responsible for PH associated with SERT overexpression. Underlying these issues is an ongoing debate on the relative contributions of SERT versus serotonin receptor signaling in mediating pulmonary vascular effects of serotonin. Studies from the Eddahibi and Adnot laboratory suggest that SERT signaling is both necessary and sufficient to mediate serotonin-induced proliferation of pulmonary vascular smooth muscle cells. Furthermore, in vivo studies from the same group indicate that SERT signaling accounts for most, if not all, of the serotonin-dependent remodeling and vascular smooth muscle proliferation seen in hypoxic and monocrotaline-induced models of PH. In contrast, other groups have shown that the same in vitro and in vivo responses can be modified through pharmacological or genetic inhibition of different serotonin receptors, including 5-HT1B/D, 5-HT2A, or 5-HT2B. These effects may result from cross-talk between signaling pathways that are activated independently by serotonin at the cell surface could enhance 5-HT1B/D signaling and promote Rho kinase-dependent vasoconstriction.

For example, recent studies have demonstrated functional evaluation of serotonin receptors in the pulmonary vasculature has yet to be performed (more than 15 serotonin receptors have been identified). In addition, the impact of other non-serotonin signaling pathways has to be taken into account as these are likely to have an impact on the balance of serotonin-dependent responses in the intact vasculature. For example, recent studies have demonstrated functional interaction between serotonin and BMP signaling in PH. Mice carrying heterozygous null mutations of the BMP type II receptor exhibit increased pulmonary vascular contractility in response to serotonin. This is associated with enhanced serotonin-induced proliferation and phosphorylation of p42/44 ERK MAP Kinases. However, serotonin-induced proliferation also requires activation Rho Kinase by the 5-HT1B/D receptors. This enables nuclear translocation of ERK and activation of GATA4-dependent transcriptional pathways involved in promoting cell proliferation. As Rho kinase activation also promotes hypoxic pulmonary vasoconstriction, these findings suggest a mechanism that could explain the observation that loss of SERT expression in SERT null mutant mice enhances acute hypoxic pulmonary vasoconstriction. In the absence of the serotonin transporter, increased levels of serotonin at the cell surface could enhance 5-HT1B/D signaling and promote Rho kinase-dependent vasoconstriction.

While these observations provide a model to illustrate the nature and functional impact of cross talk between these signaling pathways, the complexity of these interactions is only beginning to be explored. Detailed mapping and functional evaluation of serotonin receptors in the pulmonary vasculature has yet to be performed (more than 15 serotonin receptors have been identified). In addition, the impact of other non-serotonin signaling pathways has to be taken into account as these are likely to have an impact on the balance of serotonin-dependent responses in the intact vasculature. For example, recent studies have demonstrated functional interaction between serotonin and BMP signaling in PH. Mice carrying heterozygous null mutations of the BMP type II receptor exhibit increased pulmonary vascular contractility in response to serotonin. This is associated with enhanced serotonin-induced proliferation and phosphorylation of p42/44 ERK MAP Kinases in isolated PASMCs, indicating that defective BMP signaling enhances serotonin-dependent responses in these cells. To complicate matters further, there is evidence that the nature of serotonin receptor and transporter signaling may differ significantly in cells derived from different species. This is exemplified by the recent observation that phosphorylation of ERK MAPK in human PASMCs is dependent on 5-HT1B/D, whereas nuclear translocation of

Schematic representation of signaling pathways and cross-talk between SERT and the serotonin receptor 5-HT1B/D involved in regulating serotonin induced proliferation of bovine PASMCs.
phosphorylated ERK is dependent on the generation of ROS by SERT. This contrasts with studies outlined above using bovine PASMCs (Figure). These findings serve to illustrate the fact that we still have a long way to go before we can pretend to understand the complexity of serotonin signaling in the context of human disease. However, the body of published work on serotonin signaling along with the elegant in vivo studies reported in this issue of the Journal establishes the basic paradigms of serotonin signaling that are likely to be involved in the pathogenesis of pulmonary vascular disease in patients with PH.

Acknowledgments

The author thanks Regina Day from The Uniformed Services University of the Health Sciences for comments and assistance in formulating this manuscript.

References


Key Words: serotonin transporter (SERT5-HTT) ■ pulmonary arterial hypertension ■ vascular smooth muscle cells ■ signal transduction
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Circ Res. 2006;98:1229-1231
doi: 10.1161/01.RES.0000225927.04710.33
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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