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A role for the serotonin transporter in hypoxia-induced pulmonary hypertension

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Commentary

Serotonin (5-hydroxytryptamine; 5-HT) is one of many vasoactive substances postulated to participate in the development of hypoxia-induced pulmonary hypertension. Pulmonary vasoactive responses to hypoxia are intensified by 5-HT (1), but attempts to block hypoxia-induced pulmonary hypertension with 5-HT receptor antagonists have met with mixed success. Furthermore, it has been difficult to establish a causal relationship between 5-HT and the physiological response to hypoxia. Lacking in much of this work is a clear distinction between two classes of molecules, 5-HT transporters and 5-HT receptors, either or both of which may participate in the response of pulmonary vascular smooth muscle cells to 5-HT. High levels of 5-HT have been associated with pulmonary hypertension in several systems. Herve et al. (2) described a patient with pulmonary hypertension who had high levels of circulating 5-HT due to a platelet storage disease, and other individuals have been described with primary pulmonary hypertension associated with elevated serum 5-HT levels (3). In the fawn-hooded rat, an animal model for platelet storage disease that exhibits high circulating levels of 5-HT, mild hypoxia also leads to pulmonary hypertension (4, 5). Finally, pulmonary hypertension is also associated with appetite suppressants, such as fenfluramine or dexfenfluramine, which block reuptake of 5-HT. Transduction of 5-HT signals through the serotonin transporter and the 5-HT receptors Several subtypes of signal-transducing 5-HT receptors have been […]

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Serotonin (5-hydroxytryptamine; 5-HT) is one of many vasoactive substances postulated to participate in the development of hypoxia-induced pulmonary hypertension. Pulmonary vasoactive responses to hypoxia are intensified by 5-HT (1), but attempts to block hypoxia-induced pulmonary hypertension with 5-HT receptor antagonists have met with mixed success. Furthermore, it has been difficult to establish a causal relationship between 5-HT and the physiological response to hypoxia. Lacking in much of this work is a clear distinction between two classes of molecules, 5-HT transporters and 5-HT receptors, either or both of which may participate in the response of pulmonary vascular smooth muscle cells to 5-HT.

High levels of 5-HT have been associated with pulmonary hypertension in several systems. Herve et al. (2) described a patient with pulmonary hypertension who had high levels of circulating 5-HT due to a platelet storage disease, and other individuals have been described with primary pulmonary hypertension associated with elevated serum 5-HT levels (3). In the fawn-hooded rat, an animal model for platelet storage disease that exhibits high circulating levels of 5-HT, mild hypoxia also leads to pulmonary hypertension (4, 5). Finally, pulmonary hypertension is also associated with appetite suppressants, such as fenfluramine or dexfenfluramine, which block reuptake of 5-HT.

Transduction of 5-HT signals through the serotonin transporter and the 5-HT receptors

Several subtypes of signal-transducing 5-HT receptors have been characterized pharmacologically and cloned (see review in ref. 6). Depending on their subtype, these receptors may act on G-proteins and thereby activate phospholipase C or adenylate cyclase (6). By analogy with other signaling molecules, it is generally assumed that these receptors operate at the cell surface, without necessarily mediating the uptake of 5-HT. In addition, 5-HT may be internalized into a variety of cell types, including platelets, neurons, mast cells, endothelial cells, and smooth muscle cells, through an active transport mechanism that is powered by a transmembrane Na^+/Cl^- gradient (7). Transporter genes have been cloned in rat, human, mouse, bovine, and Drosophilia (8–13). The serotonin transporter (SERT) belongs to a large family of sodium chloride–dependent γ-aminobutyric acid/norepinephrine transporters. The precise relationship between 5-HT receptors and the transporter has never been adequately defined. Some cells appear to contain receptors, others transporters, and some both.

Both bovine pulmonary artery endothelial and smooth muscle cells actively transport 5-HT (14, 15), and this transport is enhanced by exposure of cells to hypoxia (15, 16). Hypoxia also activates the mitogen-activated protein (MAP) kinase (17), thereby inducing c-fos transcription, which leads to activation of hypoxia-inducible factor-1 (HIF-1) (18). In the bovine pulmonary artery smooth muscle cell, only one 5-HT receptor has been identified, which resembles the 5-HT1A or 5-HT4 receptor (19, 20). Stimulation of this receptor in the presence of a phosphodiesterase inhibitor elevates cAMP and inhibits cellular proliferation (21). On the other hand, in the absence of phosphodiesterase inhibition, cellular cAMP does not rise, and 5-HT transport predominates over transmembrane signaling, resulting in cellular proliferation and hypertrophy at concentrations of 5-HT in the micromolar range (22). Monoamine oxidase inhibitors, which block the intracellular degradation of 5-HT, augment this proliferative response. Thus, in this case, it appears that the intracellular accumulation of 5-HT drives the proliferative process, since it is doubtful that monoamine oxidase affects 5-HT metabolism at the cell surface.

5-HT signaling through SERT also acts through a variety of other signaling molecules — tyrosine phosphorylation of GTPase-activating protein, Ras activation, activation of NAD(P)H oxidase to produce reactive oxygen species (ROSs), and activation of ERK1/ERK2

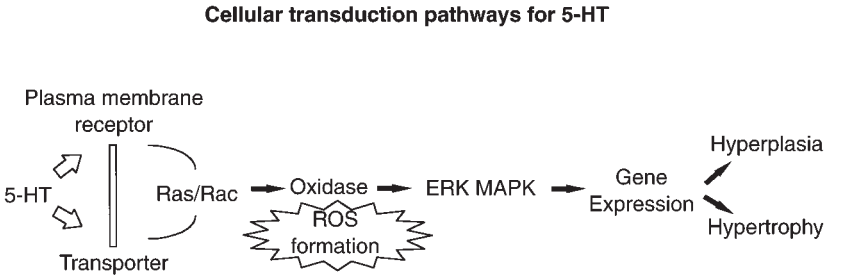

Figure 1

Stimulation begins with contact between 5-HT and either a transporter or receptor, which may lead to activation of Ras or Rac or both. The signal then activates a NAD(P)H oxidase that has not been fully characterized, leading to release of reactive oxygen species (ROS) and the activation of the ERK1 or ERK2 MAP kinases. The consequent changes in gene expression culminate in both cellular hyperplasia and hypertrophy.

(extracellular signal-regulated kinase-1/-2) MAP kinase — to induce smooth muscle cell hyperplasia or hypertrophy (Figure 1) (23–25). These signals occur only in intact cells, not in cellular homogenates (23). Although bovine pulmonary artery endothelial cells actively transport 5-HT, they neither proliferate nor induce protein tyrosine phosphorylation in response to this stimulus (23). We previously demonstrated that Chinese hamster lung fibroblasts respond to 5-HT through both SERT and a 5-HT2 receptor, leading to both transmembrane signaling and proliferation (25). Similarly, in rat mesangial cells, 5-HT activates a 5-HT2A receptor through a Gq-protein–coupled pathway, leading to activation of phospholipase C, NAD(P)H oxidase, and ERK MAP kinase (26, 27).

In Chinese ovary fibroblasts expressing an exogenous 5-HT1A receptor, 5-HT also activates NAD(P)H oxidase, ROS formation, and the ERK MAP kinase through a Gi-protein–mediated pathway (28). Humblot et al. reported that, in PC12 cells, 5-HT induces tyrosine phosphorylation and subsequently activates TIS8/egr-1 and c-fos expression (29). Furthermore, Nebigil and colleagues recently showed that the 5-HT2B receptor stimulates cyclin-dependent kinase activities by at least two mechanisms: it acts through c-Src and the receptor tyrosine kinase platelet-derived growth factor receptor (PDGFR) to stimulate cyclin D1/cdk4 activity, and it acts through c-Src alone to stimulate cyclin E/cdk2 (30). However, since SERT and 5-HT receptors may coexist in a single cell type, it remains possible that some of these effects require SERT rather than the 5-HT receptor. Nevertheless, it appears that activation of either SERT or receptor by 5-HT, depending upon cell type, can initiate a signaling process that activates cell proliferation (Figure 1).

SERT in the pulmonary response to hypoxia

These observations raise fundamental questions about the functions of 5-HT receptors and transporters: Do 5-HT receptors internalize 5-HT? Do receptors and transporters expressed in the same cell interact, and, if so, how? Resolution of these issues is complicated by the long-standing assumption in the literature that 5-HT receptors, rather than transporters, stimulate proliferation (6). The article by Eddahibi and associates in this issue of the *JCI* (31) follows closely on their recent work (32) showing that hypoxia in rats upregulates SERT mRNA. Now these authors find that mutant mice lacking this transporter are protected from developing pulmonary hypertension. After excluding the possibility that hypoxic pulmonary hypertension is reduced in mutant mice because of attenuation of acute pressure changes, Eddahibi et al. argue that the 5-HT transporter is required directly in the development of this condition (31). The present data are consistent with the thesis that remodeling of the pulmonary circulation, either as a result of or contributor to increased pulmonary vascular pressure, depends on the presence of SERT. They also fit with in vitro data showing that 5-HT causes vascular smooth muscle cell proliferation and that hypoxia enhances the uptake of 5-HT (15, 21, 22). 5-HT in the circulation, probably released from platelets, likely provides the substrate for the 5-HT transporter. This hypothesis can also account for dexfenfluramine-associated pulmonary hypertension, since this drug stimulates release of 5-HT from platelets and neurons, inhibits 5-HT reuptake by platelets (33), and acts through similar signaling pathways as 5-HT to stimulate smooth muscle cell proliferation directly (S.-L. Lee et al., unpublished data).

A better conceptual framework is still needed regarding the structural identities of or relationships between the 5-HT receptor and the 5-HT transporter. More complete information is needed about the precise cellular signaling pathways of 5-HT. Perhaps most importantly, the oxidase that serves as an intermediate in this pathway and the specific ROS it generates must be characterized more fully. Finally, as a corollary, it should be determined whether any of the components of the signaling pathways could be blocked to prevent or reverse pulmonary hypertension for therapeutic benefit.

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